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***Note from the Editor**: As a consequence of the manuscripts not being delivered in due time, some communications presented at ASIC Conference in Trieste could not be included in these Proceedings.*

Coffea Genome Structure and Relationship with Evolution

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SUMMARY

Among the numerous species constituting the *Coffea* genus, only one, *C. arabica* is tetraploid (2n=4X=44) all the others are diploid but their DNA content is quite variable. Originating from East Africa and Mascarene Islands, coffee trees are found all along the forest belt that crosses the continent. Interestingly, the genome of the species found closely to the origin center have the lowest amount of DNA and oppositely, the species that "migrated" have a much bigger genome in terms of DNA content. The main reason to explain this difference can be attributed to the nature and amount of repeated sequences. It has been shown that, as all the plants so far analyzed, coffee contains many different retrotransposons. It is assumed that the movement of some of these elements and the multiplication of copies in the genome can be related to the migration of the coffee trees and the stresses encountered. The augmentation of the DNA content also define new genetic barriers as the level of sterility in F1 hybrids issued from interspecific crosses is higher when the two parental species have bigger difference in DNA content. This sterility level is tightly related to the presence of univalent chromosomes during meiosis in the gamete precursor cells.

INTRODUCTION

Coffea genus comprises over 80 species and taxons all sharing essentially the same genome with a basic chromosome number of 11 (2x=2n=22). Only one species, *C. arabica* is tetraploid (2x=4n=44). It is generally accepted to consider that *Coffea* genus is originated from East Africa (region of North Kenya, Somalia, Ethiopia) and that it spread to West Africa on one side and to Madagascar and the Mascarene Islands on the other side. In each of these regions, secondary spreads took part, increasing the genetic variability of the genus and originating new species. A survey made on some species covering the major regions of the genus extent, showed that the nuclear DNA content is quite variable, it ranges from 0,95±0,13 pg to 1,78±0.33 pg for a diploid genome (2C value). *C. arabica* has of course a higher nuclear DNA content, with 2C=2.61±0.23 pg (Cros et al., 1995). This variation in DNA content can hardly be explained by an important interspecific difference in gene number. Even if such differences exist they can only account for a very low percentage of the total variation, instead, it was shown in many plants, but also in animals, that the major part of a genome is constituted by repetitive DNA which role is not always clearly defined.(Schmidt and Heslop-Harrison, 1998). This repetitive DNA is constituted by different kind of repeated sequences, among which: the telomeric and centromeric ones, necessary for chromosome stability and replication, various long or short repeats, satellites, microsatellites, tandems or palindromes which role needs still to be elucidated, transposons and retrotransposons which role in evolution has been shown (Lyubomirskaya and Ilyin, 1999) and the ribosomal RNA coding regions.

In coffee trees, interspecific hybrids can be obtained, but the more the DNA content varies between the parents the most sterile these hybrids are because of the increasing number of univalent chromosomes appearing during meiosis. Segregation distortions are also frequent

in interspecific hybrids, and the number of distorted alleles is more important when the difference in DNA content of the parents is most important.

It is very interesting to understand the origin of the diversity of *Coffea* genomes, identify the different repeated sequences present in each or all genomes, quantify and localize them. It could be possible to identify species specific, or even chromosome specific, sequences, follow the evolution of transposons and retrotransposons among the different species and relate their presence and frequency to the evolution of the genus.

RESULTS AND DISCUSSION

A *Sau* 3A genomic library enriched in repeated sequences was constructed using nuclear DNA from a F1 hybrid issued from a cross between *C. liberica* var Dewevrei and *C. pseudozanguebariae*. 193 clones were recovered and sequencing of the clones is underway. Up to date 36 sequences have been obtained and similitude searches (BLAST) have been conducted on data banks. The length of the sequenced fragment varies from 24 to 569 bp. The majority of the sequences so far analyzed (55%), showed no similarity with any data recorded in the banks. Very few coding sequences (5%) where detected showing significant similarity with known proteins or putative proteins. 33% of the fragments were similar to recognized repeated sequences including transposable elements which represented 33% of the repeated sequences (11% of the total). In Furthermore, the two major groups of retrotransposons, namely copia and Ty3-gypsy like, were found in our library. All these results are summarized in Table 1.

Table 1. Identification of some repeated sequences found in nuclear DNA of a F1 hybrid from a cross between *C. liberica* var Dewevrei and *C. pseudozanguebariae*

Seq #	length pb.	%AT	Comments, similitudes	Seq #	length pb.	%AT	Comments, similitudes
4	266	64	Copia-like, gag-pol polyprotein	15a	29	52	cp DNA rDNA spacer
6	569	58	H.s. ?	15b	132	62	?
7a	153	64	?	16b	43	67	?
7b	140	55	URF?	17a	116	65	Ty3/gypsy like, gag-pol polyprotein
8	469	61	?	17c	47	62	?
9	70	53	?	18	90	60	?
10	186	63	?	20	182	58	rDNA spacer
11a	53	60	?	21a	45	58	?
11b	185	53	Ty3/gypsy like, Integrase	21b	58	57	?
12	513	61	Rep. Seq.	21c	132	70	?
12a	339	62	?	21d	143	59	Protein CLB1 (L. esc.)
12b	185	60	Athila like?	33	563	61	Rep. Seq.
12c	24	67	?	35	265	68	L. esculentum Rep. Seq.
12d	35	57	?	36	527	63	
13a	136	68	?	38	444	65	ADNcp?
13b	25	60	?	40	466	60	H. sapiens, seq. Rep.
13c	206	80	?	54	448	61	Rep. Seq.
14	257	65	?	55	528	64	H. sapiens

Interestingly, it appears to be very promising to study the distribution and the organization of these retrotransposons in *Coffea* genomes and relate them to the spread and species differentiation within the genus. Indeed, it has been reported that the replication of retrotransposons might be induced by stress conditions (Grandbastien, 1998). The spread of an organism to a new environment is a stressful situation for that organism, retrotransposons might be activated under these conditions, in addition to the accumulation of mutations occurring during the retrotransposons insertion in new loci, the size of the genome will increase.

These events can be repeated several times on a period of time covering hundreds thousand years leading to new species with different coding and regulating capabilities and with genomes of very different sizes in terms of DNA content (not necessarily chromosomic numbers). This situation is encountered for the *Coffea* genus, as shown on Figure 1, the species with the lowest DNA content are found in East Africa, the alleged region of origin of the genus, and the species with the higher DNA content were identified is West Africa where they spread after a long migration across the African continent. Genome changes can also occur on much lower scale due to local variation in climatic conditions (Kalendar et al., 2000), which can explain, or at least be part of, secondary speciation.

It appears very important for the understanding of Coffea evolution to better characterize the nature and the amount of the retrotransposons present.

Figure 1. spreading of the *Coffea* genus in the African continent and the Indian Ocean slands

To identify species specific repeated sequences in order to follow introgressions. Survey the distribution of repeated sequences on the chromosomes to understand segregation distortions in interspecific progenies and try to estimate recombination possibilities. Design of new strategies for *Coffea* species sustainable improvement can largely benefit from this studies.

BIBLIOGRAPHY

Cros J, Combes MC, Chabrillange N, Duperray C, Monnot des Angles A, Hamon S (1995) Nuclear DNA content in the subgenus *Coffea* (*Rubiaceae*): inter- and intra-specific variation in African species. Canadian Journal of Botany **73**: 14-20

Grandbastien M (1998) Activation of plant retrotransposons under stress conditions. Trends in Plant Science **3**: 181-187

Kalendar R, Transkanen J, Immonen S, Nevo S, Schulman AH (2000) Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proceedings of the National Academy of Sciences of the USA **97**: 6603-6607

Lyubomirskaya NV, Ilyin YV (1999) Eukaryotic mobile genetic elements: the past, the present, and the future. Molecular Biology **33**: 958-968

Schmidt T, Heslop-Harrison JS (1998) Genomes, genes and junk: the large-scale organization of plant chromosomes. Trends in Plant Science **3**: 195-199

Update on Coffee Biochemical Compounds, Protein and Gene Expression during Bean Maturation and in other Tissues

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SUMMARY

Although coffee is an important economic crop, little work has been carried out on the bean during its maturation. Through a limited number of examples, the aim of this presentation is to make an overview about changes of biochemical compounds, protein and gene expression occurring mainly during coffee bean maturation but also during germination and in other tissues.

The kinetic of accumulation of the main compounds present in the mature bean was performed separately on the three main tissues of the cherry (pericarp, perisperm and endosperm) from 15 weeks after flowering (WAF) to maturity. The relative importance of these three tissues varies during maturation principally. For example the perisperm (maternal tissue), which is the main tissue in the early stages of the bean, is progressively replaced by the endosperm. The evolution of the compounds analyzed by HPLC (i.e quinic acids, 3,5CQA, 5CQA, sucrose glucose, fructose…) is different in these three tissues, it looks similar for several genotypes of *C. canephora* provided by ICCRI, although some differences in the grain filling are observed. These results confirmed that major changes occurred during coffee bean maturation and that exchanges occurred between the main tissues of the cherry.

This point is also confirmed by 2-D gel electrophoresis, which shows significant differences in protein composition during maturation. By the 2-D approach, N-terminal sequencing of proteins and database searching, we also identify several coffee proteins, which led us to clone their corresponding cDNAs. Some of them were used as specific probes to check the expression of the corresponding genes during grain maturation and in other coffee tissue.

For example, the *csp1* gene, encoding 11S storage proteins, and the α-galactosidase gene are expressed in the endosperm but are silent in the perisperm. On the other hand, the two endo-β-mannanase genes, *manA* and *manB*, are only expressed during bean germination. Other examples of coffee genes expressed only in defined tissues like in leaves or perisperm, or on the contrary expressed in all coffee (*C. arabica* and *canephora*) tissues tested, will be shown.

For some of the cDNAs presented in this study, corresponding genes and promoters where cloned and tested in tobacco plants to check their specificity. These promoters could represent useful molecular tools to study tissue-specific gene expression in coffee plants.

INTRODUCTION

Although coffee is an important economic crop, little work has been carried out on the fruit during its maturation (Wormer, 1964; Ramaiah and Vaudeva, 1969; Dentan, 1985; Guyot et al., 1988; Rogers et al., 1999b). The filling up of the grain occurs through a sequential pattern of growth; it is associated with the biosynthesis of the compounds that are present in the mature grain. Some of these compounds, like proteins and lipids, are synthesized with the major tissue of the mature grain, i.e. the endosperm. Other compounds, like caffeine, are synthesized in the chlorophyll tissues, mainly the leaves (Ogawa et al., 2001) but also the pericarp, a maternal tissue that constitutes the major part of the cherry. Thus, the compounds synthesized in the chlorophyll tissues are transported to the grain. This process involves not only a transport in the vascular vessels, but also an important phenomenon of exchanges between the various tissues of the cherry, the pericarp, the perisperm and finally the endosperm (Figure 1).

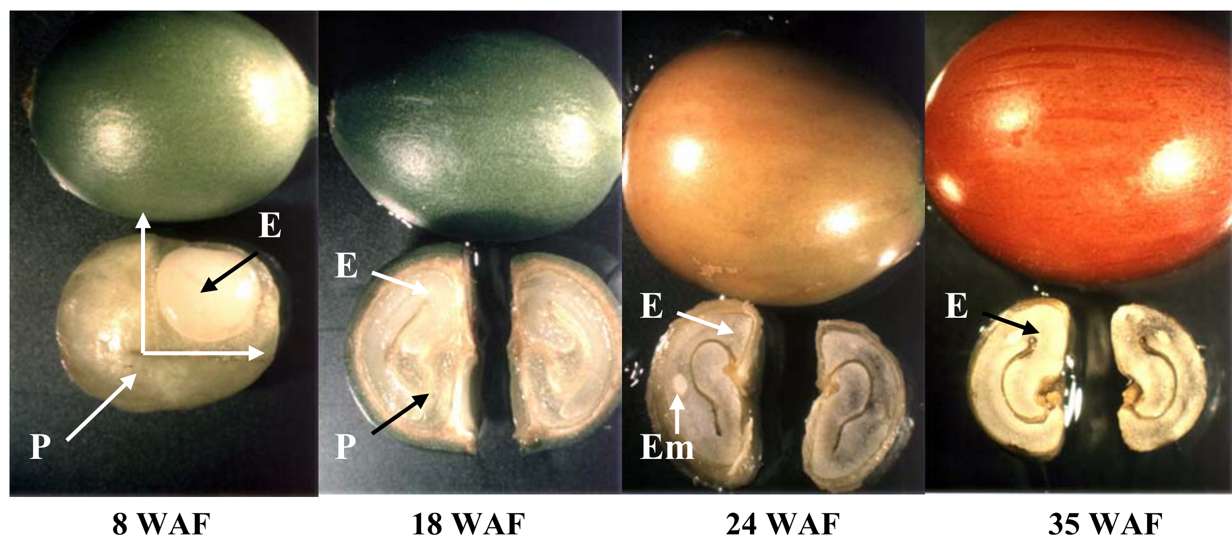

Figure 1. Tissue changes during coffee bean development. (E) endosperm; (P) perisperm, (Em) zygotic embryo. Arrows indicate the position of each tissue. White arrows at 8 WAF indicate how the perisperm has been dissected to reveal the growing endosperm. Length of the cherries: 0,8 to 1 cm at 8 WAF and 1,2 to 1,6 cm at 18, 24 and 35 WAF. Cherries were harvested from *C. arabica* var. Caturra grown in greenhouse

A better knowledge concerning to the process of transport, metabolic flux between tissues and accumulation within the endosperm is critical to identify targets for the improvement of the technological and quality traits of the mature bean. Furthermore it is a prerequisite for the identification of the genes that are involved in the elaboration of the bean characteristics.

Starting some years ago, we progressively implemented the condition for the study of these aspects. Through a limited number of examples, the aim of this paper is to make an overview about the evolution of the some morphological and chemical characteristics, as well as of some gene expression, during the maturation of the fruit, but also during the bean germination and in other tissues.

MATERIAL AND METHODS

Plant material and conditions of germination

Ripe fruit of *Coffea arabica* var Caturra 2308 were obtained from Nestlé greenhouse-grown trees (for conditions of culture see Rogers et al., 1999a).

Biochemical analysis

Sugars and organic acids were analyzed as described in Rogers et al. (1999b). Caffeine, trigonelline and chlorogenic acids were analyzed as described by Balyaya and Clifford (1995).

Measurement of protein concentration

Concentration of total protein in solution was routinely measured by the method of Bradford (1976) using BSA as a standard.

2-D electrophoresis, SDS-PAGE, gel staining and electro-blotting in preparation for amino acid sequencing

Protocols were identical to those described previously (Rogers et al., 1999a).

Isolation of RNA and construction of the cDNA library

Total RNA was extracted from different tissues from greenhouse-cultivated trees of *Coffea arabica* var. Caturra 2308 as described previously (Rogers et al., 1999a).

Northern- blotting

Total RNA (20 µg) was used to perform northern blotting as described previously (Rogers et al., 1999a). Even loading of the various RNA samples was controlled by measuring OD at 260 nm and by verification of the equal abundance of 18S and 26S rRNA (not shown). Filters were prehybridized and hybridized with the [^{32}P]-labeled probe. Radioactivity was revealed with Hyperfilm MP (Amersham Pharmacia Biotech).

EVOLUTION OF MORPHOLOGICAL CHARACTERISTICS AND MAJOR COMPOUNDS DURING COFFEE FRUIT DEVELOPMENT

In the framework of its *C. canephora* breeding program, the Indonesian Coffee and Cocoa Research Institute (ICCRI), located in East Java, has selected 12 clones for their agronomic and pest resistant characteristics.

At the pick of flowering, plagiotrops from parental plants were double tagged, on both sides of 5-7 flowering nodes. In order to get only fruits with a synchronized growth, secondary flowers were regularly removed. Starting 7 weeks after flowering (WAF) until the maturity, cherries from the tagged branches were picked each five weeks, immediately put in liquid nitrogen and shipped to France in dry ice. Grain tissues were separated, weighted and analyzed as previously described (Rogers et al., 1999b).

1-1)

As regard the evolution of the fresh weight of the cherries, the 12 clones can be classified in four groups (Figure 2).

For 8 clones, the development of the fruit is significant after 7 WAF, where as the four others show a delay of 4-5 weeks. Within each group, two sub-groups can be identified as regard the kinetic of the fruit growth. However, the maturity of the cherries from all clones occurs during the same period, between 43 and 50 WAF. When the cherries are mature, the difference between the four groups is characterized by the relative weight of the various tissues. In fact, the fresh weight of the endosperm is more or less constant, 0.4-0.5 g, where as the weight of the pericarp varies from 0.92 (group 4) to 1.46 (group 1), which corresponds to 55.4 (group 4) to 65.0% (group 1) of the weight of the cherries (Figure 3). At this stage we can notice that there is no clear evidence that a difference exists in the composition of the mature bean.

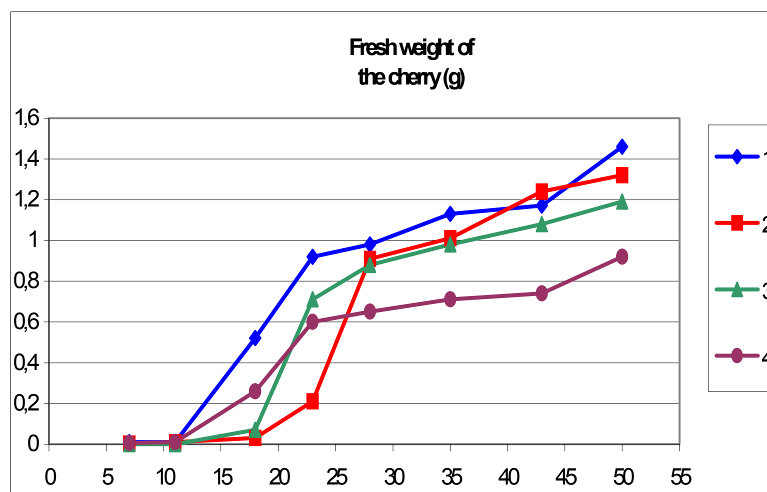

Figure 2. Evolution of weight during the maturation of the cherries Cherries are harvested from 12 different clones of *C. canephora* coming from ICCRI (Indonesia) at regular weeks after flowering (WAF)

However, three aspects, which have to be confirmed by ongoing new analysis, can be highlighted (Figure 4): (i) the endosperm of the cherries from the two groups of plants with a delay in their development, has less free sucrose; (ii) the endosperm of the cherries with the less developed pericarp has more chlorogenic acids; and (iii) in the mature endosperm, the ratio CQA/diCQA is different between the two groups of clones with an early development and the two groups of clones with a late development (data not shown).

1-2)

All compounds that were analyzed (caffeine, trigonelline, chlorogenic acids, organic acids and free sugars) are found in all tissues at any stage of the fruit development (Figure 5).

All together, their weight expressed in % of dry matter (Figure 5A), decreases during the fruit development, excepted in the pericarp where an important synthesis of sucrose occurs after 35 WAF. In the mature endosperm these compounds represent only 20% of the dry matter due to the fact that other compounds, like proteins, lipids and polysaccharides, are actively synthesized after 23-28 WAF. However, if the data are expressed in µMole per dry tissue (Figure 5B), the compounds analyzed significantly increase in the pericarp and the endosperm.

1-3)

The kinetic of evolution of the individual compounds in the fruit tissues is highly variable as shown in the four examples presented in Figure 6.

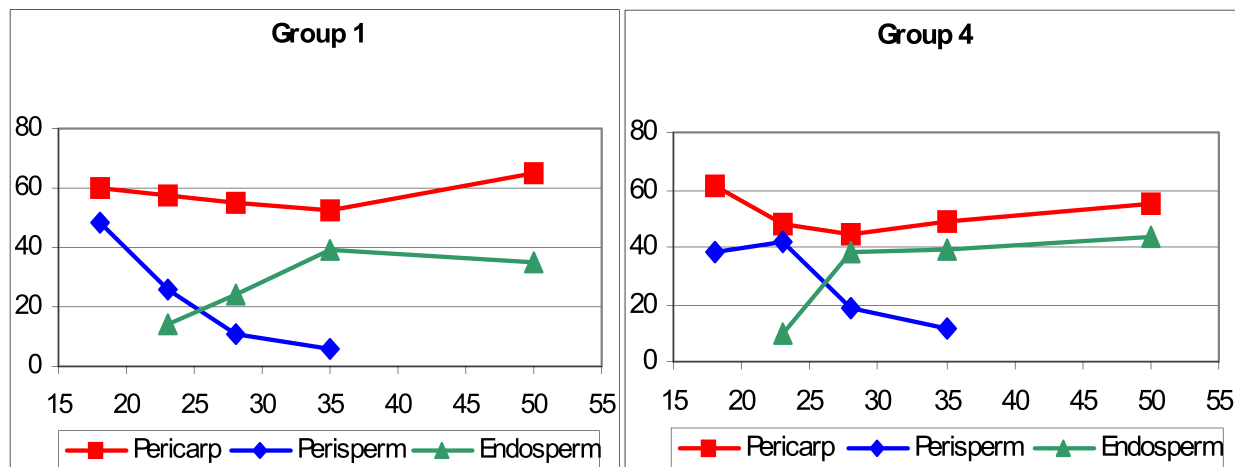

Figure 3. Evolution of tissues during the maturation of *C. canephora* cherries. Values indicate the percentage of each tissue at regular weeks after flowering (WAF) for the group 1 and 4 of *C. canephora* identified in Figure 2

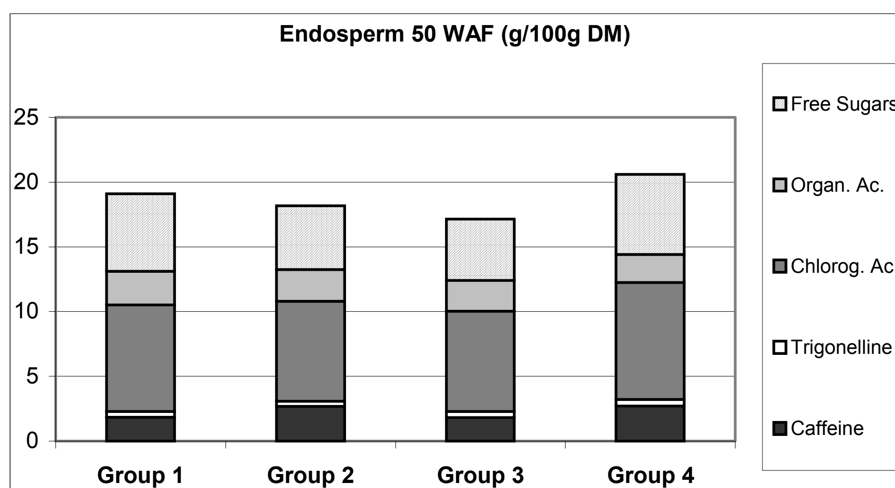

Figure 4. Amount of some compounds in the mature beans (50 WAF) of the different groups of *C. canephora* (Figure 2). Values are indicated in g. $100g^{-1}$ of dry material (DM)

Expressed in µMole per dry tissue, the caffeine continuously accumulates in the endosperm, while it is decreasing in the pericarp after 28 WAF, and it is more or less constant in the perisperm (Figure 6A). Such a situation could mean that caffeine is transported directly from the chlorophyll tissues to the endosperm. Quinic acid, which is one of the precursors for all chlorogenic acids, slightly increase in the pericarp and the endosperm, while it is dramatically and continuously decreasing in the perisperm (Figure 6B). It could suggest that quinic acid is transported through the perisperm to the endosperm where chlorogenic acids could be synthesized, as shown by the significant increase of 5CQA in the endosperm (Figure 6C). At last, sucrose, which is the most important free sugar in the fruit, slightly increases in all tissues from 18 to 35 WAF, and dramatically increases until the fruit maturity (Figure 6D). It could suggest that sucrose is transported directly to the endosperm from the chlorophyll

tissues.Whatever the diversity of situations concerning the evolution of the compounds analyzed, the key steps in the fruit development were clearly identified in our material.

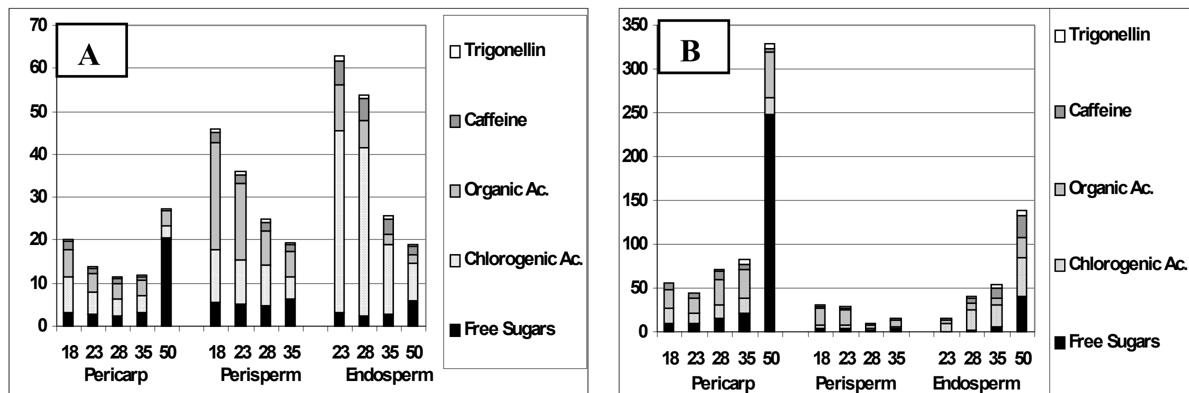

Figure 5. A and B. Analysis of compounds found in each tissue of *C. canephora* cherries during maturation. Values are indicated in % of dry material (DM) (Figure 5A) or in µM per dry tissue (Figure 5B)

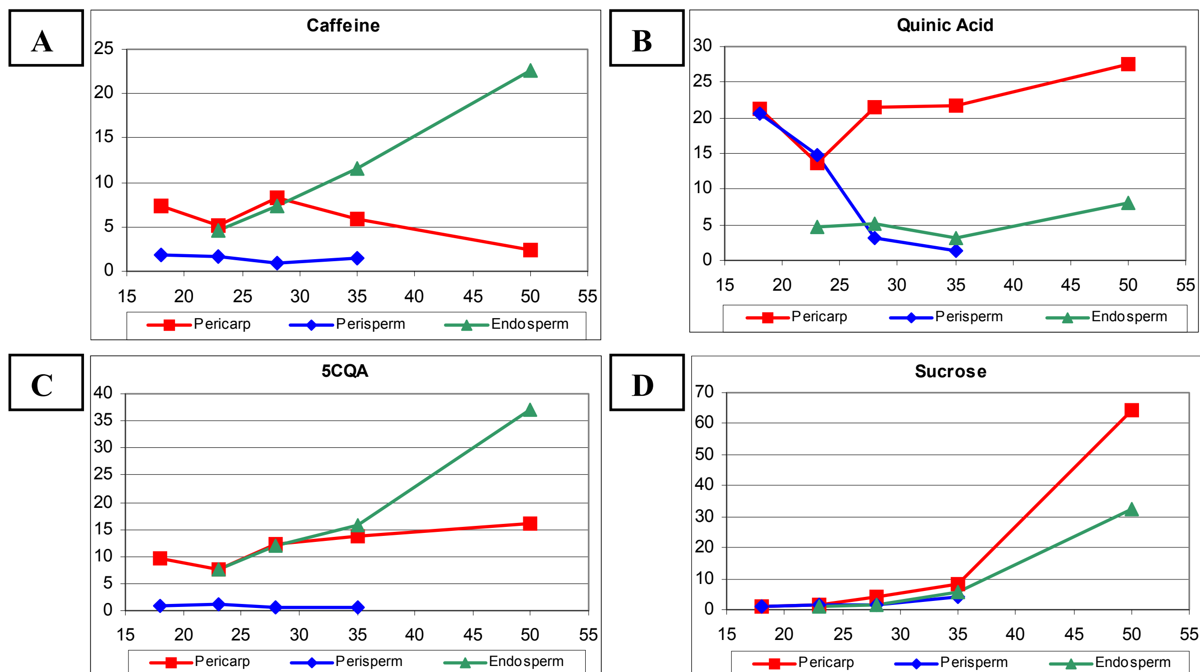

Figure 6. Kinetics of some compounds in different tissue of *C. canephora* (Group 1) cherries during maturation. Values are in µM per dry tissue at regular weeks after flowering (WAF)

From all these observation and others not shown, it make obvious that the filling up of the grain is a complex phenomenon that involves the sequential expression of a lot of genes, still to be identified.

TISSUE AND PROTEIN EVOLUTIONS DURING THE DEVELOPMENT OF COFFEE GRAIN

We have also been interested in describing the evolution of the protein profiles within the grain during its development. For that work we used the fruits of a *C. arabica* plants grown in

our tropical greenhouse. The duration of the fruit development is usually shorter for *C. arabica* and the date of occurrence of the key steps cannot be compared to *C. canephora.* In our condition, the breaking point characterizing the change between the perisperm and the endosperm in *C. arabica* takes place 12 to 16 WAF, where as it was 28 to 35 WAF for the Indonesian *C. canephora* clones. As shown by 2-D gel electrophoresis, the protein profiles are clearly specific to each stage of development. In particular, several abundant proteins observed when the perisperm predominates are absent in the endosperm (Figure 7) (see also 3-3). On the other hand, legumin (11S) storage proteins begin to accumulate when the endosperm is growing up and they account for approximately 45% of the total proteins of the mature coffee bean) (Rogers et al., 1999a). Similar observations were also made for *C. canephora* (data not shown).

ANALYSIS OF GENE EXPRESSION IN DIFFERENT COFFEE TISSUES

Another objective of our research programs is to analyze gene expression in different coffee tissues supposed to play an essential role in the synthesis of major component found in the mature bean. To do that, proteins were extracted from various coffee tissues, separated by 2-D gel electrophoresis, and some of them were characterized by N-terminal and internal sequencing (Rogers et al., 1999a). Beside this proteomic approach, RNA extractions from different tissues of a *C. arabica* var. Caturra grown in our greenhouse, were also performed and in some cases corresponding cDNA libraries were constructed (i.e. bean at regular maturation stages, leaves, grain during *in vitro* germination). This led us to clone and sequence several cDNAs that have been used for example to characterize major changes occurring during bean development.

Coffee storage proteins

In coffee beans, storage proteins are legumin (11S) like proteins encoded by a gene family. A coffee storage protein encoding cDNA and its corresponding gene (*csp1*), were cloned in our laboratory (Rogers et al., 1999a, Marraccini et al., 1999). We shown that 11S-specific mRNAs are not detected in the perisperm of *C. arabica*, but accumulate to high levels during the first stages of endosperm development (Figure 8A). RT-PCR experiments also confirmed that 11S-specific mRNAs are not found in other coffee tissues like leaves, roots, stems, flower buds, and stems for example, but are detected in *C. canephora* somatic embryos at the torpedo stage (data not shown). This latter point agrees with the fact that storage proteins are indeed detected in somatic embryos of *C. arabica* cv. Catimor at the torpedo stage (Yuffa et al., 1994).

α-D-galactosidase (EC: 3.2.1.22)

This enzyme is highly expressed in coffee beans (Courtois and Petek, 1966) and its corresponding cDNA as been cloned (Zhu and Goldstein, 1994). Using it as a probe, we showed that the α-galactosidase encoding gene is expressed during the endosperm expansion (Figure 8B) where a high α-galactosidase enzymatic activity is also detected (data not shown). No α-galactosidase enzymatic activity and mRNAs are found in the perisperm of the young bean. However, both α-galactosidase activity and their corresponding transcripts are detected in various tissues (leaves, roots, embryos, pericarp…) of *C. arabica* and *canephora* from different origins (Marraccini and Rogers, unpublished).

Perisperm-specific expression

By comparison of 2-DE profiles of different coffee tissues, we identified highly expressed perisperm-specific proteins (Figure 7), which are not present in the endosperm, pericarp and leaves from our *C. arabica* grown in greenhouse. A cDNA encoding one of these proteins has been cloned (Marraccini, unpublished). When we used it as a probe, we showed that the expression of its corresponding gene is perisperm-specific (Figure 8C), a result confirming our 2-DE observations.

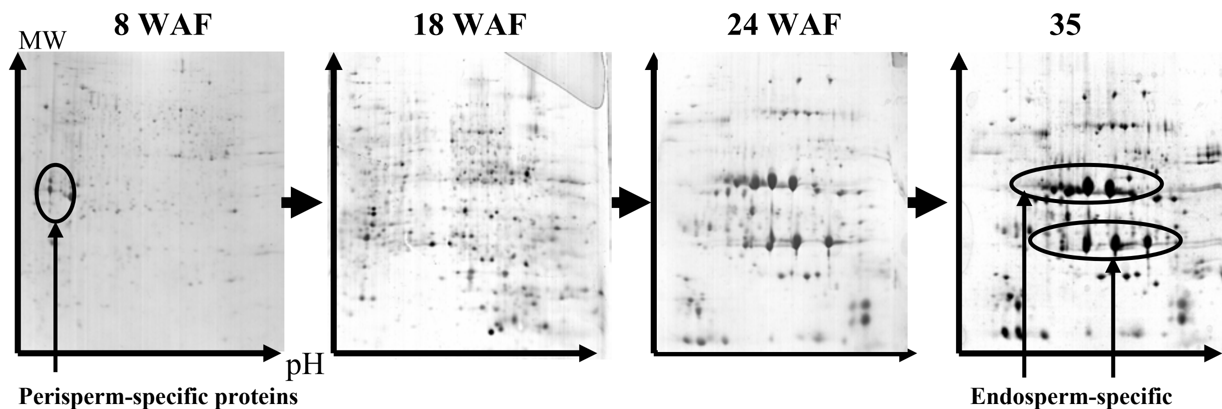

Figure 7. Evolution of proteins during coffee bean development. Proteins were extracted from bean of *C. arabica* var. Caturra grown in greenhouse, analyzed by 2-dimensional gel electrophoresis as described before (Rogers *et al.* 1999a) and gels (pI 3-10 linear) were silver-stained

Mannanase (EC 3.2.1.78: [1->4]-β-mannan endohydrolase)

We were also interested to characterize the endo- -mannanase activity during grain germination and to clone its corresponding mRNAs during coffee bean germination. The endo-β-mannanase activity is likely to be central to the metabolism of cell wall mannans during the germination of grains of coffee. Two endo-β-mannanase cDNAs (termed *manA* and *manB*) were cloned by different strategies from *C. arabica* (Marraccini et al., 2001). ManA (Genebank: AJ293305) and B (Genebank: AJ278996) proteins share about 56% sequence identity. During germination, the peak endo-β-mannanase activity occurred at approximately 28 DAI (days after imbibition) and was not detected in grains prior to imbibition (i.e mature beans) (data not shown). Northern hybridizations with *manA*- and *manB*-specific probes showed that mRNA transcripts for both cDNAs were present at the same periods of bean germination with transcript peaks at 22 days after imbibition of water (Figure 9). In addition, the expression of coffee mannanase encoding genes appears to be specific of the germination step because corresponding transcripts were not detected during grain maturation or in the other tissues such as roots, stems, flowers and leaves (Figure 10). The identification of these two coffee mannanase-encoding cDNA now open the way to further characterization of these enzymes (kinetic analyses, substrate specificity…) after expression in heterologous systems.

Leaf-specific expression

As performed earlier, comparison of 2-DE profiles also led us to identify a leaf-specific protein corresponding to the small subunit of the ribulose1,5-bisphosphate carboxylase (Rubisco: EC 4.1.1.39) (data not shown). To a lesser extend, this protein is also present in green pericarps, which agrees with a recent publication (Lopez et al., 2000), but undetectable

in grain tissues (zygotic embryo, perisperm) whatever their maturation stage. Expression analysis showed that RbcS-encoding genes are highly expressed in coffee leaves of different length, but not in flowers, somatic embryos, stems and in other (non-photosynthetic) tissue tested such as in roots and mature grain for example (Figure 11).

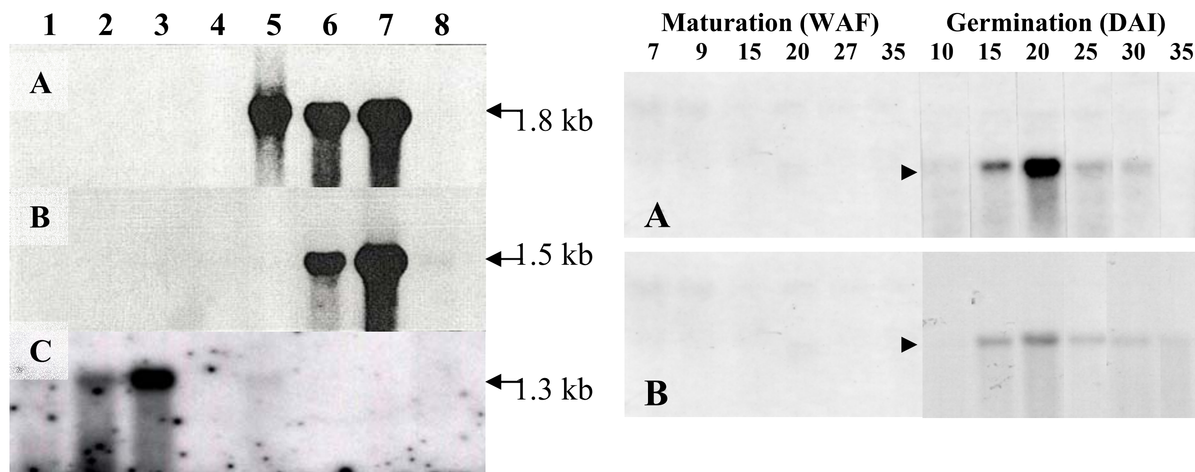

Figure 8. (Left) Northern-blot analysis of different genes expressed during grain development of *C. arabica* var. Caturra. Total RNAs were probed successively by ^{32}P-labelled cDNA encoding 11S-storage proteins (A), α-galactosidase (B) and a perisperm-specific protein (C). RNA preparations were made from grains harvested at 4 WAF (lane 1); 7 WAF (lane 2); 9 WAF (lane 3); 14 WAF (lane 4); 18 WAF (lane 5); 22 WAF (lane 6); 27 WAF (lane 7) and 35 WAF (lane 8). RNA molecular weights (in kb) are also indicated

Figure 9. (Right) Northern-blot analysis of total RNAs from various stages of grain maturation (WAF) and germination (DAI). The positions of abundant ribosomal RNA 28S and 18S are indicated (grey arrows) as well as the *manA* (A) and *manB* (B) -specific transcripts (black arrows). Grains were from *C. arabica* var. Caturra grown in greenhouse

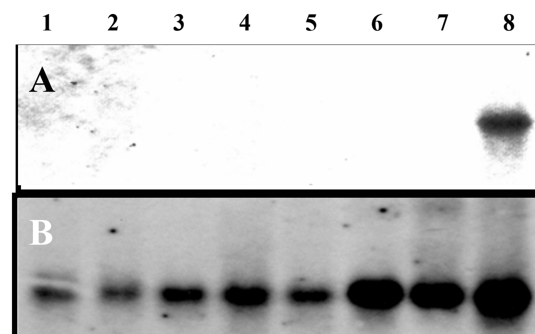

Figure 10. A, B. Northern-blot analysis of total RNAs from various coffee tissues probed with *manA* cDNA (A) and *GOS2* cDNA (B). Lanes: 1, young leaves* (< 1 cm); 2, medium leaves*; 3, old leaves*; 4, flowers buds*; 5, roots*; 6, somatic embryos (*C. canephora* 126); 7, mature grains (35 WAF); 8, germinated grains (22 DAI).*: from *C. arabica* var. Caturra grown in greenhouse

The corresponding *rbcS1* cDNA, as well as its gene and a 1-kb genomic fragment containing the promoter region were also cloned. This promoter contains several Light-Responsive Elements (LRE) well known to be implicated in the transcriptional control of gene expression by light in other higher plants (Argüello-Astorga and Herrera-Estrella 1998). It is also able to target the expression the *uidA* (GUS) reporter specifically in leaves of transformed tobacco plants and to light-regulate the expression of this reporter gene (Marraccini, unpublished).

Figure 11. Detection of *rbcS*1 transcripts by Northern-blot. Total RNAs were probed with the 3' end of the *rbcS*1 ^{32}P-labelled. Lanes (1) young leaves*; (2) medium leaves*; (3) old leaves*; (4) mature flowers*; (5) stems*; (6) flower buds*; (7) roots*; (8) mature grains* (35 WAF); (9) somatic embryos of *C. arabica* hybrid ET29xCa5 (kindly given by D^{r}. T. Leroy, CIRAD, Montpellier, France). *: from *C. arabica* var. Caturra grown in greenhouse

Housekeeping functions

We also cloned the coffee *GOS2* cDNA as well as its corresponding gene, and use its mRNA as an internal control of coffee gene expression (Figure 10). Indeed, this cDNA encodes a protein implicated in the cell translation (protein synthesis) process (de Pater et al., 1992). Expression analysis confirmed that this gene is highly transcribed in all the *C. arabica* and *canephora* tissues analyzed, suggesting that it is under the control of a strong and constitutive promoter, like described in rice (de Pater et al., 1992).

CONCLUSION

At the molecular and proteomic point of view, our analyses clearly reinforce the fact that major tissue changes occur during the development of coffee grain. In the example described here, we show that expression of genes as well as the presence of corresponding proteins is clearly compartmentalized to the perisperm or endosperm tissues. We showed the existence of very well separated kinetics of gene expression for the genes encoding major proteins of the coffee bean and that there is a tight coupling between gene transcription and translation. Despite the fact that the coffee bean development is very long, the situation that we describe here is like observed in other plants (Shirsat, 1991; Parcy et al., 1994). With our examples, we also observed that gene expression is very reduced in the last weeks of bean maturation (i.e. red cherries) probably because this developmental stage corresponds to an active dehydration phase. This is in agreement with other work showing a rapid and important increase of this RNA synthesis in coffee endosperm over the first days after imbibition (Giorgini, 1988). Since this information was not yet available, it could now help us to define better the bean developmental stages for the construction of cDNA libraries.

For some of the cDNAs presented in this study, corresponding genes and promoters were cloned and sequenced. Some of these promoters were also tested in tobacco plants to check their relative strength by comparison to the CaMV 35S promoter as well as their tissue-specificity, as it has been done previously (Gaitan, 1998; Marraccini et al., 1999). They could

represent useful molecular tools to study further tissue-specific gene expression in coffee plants.

REFERENCES

Argüello-Astorga G., Herrera-Estrella L. (1998) Evolution of light-regulated plant promoters. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49**: 525-555.

Balyaya K.J., Clifford M.N. (1995) Individual chlorogenic acids and caffeine contents in commercial grades of wet and dry processed Indian green robusta coffee. Food Sci. Technol. **32**: 104-108.

Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem **2**: 248-254.

Courtois J. E., Petek F. (1966) α-Galactosidase from coffee beans. Meth Enzymol **8**, 565-571.

Dentan E. (1985) Etude microscopique du développement et de la maturation du grain de café. ASIC, 11° Colloque, Lomé 381-389.

Gaitan A.L. (1998) Cloning and evaluation of gene promoters in *Coffea* spp. Ph.D dissertation Cornell University.

Giorgini J.F (1988) Ribonucleic acid characterization and synthesis in germinating coffee seed endosperm. Braz. J. Med. Biol. Res. **21**: 811-824.

Guyot B., Petnga E., Vincent J.C. (1988) Analyse qualitative d'un café, *Coffea canephora* var Robusta, en fonction de la maturité. 1; Evolution des caractéristiques physiques, chimiques et organoleptiques. Café Cacao Thé **32**: 127-140.

Lopez Y., Riano N., Mosquera P., Cadavid A., Arcila J. (2000) Activities of phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase/oxygenase in leaves and fruit pericarp tissue of different coffee (*Coffea* sp.) genotypes. Photosynthetica **38**: 215-220.

Marraccini P., Deshayes A., Pétiard V., Rogers W. J. (1999*)* Molecular cloning of the complete 11S seed storage protein gene of *Coffea arabica* and promoter analysis in transgenic tobacco plants. 1999. Plant Physiol. Biochem. **37**: 273-282.

Marraccini P., Rogers W.J., Allard C., André M-L., Caillet V., Lacoste N., Lausanne F., Michaux S. (2001) Molecular and biochemical characterization of endo-β-mannanases from germinating coffee (*Coffea arabica*) grain. Planta (in press).

Ogawa M., Herai Y., Koizumi N., Kusano T., Sano H. (2001) 7-methylxanthine methyltransferase of coffee plants. J. Biol. Chem. **276**: 8213-8218.

Parcy F., Valon C., Raynal M., Gaubier-Comella P., Delseny M., Giraudat J. (1994) Regulation of gene expression programs during *Arabidopsis* seed development: roles of the *ABI3* locus and endogenous abscisic acid. Plant J. **6**: 1567-1582.

de Pater S., van der Mark F., Rueb S., Katagiri F., Chua N-H., Schilperoort R.A., Hensgens L.A.M. (1992) The promoter of the rice gene *GOS2* is active in various different monocot tissues and binds rice nuclear factor ASF-1. Plant J. **2**: 837-844.

Ramaiah P.K., Vasudeva N. (1969) Observations on the growth of coffee berries in South India. Turrialba **19**: 455-464.

Rogers W. J., Bézard G., Deshayes A., Pétiard V., Marraccini P. (1999a) Biochemical and molecular characterization and expression of the 11S-type storage protein from *Coffea arabica* endosperm. Plant Physiol. Biochem. **37**: 261-272.

Rogers W. J., Michaux S., Bastin M., Bucheli P. (1999b) Changes to the content of sugars, sugar alcohols, myo-inositol, carboxylic acids and organic anions in developing grains from different varieties of Robusta (*Coffea canephora*) and Arabica (*C.arabica*) coffees. Plant Sci. **149**: 115-123.

Shirsat A.H. (1991) Control of gene expression in the developing seed, *in*: Grierson D. (Ed), Developmental regulation of plant gene expression, Chapman and Hall, New York, pp 153-181.

Wormer T.M. (1964) The growth of the coffee berry. Ann. Bot. **28**: 47-55.

Yuffa A.M., de Garcia E.G., Nieto M.S. (1994) Comparative study of protein electrophoretic patterns during embryogenesis in *Coffea arabica* cv Catimor. Plant Cell Rep. **13**: 197-202.

Zhu A., Goldstein J. (1994) Cloning and functional expression of a cDNA encoding coffee bean α-galactosidase. Gene **140**: 227-231.

Towards Identification and Characterization of Candidate Genes Involved in Coffee Cup Quality

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SUMMARY

A project on the genetics of biochemical compounds involved in the cup quality of coffee was initiated by IRD (ex ORSTOM) in 1994. The major compounds studied were: sucrose, chlorogenic acids (CGA), caffeine and trigonelline. The analysis was done on the offspring of an interspecific cross between *C. pseudozanguebariae* (PSE) and *C. liberica* var. *dewevrei* (DEW). PSE is a wild species with a short fruit maturation period (2.5 months) and differs from DEW for some biochemical compounds related to the cup quality: no caffeine and low content in CGA was evaluated in PSE green beans, whereas high contents of sugar and trigonelline was observed. The role of environment and genetic effects has been shown by traditional quantitative genetics. An AFLP genetics map was obtained using this cross and QTL were located for caffeine, CGA and trigonelline accumulation.

The project currently focuses on CGA and caffeine, both responsible for bitterness. A new cross between PSE and *C. canephora* (CAN), a highly caffeine and CGA producing species, is now being mapped. A biochemical evaluation is also underway. Two cDNA libraries have been obtained from CAN leaves and beans harvested at different maturation stages. Genes involved in the control of caffeine and CGA content are being investigated. The first approach used consists in looking for equivalent heterologous sequences to design specific primers deduced from conserved domains of such genes. The corresponding fragments will be amplified and used as probes to find a possible correspondence with the QTL. If such an equivalence was found, the corresponding genes will be isolated and characterized. An alternate approach is also planed by using differential screening of cDNA libraries established from tissues of different species at different developmental stages.

RÉSUMÉ

Un projet sur la génétique des composés biochimiques impliqués dans la qualité à la tasse du café a été initié à l'IRD (ex ORSTOM) en 1994. Les principaux composés étudiés sont: le saccharose, les acides chlorogéniques (ACG), la caféine et la trigonelline. L'analyse a été faite sur la descendance d'un croisement interspécifique entre *C. pseudozanguebariae* (PSE) et *C. liberica* var. *dewevrei* (DEW). PSE est une espèce sauvage possédant une courte période de maturation des graines (2 mois et demi). Elle diffère de DEW pour la composition biochimique de ces graines en ce qui concerne les composés liés à la qualité du café-boisson. Si l'on ne trouve pas de caféine et si la teneur en ACG y est faible, en revanche, les teneurs en sucres et en trigonelline sont élevées. L'impact relatif du milieu et des gènes a été montré par génétique quantitative. Une carte génétique AFLP a été obtenue en utilisant le croisement (PSExDEW)xDEW et des QTLs ont été localisés pour les teneurs en caféine, en ACG et en trigonelline.

Actuellement, le projet s'intéresse particulièrement aux ACG et à la caféine, tous deux impliqués dans l'amertume du café-boisson. Un nouveau croisement entre PSE et *C. canephora* (CAN), fort producteur de caféine et d'ACG, est en cours de cartographie. Une évaluation biochimique est également commencée. Deux banques d'ADNc ont été obtenues à partir de feuilles de CAN et de graines récoltées à différents stades de maturation. Les gènes impliqués dans le contrôle de la teneur en caféine et ACG sont recherchés. La première approche utilisée consiste à trouver des séquences hétérologues équivalentes pour définir des amorces spécifiques déduites des domaines conservés de ces gènes. Les fragments correspondants seront amplifiés et utilisés comme sondes pour trouver une correspondance possible avec des QTLs. Si une telle co-localisation est trouvée, les gènes correspondants seront isolés et caractérisés. Une autre approche envisagée consiste à utiliser une double comparaison soustractive de banques d'ADNc, établies à partir de tissus de deux espèces différentes à deux stades différents de développement.

INTRODUCTION

Coffee cup quality is based on the characterization of a large number of factors including taste and aroma. These factors are related to the biochemical content of roasted beans. A thousand of compounds, appearing during roasting, are involved in coffee cup quality. These compounds rise from a smaller number of biochemical compounds present in green beans. Their presence could have a favorable effect on the coffee cup quality, as for trigonelline and sugars, or an unfavorable one, as for chlorogenic acids and caffeine (Clifford, 1985; Macrae, 1985).

A modification of these compounds in the bean may have an effect, positive or negative, on the coffee cup quality. To improve coffee cup quality, we could increase the synthesis of favorable compounds or decrease the synthesis of negative compounds. In this work, we were only interested in the decrease of negative compounds. Compound level vary according to enzyme activity involved in the biochemical pathways. Corresponding enzymes are themselves regulated by genes for their structure and regulation. Finally, modifying gene expression, particularly for genes implicated in the biochemical compound content, may be useful for the improvement of the coffee cup quality. For doing this, two alternative proceedings are available: reducing gene expression involved in the biosynthesis of unfavorable compounds, which will limit their synthesis, or enhancing the expression of genes involved in their catabolism, which will increase their degradation.

The identification of such genes, candidate genes, requires knowledge of the compound content inheritance. In other words, it firstly requires a biochemical analysis in order to evaluate the biochemical diversity in the genus. Secondly, a genetic analysis of interspecific cross offspring will indicate inheritance. Parental species were those that appeared interesting during the biochemical analysis. Thirdly, genetic mapping and QTL location will be carried out.

RESULTS

Biochemical analysis

Compound content analysis was carried out on green beans harvested on the cultivated species, *C. canephora* and *C. arabica* (Figure 1).

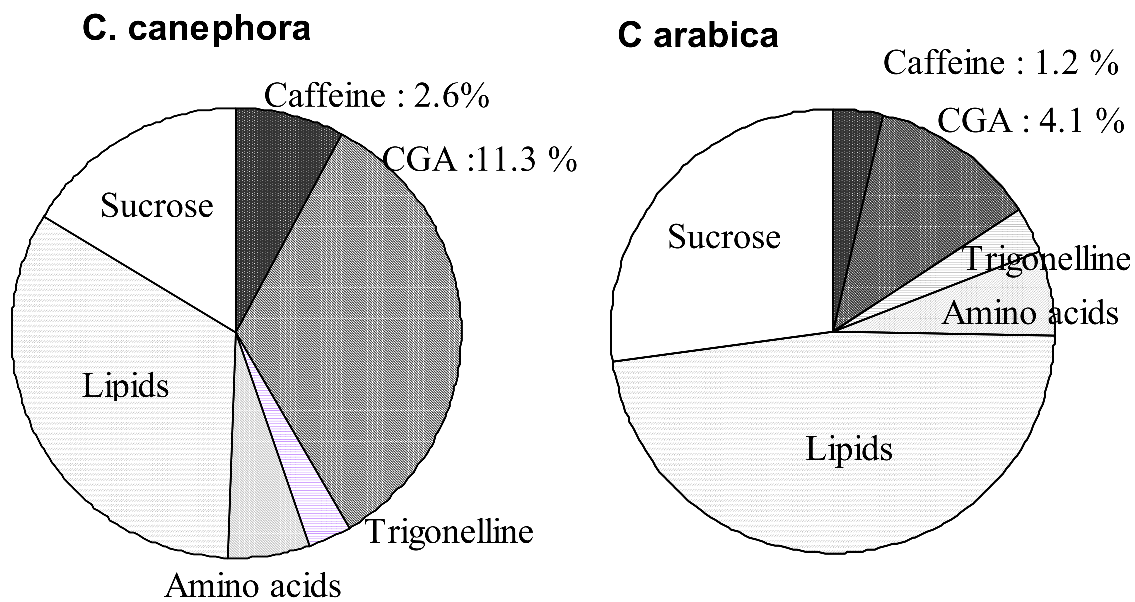

Figure 1. Biochemical content of green beans of *Coffea arabica* and *Coffea canephora*

The major studied compounds were sucrose, lipids, chlorogenic acids (CGA), caffeine and trigonelline. More than 80% of the *C. arabica* bean content (on dry matter basis: dmb) consisted in compounds such as sucrose, lipids, amino acids and trigonelline showing positive effect on coffee cup quality. In *C. canephora* beans, these compounds represented only 58% (dmb) of the biochemical content. At the opposite, the negative compounds content, as caffeine and chlorogenic acids, was more than two-fold in *C. canephora* beans.

The biochemical diversity in the genus *Coffea* was studied for chlorogenic acids and caffeine in green beans (Anthony et al., 1993). Compared to the cultivated species, several wild species exhibited large differences in caffeine and chlorogenic acid contents. Two species were interesting (Figure 2): *C. pseudozanguebariae* (PSE) (low levels in chlorogenic acids and caffeine) and *C. liberica* var. *dewevrei* (DEW) (high chlorogenic acid content and a caffeine level close to *C. arabica*). To develop the genetics of corresponding biochemical compounds, the interspecific offspring (PSE x DEW or PSE x CAN) were used.

Genetic analysis

Inheritance of compound content has been evaluated using the cross between PSE and DEW. Only the caffeine and chlorogenic acid content studies are reported here. Caffeine content was under a polygenic control with a recessive Mendelian gene coding for the absence. Barre et al. (1998) reported a weak effect of environment (6%) on the bean caffeine content. The polygenic control and additivity were also observed for chlorogenic acid content. Ky et al. (1999) showed that inheritance was polygenic and influenced by environment (20-50%). The same type of analysis was done in the offspring for sugar and trigonelline (Ky et al., 2000a; Ky et al., 2001). An AFLP genetic map has been obtained and quantitative trait loci (QTL) have been located for caffeine, CGA and trigonelline accumulation (Ky et al., 2000b).

Identification of candidate genes

Our project focuses on CGA and caffeine, both involved in bitterness and the cross between *C. pseudozanguebariae* (PSE) and *C. canephora* (CAN), very different for bean compound contents (Figure 2). This cross is also being mapped and a biochemical evaluation is also underway.

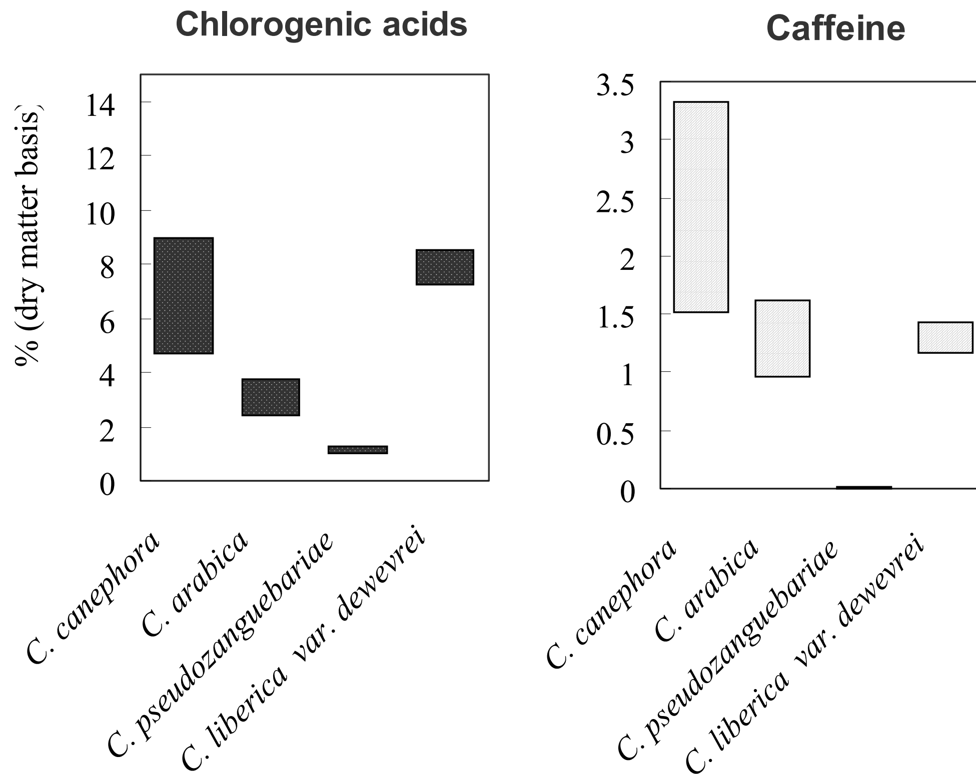

Figure 2. Variations in chlorogenic acid and caffeine content in green beans of different *Coffee* species

A first approach was developed to investigate genes involved in the control of caffeine and CGA content. It consists into the search of heterologous sequences on data libraries. Consensus primers were obtained by comparing gene sequences available from other plants and by identifying their conserved domains. These primers will be used to amplify genomic DNA with different PCR techniques and the corresponding fragments will be used as probes. Gene was then mapped in order to find a possible co-location with the QTL yet described. In case of co-location, the corresponding genes will be considered as candidate gene, then isolated and characterized. The corresponding enzymes will be studied and the modification of gene expression will be checked by genetic transformation.

CGA and caffeine have negative effect on coffee cup quality, but they have a positive role in resistance to diseases. Then, modification must be done only in seeds. For this, the best tool seems to plan a genetic transformation *via Agrobacterium tumefaciens* carrying a plasmid containing a cassette with a fruit-specific promotor gene, the gene of interest and a terminator. In conclusion, preliminary results for five enzymes of the biosynthesis pathway, the amplification of genomic DNA from *C. canephora* with consensus primers conduced to the isolation of homologous probes. Analysis of genetic diversity and mapping are now initiated.

A double differential screening of cDNA libraries (or protein profiles obtained by two dimensional electrophoresis) was also developed. Two cDNA libraries were obtained from CAN, one from leaves and the other from beans at different maturation stages. The same procedure will be done for PSE.

REFERENCES

Anthony F., Clifford M.N., Noirot M., 1993 Biochemical diversity in the genus Coffea L.: chlorogenic acids, caffeine and mozambioside contents. Genetic Resources and Crop Evolution, **40**: 61-70.

Barre P., Akaffou S., Louarn J., Charrier A., Hamon S., Noirot M., 1998. Inheritance of caffeine and heteroside contents in an interspecific cross between a cultivated coffee species *Coffea liberica* var "*dewevrei*" and a wild species caffeine free *C. pseudozanguebariae*. Theor. Appl. Genet., **96**: 306-311.

Clifford M.N., 1985. Chlorogenic acids. In : R.J. Clarke and R. Macrae (eds), Coffee, Volume **1**: Chemistry, pp. 153-202. Elsevier Applied Science, London.

Ky C.L., Barre P., Lorieux M., Trouslot P., Akaffou S., Louarn J., Charrier A., Hamon S., Noirot M., 2000b. Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (Coffea sp.). Theor. Appl. Genet.,..

Ky C.L., Doulbeau S., Guyot B., Charrier A., Hamon S., Louarn J., Noirot M., 2000a. Inheritance of sucrose content in the interspecific cross: *Coffea pseudozanguebariae* x *Coffea liberica* var "*dewevrei*". Plant Breeding, **119**: 165-168.

Ky C.L., Guyot B., Louarn J., Hamon S., Noirot M., 2001. Trigonelline inheritance in the interspecific cross between *Coffea pseudozanguebariae* x *Coffea liberica* var "*dewevrei*". Theor. Appl. Genet.,.

Ky C.L., Louarn J., Guyot B., Charrier A., Hamon S., Noirot M., 1999. Relations between and inheritance of chlorogenic acid content in an interspecific cross between *Coffea pseudozanguebariae* and *Coffea liberica* var "*dewevrei*". Theor. Appl. Genet., **98**: 628-637.

Macrae R., 1985. Nitrogenous compounds. In : R. J. Clarke and R. Macrae (eds), Coffee, Volume **1**: Chemistry, pp. 115-152. Elsevier Applied Science, London.

Potential use of SSR markers for *Coffea* spp. genetic mapping

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SUMMARY

Microsatellites, also called Simple Sequence Repeats (SSRs), are versatile molecular markers that are widely spread in plant and animal genomes. Their interest resides in the fact that they are co-dominant and locus-specific.

Among plants, they have been used to establish and saturate genetic maps for less than 10 years, in complement to RFLP, AFLP, RAPD and isozymes. SSRs have already been used for coffee genetic studies; here, they will also allow good definition of QTLs that will be looked for in different progenies.

A *Coffea canephora,* Clone 126, genomic DNA library has been obtained using *Rsa* 1 endonuclease and enriched with GA and GT sequences using streptavidin-coated magnetic particles.

More than 800 clones have been obtained and sequenced. After elimination of redundants, several hundreds of primer pairs are expected to be designed and effectively available for detecting SSR loci by PCR amplification. Those loci will be mapped for *Coffea* inter and intra-specific crosses.

RÉSUMÉ

Les microsatellites, encore appelés SSRs (Simple Sequence Repeats), sont des marqueurs moléculaires pratiques qui sont largement répandus dans les génomes animaux et végétaux. Leur intérêt réside dans le fait qu'ils sont locus-spécifiques et co-dominants.

Chez les plantes, ils ont été utilisés pour établir et saturer des cartes génétiques depuis moins de 10 ans, en complément des marqueurs RFLP, AFLP, RAPD et isoenzymatiques. Les microsatellites ont déjà été utilisés pour des études génétiques du caféier. Dans ce travail, ils nous permettront une bonne définition des QTL que l'on recherchera dans différentes descendances.

Une banque génomique de *Coffea canephora* (Clone 126) a été obtenue avec l'endonucléase *Rsa* 1 et enrichie en séquences GA et GT avec des particules magnétiques recouvertes de streptavidine. Plus de 800 clones ont été obtenus et séquencés. Après élimination des redondances, on espère construire plusieurs centaines de paires d'amorces et les utiliser pour

la détection des loci SSR par amplification PCR. Ces loci seront placés sur des cartes de croisements inter et intra-spécifiques de *Coffea.*

INTRODUCTION

Microsatellites, also called Simple Sequence Repeats (SSRs), or Variable Number of Tandem Repeats (VNTRs) are versatile molecular markers that are widely spread in plant and animal genomes. Their interest resides in the fact that they are co-dominant and locus-specific and also that they seem to be evenly spread out along coding (to a lesser extent) and non-coding regions of most Eukaryots and therefore of plants (Wang et al., 1994). They are also found in mitochondrial and chloroplastic genomes (Ennos et al., 1999) and comprise a variable number of tandem repeats from one to six nucleotides long (Tautz, 1989). They are highly polymorphic due to variations in the number of repeated monomers; dinucleotide repeats are most abundant in plants, (GA) and (AT) being predominant (Wang et al., 1994; Primmer et al., 1997).

Another interest is that they are PCR-based, that is, they can be detected by the Polymerase Chain Reaction using primer pairs complementary to their flanking regions.

Reports about microsatellite analysis abound among annuals but are starting to be more frequent for woody perennials. They are useful in population genetics where they can be used for: determining genetic structure, identify clones, parenthood and genetic distances calculation (Ashkenazi et al., 2001), or gene flow analysis (Steinkellener et al., 1997).

For about 10 years, they have been recommended for use in the construction of highly saturated genetic maps in complement to RFLP, AFLP, RAPD and isozymes (Beckmann and Soller, 1990) and QTL detection. They have actually been used in mapping a large number of plant species, for example: maize (Senior et al., 1996), rice (Wu and Tanksley, 1993), soybean (Akkaya et al., 1995), and of course Arabidopsis (Brandes et al., 1997).

SSRs have already been used for coffee genetic studies. First, Metullio et al. (1999) and Rovelli et al. (2000) identified 69 $(ATC)_n$ microsatellite loci and 180 $(TG)_n$ loci in *C. arabica* enriched genomic libraries. Not all of them were polymorphic, but it was the first work of this kind described on coffee.

Another work dealing with the application of SSR markers for genetic and diversity studies in coffee was presented by Combes et al. (2000). They used $(TG)_n$ microsatellites in *C. arabica* and related species. This work reveals that although developped in *C. arabica*, most of 11 primers used cross-amplify with diploid species.

Recently, SSRs have also be used to assess genetic stability of somatic embryos-derived plants (Rani et al., 2000), especially at the mitochondrial level.

In the work we present here, our aim is to develop a set of molecular markers (SSRs) suitable for genetic mapping of *Coffea canephora*

MATERIALS AND METHODS

Coffea canephora L., clone 126 has been used in this study.

DNA was extracted from fresh leaves from greenhouse grown plants according to the protocole described by Risterucci et al. (2000).

A genomic DNA library has been obtained using *Rsa* 1 endonuclease and enriched with $(GA)_n$ and $(GT)_n$ sequences using streptavidin-coated magnetic particles and biotin-labelled microsatellite oligoprobes. The clones were screened with 32P-labelled probes.

768 detected clones have been sequenced using automated sequencerS (Licor, Abi 3700, Megabace). After elimination of redundants and unusable sequences, presence of SSR has been checked.

RESULTS AND DISCUSSION

We succeeded in creating a *Coffea canephora* genomic library enriched with GA and GT oligonucleotides. The percentage of enriched clones was about 58%.

A total of 2016 clones were screened and more than 900 clones gave a positive response. 768 positive clones were sequenced.

About 545 non redondant sequences containing SSRs were obtained.

Oligonucleotide primers complementary to flanking regions of identified repeats must be designed in order to reliably detect microsatellites loci. To that respects, we will use Primer 3 software. They will be first tested on genomic DNA of *C. canephora*.

Potentially 200 to 300 primer pairs can reveal polymorphic SSR markers.

CONCLUSIONS

SSRs are very versatile markers that require small amounts of DNA. Therefore they can be useful for analyses of young plants, allowing to save time in the study of perennial crops with long growing cycle like coffee.

We plan to use the markers we will obtain for construction of *C. canephora* intra- and inter-specific linkage maps or saturation of already existing coffee maps. In return, those maps will be useful to breeders as providing a sound base for marker assisted selection (MAS - Lashermes et al., 2000) for quick identification of individuals harbouring genes of interest. Moreover, as based on the polymerase chain reaction, SSR markers will be easy to implement near coffee growing areas.

REFERENCES

Akkaya M.S., Shoemaker R.C., Specht J.E., Bhagwat A.A., Cregan P.B., 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci. **35**: 1439-1445.

Ashkenazi V., Chani E., Lavi U., Levy D., Hillel J., Veilleux R.E., 2001. Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. Genome **44**: 50-62.

Beckmann J.S. and Soller M., 1990. Toward a unified approach to genetic mapping of eucaryotes based on sequence tagged microsatellite sites. Bio/Technology (New York) **8**: 30-32.

Brandes A., Thompson H., Dean C. Heslop-Harrison J.S., 1997. Multiple repetitive DNA sequences in the paracentromeric regions of *Arabidopsis thaliana* L. Chromosome Res. **5**: 238-246.

Combes M. C., Andrzejewski S., Anthony F., Bertrand B., Rovelli P., Graziosi, G., Lashermes P., 2000. Characterization of microsatellite loci in *Coffea arabica* and related coffee species. Molecular ecology, **9**: 1171-1193.

Ennos R.A., Sinclair W.T., Hu X.S., Langdon A., 1999. Using organelle markers to elucidate the history, ecology and evolution of plant populations, pp 1-19 in: Molecular Systematics and Plant Evolution, ed. by P.M. Hollingsworth, R.M. Bateman, R.J. Gornall. Taylor & Francis limited, London.

Lashermes P., Combes M. C, Topart P., Graziosi, G., Bertrand B., Anthony F., 2000. Molecular breeding in coffee (*Coffea arabica* L.). In: Sera T., Soccol C.R., Pandey A., Roussos S. (eds.) Coffee Biotechnology and Quality, pp 101-112. Kluwer Academic Publishers, Dordrecht (The Netherlands).

Mettulio R., Rovelli P., Anthony F., Anzueto F., Lashermes P., Graziosi G., 1999. Polymorphic microsatellites in *Coffea arabica.* In: ASIC (eds), 18th colloquium, Helsinki (Fin), pp 344-347. ASIC, Paris (France).

Primmer C.R., Raudsepp T., Chowdhary B.P., Moller A.R., Ellegren H., 1997. Low frequency of microsatellites in the avian genome. Genome Res. **7**: 471-482.

Rani V., Singh K.P., Shiran B., Nandy S., Goel S., Devarumath R.M., Sreenath H.L., Raina S.N., 2000. Evidence for new nuclear and mitochondrial genome organizations among high-frequency somatic embryogenesis-derived plants of allotetraploid *Coffea arabica* L. (Rubiaceae). Plant Cell Reports **19**: 1013-1020.

Risterucci A.M., Grivet L., N'Goran J.A.K., Pieretti I., Flament M.H., Lanaud C., 2000. A high density linkage map of *Theobroma cacao* L., Theor. Appl. Genet., **101**: 948-955.

Rovelli P., Mettulio R., Anthony F., Anzueto F., Lashermes P., Graziosi G., 2000. Microsatellites in *Coffea arabica* L. In: Sera T., Soccol C.R., Pandey A., Roussos S. (eds.) Coffee Biotechnology and Quality, pp 123-133. Kluwer Academic Publishers, Dordrecht (The Netherlands).

Senior M.L., Chin E.C.L., Lee M., Smith JS.C., Stuber C.W., 1996. Simple sequence repeat markers developed from maize sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersico*n species. Theor. Appl. Genet. **94**: 264-272.

Steinkellener H., Fluch S., Turetschek E., Lexer C., Streiff R., Kremer A., Burg K., Gloessl J., 1997. Identification and characterization of $(GA/CT)_n$ microsatellite loci from *Quercus petraea*. Plant Mol. Biol. **33**: 1093-1096.

Tautz D., 1989. Hypervariability of simple sequences as a general source of polymorphic DNA markers. Nucleic Acid Res. **17**: 6483-6471.

Wang Z., Weber J.L., Zhong G., Tanksley, S.D., 1994. Survey of plant short tandem DNA repeats. Theor. Appl. Genet. **88**: 1-6.

Wu K.S., Tanksley S.D., 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet. **241**: 225-235.

A Catalogue of Genes Expressed in *Coffea arabica* L.

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SUMMARY

Plant synthesise more than 100,000 different compounds, many of which have high nutritional value or industrial applications. In the past, the isolation and characterisation of a relevant gene was a complex and time consuming task. More recently the EST (Expressed Sequence Tag) analysis have provided a cost-effective and rapid alternative route to the isolation of large numbers of genes. Knowledge of expressed genes in *Coffea arabica* is practically non existent and therefore we undertook an EST project.

About 30 seeds of bourbon yellow were allowed to germinate on wet blotting paper. The root (about 15 mm) were cut and immediately frozen in liquid nitrogen, Following the grinding of the tissue, the polyadenylated mRNA was extracted and purified by oligo-dT column chromatography by standard techniques. The first strand of cDNA was synthesised by reverse transcriptase and an anchored oligo-dT-NotI primer. After completion of the second strand, using the Gubler and Hoffman strategy, the cDNA fragments were repaired with T4 DNA Polymerase, ligated to BstX1 non-palindromic adaptors, digested with NotI and size-fractionated on CLB4 spun column. The cDNA was directionally cloned into a *BstX1, NotI* digested pcDNAII plasmid vector (Invitrogen) and the ligation reaction was used for transforming electro-competent *E. coli* DH10B cells. PCR tests were run on a sample of the library to determine its quality. We obtained 800,000 recombinant bacterial clones with an average insert size of 1 kb. More than 500 random clones were picked-up in the 384 wells plates and the inserts amplified by PCR. The positive PCR products were re-arrayed in new plates and sequenced on a 3,700 ABI sequencer.

The first catalogue of gene expressed in the meristemal radical tissue of *Coffea arabica*, shows a large number of genes with a high degree of homology with sequences of other plant species like *A. thaliana, L. esculentum, O. sativa* etc. About 35% of the sequences showed no homology to known sequences. As expected, we obtained a relatively large number of sequences coding for ribosomal proteins. The second most abundant class of sequences was those coding for chitinases. Most probably these genes are involved in the defense response of the young roots to bacteria and moulds. Other genes with high expression are the extensin and the acid phosphatases.

Besides the abundant classes of expressed genes mentioned above, we found some unique sequences, which are amenable for further developments. This group includes the glycoprotein EP1, a member of S locus protein family, which could be used to study the autofertility of *C. a.*; one of the many resistance genes, presumably a rust resistance gene.

INTRODUCTION

The genes expressed in an organism can be studied using two complementary approaches: on the one hand, the modes of expression and regulation of selected genes can be investigated in the context of a narrowly defined pathology or metabolic pathway; on the other, the overall expression of a tissue or an organism can be examined within a broader study. In the first

case, the field of investigation gradually widens as the characteristics of a particular gene emerge, and the metabolic pathway of the cell type within which it is expressed become known. The second approach, by contrast, entails a gradual narrowing down of the field of investigation: the entire range of genes expressed in a tissue is studied with a view to identifying its peculiarities and restricting the analysis to the more important and interesting genes. The method most commonly adopted for the second type of analysis is the systematic sequencing of cDNA clones. The sequences obtained by this method constitute unique tags for a particular transcript. For this reason, they are called EST (Expressed Sequence Tag).

On a large scale, ESTs represent a quick and cost-effective means of producing partial sequences for the majority of the genes expressed in an organism. At present, several organisms of scientific and/or economic interest, such as soybean, barrel medic, tomato and *Arabidopsis thaliana*, are investigated using ESTs. For the plants mentioned above, more than 500,000 ESTs have been deposited in a specialised database, dbEST (Boguski et al., 1993). The comparison of an unknown sequence with the vast number of ESTs kept in the database is becoming an important and powerful instrument of genetic research. In order to isolate a gene, it has become increasingly common to construct a cDNA library from a tissue expressing high levels of a specific gene, then to produce a certain number of ESTs, and finally to check whether they display any homologies with the corresponding genes in other species. (Ohlrogge and Benning, 2000; Newman et al., 1994). This article reports a cDNA library obtained from root meristem of *Coffea arabica*, the production of several ESTs, and the creation of a database for ease of reference.

MATERIAL AND METHODS

Construction of a cDNA library

About 30 seeds of of *Coffea arabica* var. Catuai red were allowed to germinate on wet blotting paper. The root (about 15 mm for a total of 3.5 g) were cut and immediately frozen in liquid nitrogen. Following the grinding of the tissue, the total RNA was extracted using the protocol described by Chomczynski e Sacchi (1986) and the polyadenylated mRNA was purified by oligo-dT column chromatography by standard techniques. The first strand of cDNA was synthesised by reverse transcriptase and an anchored oligo-dT-NotI primer. After completion of the second strand, using the Gubler and Hoffman strategy, the cDNA fragments were repaired with T4 DNA Polymerase, ligated to BstXI non-palindromic adapters, digested with NotI and size-fractionated on CLB4 spun column. The cDNA was directionally cloned into a BstXI, NotI digested pcDNAII plasmid vector and the ligation reaction product was used for transforming electro-competent *E. coli* DH10B cells.

EST preparation

Following the construction of the cDNA library, the single clones were isolated and collected. Each colony was transferred to 384 well plates and allowed to grow on SOB culture medium supplemented with ampicillin (60 µl per well), at the temperature of 37°C. Ten plates were prepared for a total of 3840 bacterial clones. About 200 µl of bacterial culture were transferred with a 384 tooth comb to a 384 well PCR plate together with 20 µl of reaction mix. The cells were lysed during the first denaturation phase at 95°C. The DNA released by the cells was amplified under the following conditions: 95°C for 5’, first cycle; 95°C x 30”, 55°C x 30”, 72°C x 2’30” for the following 35 cycles.

At the end of the reaction, 5 µl of mix underwent electrophoresis on 1% agarose gel to check the quality of the amplification and the length of the amplicons. The DNA sequencing was performed only on the amplicons longer than 500 bp.

Bioinformatic analysis of sequences and database construction

Sequences have been corrected and assembled using the Seqman programme of the informatic package DNAStar (Lasergene). The following parameters have been applied: End Trimming: High, Assembling Match Size: 50, with a Minimum Match Percentage of 90. These parameters are very high: we preferred to have a higher number of contigs rather than create informatic chimeras. The search for similarities was facilitated by a series of Perl scripts created by our team. We have used the netblast programme, in that it allowed us to use the NCBI database bypassing the graphic interface on the web. To identify the homologies, all the contigs were compared to nucleotide sequences, including the ESTs stored in specialised databases, and to polypeptide sequences. The contig sequences, the homology correspondence and the transcript annotations were organised in a data sheet (Excel, Microsoft) as reported in Figure 1.

RESULTS AND DISCUSSION

Out of the 3840 PCRs of the library's bacterial clones, 1260 were of sufficient quality and length to be sequenced. After correcting and assembling them with Seqman we obtained 1208 cluster in 901 contigs. As can be seen, sequence redundancy is rather low. Apparently the gene activity in the root meristem is fairly equilibrated and no overexpression of single genes was detected.

The contigs were designated by the abbreviation RM (Root Meristem) followed by a number referring to the first EST which gave origin to the contig. This nomenclature allows for the identification of the original clone and give access to the stored clones for further research developments. By using BLASTn and BLASTx, we compared all these sequences with those entered in public databases, but for reasons of space, the results of these comparisons cannot be reported here in full. The BLAST results are summarised in Figure 2. Notably more than 80% of the clusters show significant homology with known genes or anonymous sequences.

Below we give a brief description of the main classes of transcripts. The long list of ribosomal protein is immediately striking, but easily explicable since the library was built from a proliferating tissue and therefore under an intense activity of protein synthesis. Rather more surprising, given that the mRNA was extracted from root tips, is the presence of a certain number of proteins and enzymes engaged in photosynthesis: this is unexpected in a tissue never exposed to light. However recent data suggest the notion that light signal trasduction and pathogenesis-related gene signalling pathways are connected (Genoud et al., 1998) and, as reported below, we found a significant number of genes involved in the protection mechanism.

Another group of genes expressed in significant quantities are the chitinases. This is a particular family of enzymes specifically engaged in the degradation of chitin. Since the cell wall of fungi and the insect exoskeleton is largely made up of chitin, the reasons for the high quantity of these enzymes is clear. These proteins are crucial in defending the embryo from mould and, to a lesser extent, insects (Yeboah et al., 1998). Other important proteins for the protection of the embryo are those involved in the so-called 'oxidative burst' (Schweitzer et al., 1999). This term describes one of the first reactions to infection which can be identified in plants: great quantities of ROS (Reactive Oxygen Species), especially H_2O_2 (produced by superoxide dismutase, which was found in the library) and superoxyde radical $^{\bullet}O_2$, are quickly released into the extracellular matrix. These are toxic molecules, in that they trigger uncontrolled oxidative and radical reactions. A comparable reaction can be detected in the

immune system of mammals in which there is a release of these molecules in the phagocytosis vesicles of neutrophils, eosinophils and macrophages.

	A	C	F	G	H	I	J
1	Cluster	Annotation	Chromatogram	Contig	BLASTx-nr	BLASTn-nr	BLASTn-est
2	RM-0-A01	unknown	RM-0-A01.abi	RM-0-A01c.txt	RM-0-A01cxnr.htm	RM-0-A01crnr.htm	RM-0-A01cnest.htm
3	RM-0-A02	ripening-related protein-like	RM-0-A02.abi	RM-0-A02c.txt	RM-0-A02cxnr.htm	RM-0-A02crnr.htm	RM-0-A02cnest.htm
4	RM-0-A03	18S rRNA gene	RM-0-A03.abi	RM-0-A03c.txt	RM-0-A03cxnr.htm	RM-0-A03crnr.htm	RM-0-A03cnest.htm
5	RM-0-A04	eukaryotic initiation factor 3H1	RM-0-A04.abi	RM-0-A04c.txt	RM-0-A04cxnr.htm	RM-0-A04crnr.htm	RM-0-A04cnest.htm
6	RM-0-A05	DEHYDRATION-RESPONSIVI	RM-0-A05.abi	RM-0-A05c.txt	RM-0-A05cxnr.htm	RM-0-A05crnr.htm	RM-0-A05cnest.htm
7	RM-0-A06	ribosomal protein putative	RM-0-A06.abi	RM-0-A06c.txt	RM-0-A06cxnr.htm	RM-0-A06crnr.htm	RM-0-A06cnest.htm
8	RM-0-A07	apyrase	RM-0-A07.abi	RM-0-A07c.txt	RM-0-A07cxnr.htm	RM-0-A07crnr.htm	RM-0-A07cnest.htm
9	RM-0-A08	chitinase	RM-0-A08.abi	RM-0-A08c.txt	RM-0-A08cxnr.htm	RM-0-A08crnr.htm	RM-0-A08cnest.htm
10	RM-0-A09	proteinase putative	RM-0-A09.abi	RM-0-A09c.txt	RM-0-A09cxnr.htm	RM-0-A09crnr.htm	RM-0-A09cnest.htm
11	RM-0-A10	ribosomal protein L35	RM-0-A10.abi	RM-0-A10c.txt	RM-0-A10cxnr.htm	RM-0-A10crnr.htm	RM-0-A10cnest.htm
12	RM-0-A11	NBS-LRR disease resistance	RM-0-A11.abi	RM-0-A11c.txt	RM-0-A11cxnr.htm	RM-0-A11crnr.htm	RM-0-A11cnest.htm
13	RM-0-A12	unknown	RM-0-A12.abi	RM-0-A12c.txt	RM-0-A12cxnr.htm	RM-0-A12crnr.htm	RM-0-A12cnest.htm
14	RM-0-A13	elongation factor 1B gamma	RM-0-A13.abi	RM-0-A13c.txt	RM-0-A13cxnr.htm	RM-0-A13crnr.htm	RM-0-A13cnest.htm
15	RM-0-A14	unknown	RM-0-A14.abi	RM-0-A14c.txt	RM-0-A14cxnr.htm	RM-0-A14crnr.htm	RM-0-A14cnest.htm
16	RM-0-A15	unknown	RM-0-A15.abi	RM-0-A15c.txt	RM-0-A15cxnr.htm	RM-0-A15crnr.htm	RM-0-A15cnest.htm
17	RM-0-A16	unknown	RM-0-A16.abi	RM-0-A16c.txt	RM-0-A16cxnr.htm	RM-0-A16crnr.htm	RM-0-A16cnest.htm
18	RM-0-A17	root-specific protein RCc3	RM-0-A17.abi	RM-0-A17c.txt	RM-0-A17cxnr.htm	RM-0-A17crnr.htm	RM-0-A17cnest.htm
19	RM-0-A18	ATP/ADP-transporter, chlorop	RM-0-A18.abi	RM-0-A18c.txt	RM-0-A18cxnr.htm	RM-0-A18crnr.htm	RM-0-A18cnest.htm
20	RM-0-A19	phosphate transport protein, r	RM-0-A19.abi	RM-0-A19c.txt	RM-0-A19cxnr.htm	RM-0-A19crnr.htm	RM-0-A19cnest.htm
21	RM-0-A20	ascorbate peroxidase	RM-0-A20.abi	RM-0-A20c.txt	RM-0-A20cxnr.htm	RM-0-A20crnr.htm	RM-0-A20cnest.htm
22	RM-0-A21	unknown	RM-0-A21.abi	RM-0-A21c.txt	RM-0-A21cxnr.htm	RM-0-A21crnr.htm	RM-0-A21cnest.htm
23	RM-0-A22	acid phosphatase/vegetative	RM-0-A22.abi	RM-0-A22c.txt	RM-0-A22cxnr.htm	RM-0-A22crnr.htm	RM-0-A22cnest.htm
24	RM-0-A23	AMINOPEPTIDASE	RM-0-A23.abi	RM-0-A23c.txt	RM-0-A23cxnr.htm	RM-0-A23cxnr.htm	RM-0-A23cnest.htm
25	RM-0-A24	peroxidase	RM-0-A24.abi	RM-0-A24c.txt	RM-0-A24cxnr.htm	RM-0-A24crnr.htm	RM-0-A24cnest.htm

Figure 1. Organisation of the EST database. The name of the contig was established by the first sequence which originated the cluster as reported in column (A). The putative function of each contig was assigned on the bases of the homology to known sequences of other plants (column C). In the following columns we reported the hypertext links to the chromatogram (F), the contig consensus sequence which was used for the homology analysis (G) and the results of the analysis performed with the programmes BlastX (H), BlastN (I) and dbEST (J)

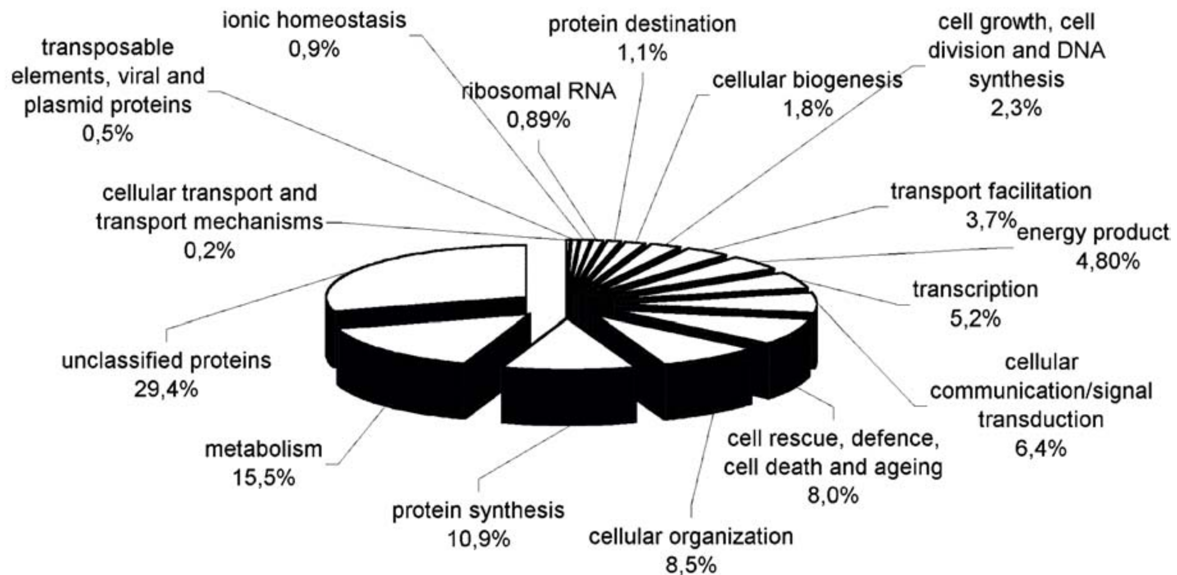
Coffea arabica transcripts	
Number of clusters after EST	901
Cluster similar to known genes	576 (63%)
Cluster similar to other ESTs or	176 (19%)
Cluster without any similarity	149 (18%)

Figure 2. **Summary of the work on the systematic sequencing of cDNA libraries of *Coffea arabica* L.**

A ubiquitous protein in plants, especially in embryos, is the germin, which was indeed found in many library clones. It has been reported (Lane, 1994) that the expression of germin-like proteins increases in the case of infections and that these proteins are involved in the oxidative burst. Normally the intracellular H_2O_2 is eliminated by catalases, peroxidases, ascorbate peroxidases, dehydroascorbate and gluthatione. All these proteins are represented in our library by at least one clone. Besides the defence mechanism H_2O_2 contributes also to the formation of the cell wall. Lane (1994) reported that a high level of H_2O_2 in the extracellular matrix is associated to the insolubilisation of the cell wall glycoproteins, especially those rich

in proline (PRP) and hydroxyproline (HRGP) which confer mechanical resistance to the cell wall. Again these proteins were well represented in our EST library.

Figure 3. **Functional classification of transcripts expressed in root meritematic tissue of *Coffea arabica***



We have also identified a gene which, without doubt, codes for a NBS-LRR-type resistance protein, a family which includes all known genes involved in the resistance to rust and other infection.

We found also two peculiar sequences: one was homologous to the transposase IS10 carried by the Tn10 transposone of *E.coli*; the other was homologous to the polyprotein pol of the retroelement "Copia" of *Drosophila melanogaster*. These sequences could be very useful as markers for constructing a genetic map of coffee. Since they are carried by transposable elements, they should be present in multiple copies throughout the genome and in different locations for different varieties and plants. For this reason, they could be more useful than the classical DNA polymorphic sequences which have so far been used. In facts the extreme genetic homogeneity and the autogamy of *C. arabica* did not allow for the identification of large numbers of polymorphic sequences.

Apart from the proteins and genetic families mentioned above, some single genes may be of great interest for future research. We found the sequence of a sucrose synthase which regulates the synthesis of this type of sugar and whose concentration in the bean is crucial to the taste of coffee. An other interesting EST sequence was that coding for the abscisic stress ripening protein, interesting as one of the factors which can be used to control the ripening of the cherries. Finally we mention a sequence homologous to the dehydration-responsive protein RD22, which is also expressed during the abscisic process in responce to dehydration (Iwasaki et al., 1995).

dbEST SummarydbEST: database of "Expressed Sequence Tags"

dbEST release 050401
Summary by Organism - May 4, 2001

Number of public entries: **7,927,233**

	Organism	ESTs
01.	Glycine max (soybean)	166,233
02.	Medicago truncatula (barrel medic)	122,365
03.	Lycopersicon esculentum (tomato)	114,999
04.	Arabidopsis thaliana (thale cress)	113,000
05.	Zea mays (maize)	89,125
06.	Oryza sativa (rice)	75,057
07.	Hordeum vulgare (barley)	68,480
08.	Sorghum bicolor (sorghum)	65,040
09.	Triticum aestivum (wheat)	60,022
10.	Solanum tuberosum (potato)	38,074
11.	Pinus taeda (loblolly pine)	35,145
12.	Lotus japonicus	27,578
13.	Sorghum propinquum	21,454
14.	Gossypium arboreum	20,978
15.	Mesembryanthemum crystallinum (common ice plant)	14,033
16.	Gossypium hirsutum (upland cotton)	9,438
17.	Lycopersicon pennellii	8,346
18.	Secale cereale	8,123
19.	Triticum monococcum	5,504
20.	Populus tremula x Populus tremuloides	4,809
21.	Lycopersicon hirsutum	2,504
22.	Aegilops speltoides	2,466
23.	Cryptomeria japonica (Japanese cedar)	2,438
24.	Brassica napus (oilseed rape)	1,702
25.	Triticum turgidum subsp. durum	1,526
26.	Mentha x piperita (peppermint)	1,316
27.	Citrus unshiu	1,251
28.	**Coffea arabica**	**1,208**

Figure 4. List of the number of ESTs per organism on May 4th, 2001 as reported in the in the dbEST databank. For simplicity, only the data of Spermatophyta have been reported. (source: http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html)

CONCLUSION

The economic importance of coffee in the world market is enormous. Yet genetic studies of this plant have so far been scant. The lack of genetic research on coffee is no doubt partially explained by the relative scarcity of economic and technical resources so far allocated to this type of research. Yet there are also some intrinsic difficulties in the study of this species, in view of its allotetraploidy and the low variability of its genome. For these reasons, the genetic study of coffee, the isolation and characterisation of its genes, proceed at a very slow pace, and is supported by very few labs in the world.

The fastest and cheapest way of gaining a somewhat superficial but relatively exhaustive genetic overview of the species is the EST approach. This method yields an impressive breath of information on the expression of an organism in a short time, and moreover enables the characterisation of single tissues.

We have built a first database of transcripts of root meristem and investigated the probable function of many genes on the basis of sequence homology with genes of plants which have been better studied. This database shall soon be available to the scientific

community on the website of the Dipartimento di Biologia, Università di Trieste, (http://www.univ.trieste.it/~biologia/). Although the present article is based on early stages in our research, it is important to emphasise its contribution to our knowledge of the genetic makeup of coffee. As can be seen from Figure 4, our ESTs list *Coffea arabica* as the 28th best characterised plants from the point of view of genetic expression.

ACKNOWLEDGEMENTS

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REFERENCES

Boguski M.S., Lowe T.M.J., Tolstoshev C.M. (1993): dbEST - database for "expressed sequence tags". Nature genetics **4**, 332-333 .

Chomczynski P., Sacchi N. (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry **162**, 156-159.

Genoud T, Millar AJ, Nishizawa N, Kay SA, Schafer E, Nagatani A, Chua NH. (1998): An Arabidopsis mutant hypersensitive to red and far-red light signals. Plant Cell. **10**, 889-904.

Gubler U., Hoffman B.J. (1983): A simple and very efficient method for generating cDNA libraries. Gene **25**, 263-269.

Iwasaki T., Yamaguchi-Shinozaki K., Shinozaki K. (1995): Identification of a cis-regulatory region of a gene in Arabidopsis thaliana whose induction by dehydration is mediated by abscisic acid and requires protein synthesis. Mol Gen Genet **247**(4), 391-398.

Lane B.G. (1994): Oxalate, germin, and the extracellular matrix of higher plants. FASEB J **8** (3), 294-301.

Newman T., de Bruijn F.J., Green P., Keegstra K., Kende H., McIntosh L., Ohlrogge J., Raikhel N., Somerville S., Thomashow M. (1994): Genes galore: a summary of the methods for accessing the results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones. Plant physiology **106**, 1241-1255 .

Ohlrogge J., Benning C. (2000): Unraveling plant metabolism by EST analysis. Current opinion in plant biology **3**, 224-228.

Schweizer P., Christoffel A., Dudler R. (1999): Transient expression of members of the germin-like gene family in epidermal cells of wheat confers disease resistance. Plant J. **20** (5), 541-542.

Yeboah N.A., Arahira M., Nong V.H., Zhang D., Kadodura K., Watanabe A., Fukazawa C. (1998): A class III acidic endochitinase is specifically expressed in the developing seed of soybean (Glycine max). Plant Mol Biol **36** (3), 407-415.

From Traditional Genetics Towards Genomics: A New Approach for an Old Problem

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SUMMARY

Since about ten years, advances in genomics increased exponentially, as well as their application in breeding. Now, I would like to present the contribution genomics in the coffee research project of an IRD-CIRAD team. Other talks will be developed today by this team.

Traditionally, coffee breeding, as in other crops, depends on the mode of reproduction. In autogamous crops, as *C. arabica*, we use the genealogic selection. In contrast, in allogamous plants as *C. canephora*, the reciprocal recurrent selection is applied. The second factor acting on breeding way is the range of the intraspecific diversity in regards to the objectives and the access possibilities to the interspecific diversity. In practice, introgression of wild traits was attempted for the two cultivated species. Another breeding way consisted to obtain interspecific F1 hybrids, as in the *Arabusta* program.

The new program, we proposed, is based on genomics and presents three objectives. The first objective is to define new tools for sustainable breeding. This concerns the marker assisted selection strategy and the genetic transformation. The second objective is to improve cup quality with two steps: firstly, to locate and identify genes and, secondly to analyse their regulation of expression. The third objective concerns the cryopreservation of genetic resources.

NEW TOOLS FOR SUSTAINABLE BREEDING

In order to define tools for sustainable breeding, we have to understand relationships between gene location and genome structure an one hand, and between gene regulation and genome structure on the other hand. By this point of view, coffee genus is a model. Indeed, there are 22 chromosomes in all diploid species, whereas the genome size of diploid species varies from 0.9 pg to 1.7 pg. Secondly, in interspecific crosses, sterility of F1 hybrids rises from presence of univalents at the meiosis metaphasis and there is a relationship between F1 hybrid sterility and parental genome size difference. Since genomes are homologous, coding gene number should not vary strongly. Consequently, genome size differences could be due to repeated sequences. But, what is the role of uncoding repeated sequences in terms of evolution? Indeed, a difference of genome size should have a selective advantage to invade the population. And this advantage could concern the gene transfer through recombination limitation and distortion increasing. This leads to the notion of qualitative reproductive barriers. Some genes can be transferred by introgression, others not.

Concerning the repeated sequences, we look for their identification their location and their quantification on one hand, and an analysis of their roles on distortion of segregation, recombination, chromosome pairing and gene expression.

A second way to study the structure of genome is the within-genus synteny (synteny is the resemblance between genetic map). To do that, we will compare four interspecific maps. All maps include *C. canephora*. Other parents were:

- *C. pseudozanguebariae* selected because it is a caffeine-free species and its genome size is low (1.1 pg);
- *C. eugenioides* , selected for its affinity with *C. arabica* genome;
- *C. liberica,* chosen for its affinity with *C. canephora* but also for its seed weight and its fructification synchronisation;
- lastly, *C. heterocalyx* was selected for its high genome size (1.7 pg) and its autogamy.

We also compare these maps with an intraspecific map between Guinean and Congolese trees.

We expect to relate the segregation distortion variation and the recombination rate differences to the parental genome size differences and the gene function and fitness. Indeed, what are genes present on distorted segment and what are their role in the fitness?

COFFEE CUP QUALITY GENES

The second objective of our project is to improve cup quality. So, we have to look for implied genes. This is a five steps approach.

- the genetic variance analysis give results on the relative influence of genotype and environment;
- the QTL analysis allows to define gene number and their location;
- this will be followed by the gene identification;
- their co-location with QTL should allow to define candidate-gene;
- the last step will consist to study their expression regulation.

First results for caffeine

Studies were carried out on an interspecific cross between C. *pseudozanguebariae* (PSE) and *C. liberica dewevrei* (DEW). Parental species presents differences for caffeine: 0% in PSE, and 1% in DEW, and for fructification time: 10 weeks in PSE, and 10 months for DEW.

We decomposed caffeine content in theses components. Indeed, caffeine content resulting from an accumulation, it can be decomposed in caffeine flow by day and day number of accumulation. In addition, we were interested by the CAF/CQA ratio.

We obtained 4 independent QTL:

- one for the fructification time;
- one for the caffeine flow by day;
- one for the CAF/CQA ratio;
- and one explaining the presence/absence of caffeine in parental species.

Further studies

To identify candidate genes, we have four possible ways knowing biosynthesis pathway and using bio-informatics, we expect obtaining of gene sequences from other plants. Proteome comparison between PSE and CAN fruits using two-dimensional electrophoresis will be carry out if the first way gives no expected results. Transcriptome comparison between PSE and

CAN fruits using cDNA libraries will also be carried out. Simultaneously, EST mapping is underway.

In addition and to summarise, all these points emphasise the importance of genetic resources as gene do nor for breeding.

GENETIC RESOURCES CRYOPRESERVATION

Genetic resources cryopreservation constitutes the third part of our project. Coffee genus is again a model. Why? Because there is a large diversity for tolerance to dehydration and low temperatures. Our first objective is to analyse the physiological basis of tolerance through scavenging effects and lipid behaviour at cold temperature. Today, Stephane Dussert showed that sucrose content does not explain tolerance diversity. He developed also a model to estimate seed dessication sensitivity in various *Coffea* L. species. This led to obtain the first *C. arabica* cryopreserved gene bank at CATIE.

The second objective is to establish the genetic basis of tolerance. With two approaches:

- the quantitative inheritance approach in order to analyse additivity;
- the location of QTL for tolerance, but also for lipid composition.

To do that, the PSE DEW cross is again useed. Indeed, PSE is tolerant to cryopreservation, whereas DEW is totally sensitive to dehydration and low temperature.

CONCLUSIONS

The three objectives of our project release on a team IRD-CIRAD. The team includes ten researches, three ingeeners and five technicians.

Partnership concerns traditional partners including CNRA, BRG, IPGRI and CATIE. It also concerns new partners in India, Uganda and Spain, but the list is open. We propose to partners collaboration on genomics projects, training masters and PhD and technicians too. This will concern molecular biology and data analysis. We can also propose expertise.

Molecular Physiology and Genetics of Coffee Resistance to Parasites

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SUMMARY

Coffea arabica varieties usually display a high yielding and good coffee quality, but exhibit a high susceptibility to many pests and diseases. Enhancing their resistance to parasites have become a crucial priority toward an economic and sustainable coffee production. Research activities were therefore developped to identify and clone genes involved in the specific resistance of coffee to nematodes (*Meloidogyne* spp.) and rust (*Hemileia vastatrix*).

A positional cloning project was started to isolate a resistance gene derived from *C. canephora*, that confers resistance against *M. exigua*. Based on the analysis of progenies obtained from resistant introgressed arabica lines, 15 AFLP markers tightly linked to the resistance gene were identified. Further linkage investigations allowed the construction of a localised genetic map of the chromosome segment carrying the *M. exigua* resistance. The low rate of recombination indicated these markers could be useful landmarks for map-based cloning of the resistance gene. With this purpose, a BAC library is being constructed.

In addition, disease resistance gene analogs (RGA) were cloned in coffee (*C. arabica* and *C. canephora*) using DNA primers designed from conserved motifs (NBS) of known plant resistance (R) genes. Analysis of PCR-derived coffee NBS sequences revealed nine distinct families of RGAs, belonging to the non-TIR class type of R-genes, in both species. Sequence variation observed among coffee RGAs suggested point mutations as the primary source of diversity within RGAs families. Efforts are being pursued to explore the possibility of implication of isolated coffee RGA families in the identified nematode resistance.

In parallel, genes early expressed during the specific resistance reaction of coffee (*C. arabica*) to the fungus *H. vastatrix* were isolated from cDNA libraries constructed using the suppression subtractive hybridization method (Diatchenko et al., 1996). cDNAs clones showing specific expression in the early stages of the resistance reaction were selected and characterized. Similarities were found with plant sequences involved in plant defence reactions such as chitinases, heat shock proteins, cytochroms P450, metallothioneins and ionic channels. Further work will aim at understanding the role of selected clones in the mechanism of specific coffee resistance to parasites.

INTRODUCTION

Coffea arabica varieties usually display a high yielding and good coffee quality, but exhibit a high susceptibility to many pests and diseases like leaf rust (*Hemileia vastatrix*, Berk & Br.),

coffee berry disease (*Colletotrichum kahawae*), coffee berry borer (*Hypothenemus hampei*), stem borer (*Xylotrechus quadripes* Chevr.) and nematodes (*Meloidogyne spp.* and *Pratylenchus spp*.). *C. canephora* (Robusta coffee) is more tolerant to these diseases and pests but product quality is considered by the consumers as rather inferior. Enhancing arabica resistance to parasites has become a crucial priority toward an economic and sustainable coffee production. Hence, transfer of desirable genes in particular for disease resistance from diploid species like *C. canephora* and *C. liberica* into tetraploid arabica cultivars without affecting quality traits has been the main objective of arabica breeding.

Molecular techniques are valuable tools for improving the efficiency of conventional coffee breeding by allowing indirect selection for resistance by looking for molecular markers linked to that resistance trait (Lashermes et al., 2000a). Such strategy is especially useful when pathogenicity tests are time-consuming, often destructive for the progenies to evaluate, and difficult to interpret. Availability of tightly linked genetic markers for resistance genes are of great help in selecting plants carrying these genes without subjecting them to pathogen attacks.

In addition, molecular data derived from the sequencing of model plant genomes and from plant Expressed Sequence Tags (ESTs) projects provide a growing body of knowledge that is expected to bring up the research on coffee more pertinent and efficient. For instance, the recent cloning of genes for resistance (R) against diverse pathogens from a variety of plants has revealed that many share conserved sequence motifs (Ellis et al., 2000). This provides the possibility of isolating numerous additional resistance genes (i. e. Resistance Gene Analogs or RGA) by polymerase chain reaction (PCR) with degenerate oligonucleotide primers.

Finally, with the use of molecular techniques, it is now possible to facilitate the transfer of desirable genes among plant varieties. Coffee genetic transformation has been successfully achieved by several research groups (Hatanaka et al., 1999; Leroy et al., 1999; Spiral et al., 1999). Our research activities were therefore developped with the aim of identifying and cloning the genes that are involved in the specific resistance of coffee to parasites. We present here some results we obtained on coffee RGAs, as well as on nematodes (*Meloidogyne* spp.) and rust (*Hemileia vastatrix*) resistance genes in coffee.

RGAs

Numerous disease resistance gene analogs (RGA) were cloned in coffee using DNA primers designed from conserved motifs (NBS) of known genes of plant resistance (R). Plant material involved accessions of both species *C. arabica* and *C. canephora*. Genomic DNA as well as mRNA extracted from leaves were used. Origin, diversity and evolution of NBS-type disease-resistance genes in coffee trees were investigated (Noir et al., 2001). Efforts are being pursued to explore the possibility of implication of isolated coffee RGA families in the identified specific resistances to parasites.

NEMATODE RESISTANCE GENES

A positional cloning project was started to isolate a resistance gene derived from *C. canephora*, that confers resistance against *M. exigua* (Bertrand et al., 2001). In particular, efforts were directed to the identification of AFLP (amplified fragment length polymorphism) markers associated with the gene *Mex-1* conferring the resistance to *M. exigua.*

The AFLP procedure was performed essentially as described by Vos et al. (1995) with minor adaptations for coffee DNA (Lashermes et al., 2000b). An aliquot of 500 ng genomic DNA

was digested with restriction enzymes *Eco*RI and *Mse*I. Restriction fragments were ligated with double-strand *Eco*RI and *Mse*I-adapters. A total of 342 primer combinations derived from 16 *Eco*RI primers and 22 *Mse*I primers were employed in this stud (Table 1).

Table 1. Screening of AFLP markers associated with the resistance to *M. exigua*

Screening Material (a)	No. of primer combinations	No. of polymorphic AFLP bands	No. of AFLP markers associated with the resistance to M. exigua (b)
Timor hybrid-derived genotypes	232	403	10
F_2 individuals	110	161	5
Total	342	564	15

[a] *Screening material included either 2 resistant (T5296 and T17925) versus 2 susceptible (T17928 and T18137) Timor hybrid-derived genotypes or 2 resistant versus 2 susceptible F_2 individuals from a cross between Et6 (susceptible parent) and T5296 (resistant parent)*
[b] *AFLP makers appearing associated with the resistance to M. exigua in a set of 10 F_2 individuals (5 resistant and 5 susceptible) from a cross between Et6 (susceptible parent) and T5296 (resistant parent)*

More than 564 markers of "canephora" chromosome segments introgressed in *C. arabica* lines were generated. Screening material included either 2 resistant (T5296 and T17925) versus 2 susceptible (T17928 and T18137) Timor hybrid-derived genotypes or 2 resistant versus 2 susceptible F_2 individuals from a cross between Et6 (susceptible parent) and T5296 (resistant parent). A total of 15 AFLP makers exhibiting a strict association with the resistance to *M. exigua* in a set of 10 F_2 individuals (5 resistant and 5 susceptible) from a cross between Et6 (susceptible parent) and T5296 (resistant parent), were identified (Figure 1). Further linkage analysis was performed in the F_2 (Et6 x T5296) population and a localised genetic map of the chromosome segment carrying the *M. exigua* resistance gene was established. The low rate of recombination indicated these markers could be useful landmarks for map-based cloning of the resistance gene. With this purpose, a BAC library is being constructed.

COFFEE RUST RESISTANCE GENES

Introduction

The early recognition event between the plant R protein and the pathogen's avr gene product triggers the rapid activation of several plant defense responses that in concert lead to disease resistance. Among these responses is rapid localized cell death at the site of infection, termed the hypersensitive response (HR), which is thought to limit pathogen invasion in plants. In the specific interaction between coffee and the rust fungus *H. vastatrix*, the leaf resistance reaction appears as chlorotic flecks usually associated with punctiform tumefactions, visible 12-15 days after inoculation (Rodrigues et al., 1975). Microscopic examination of coffee leaves during a time-course of infection showed that cellular death events are commonly observed at the infection site (guard cells) by three days post-inoculation (Silva et al., in press).

The aim of our study is to characterize genes early expressed during the hypersensitive reaction of coffee (*C. arabica*) to the rust fungi. We initiated an EST (Expressed Sequence Tag) project to establish a reference catalogue of genes that are differentially expressed during

the resistance reaction. ESTs are short DNA sequences read from randomly chosen cDNA clones. In our approach, sequencing reactions are performed on selected cDNA clones obtained from a subtractive cDNA library, and the resultant ESTs are compared with sequence databases for identification (Figure 2). Cloning and functional analysis of genes involved in the *H. vastatrix* resistance pathways will not only lead to identification of some genetic components controlling disease resistance and cell death, but also allow to manipulate these non-pathogen specific genes to achieve broad spectrum resistance in coffee.

Figure 1. Example of AFLP Marker linked to the resistance to *M. exigua* derived from the Timor hybrid. Plants showing resistance to *M. exigua* are indicated by R (Resistance)

Material and methods

Plant variety, fungal strains, and inoculation

Coffea arabica accession 110/5 was inoculated with *H. vastatrix* isolates, either race II (isolate 1427) eliciting an RH (incompatible interaction) or with race XIV (isolate 178a) giving rise to rust disease (compatible interaction), using standard conditions (D'Oliveira and Rodrigues, 1961). Control plants were inoculated with water. Coffee leaves collected at various times after inoculation were immediately frozen in liquid nitrogen and stored at –80°C until RNA extraction.

RNA extraction

Total RNAs were extracted from coffee leaves using the RNeasy Plant kit (Qiagen, France) completed by a DNAse treatment. Quality and concentration of RNA were checked on denaturing agarose gel and by absorbance measurements at 230, 260 and 280 nm on a UV spectrophotometer.

Construction of the subtractive cDNA library

Based on cytochemical observations (Silva et al., 1999, and in press) which showed that 48 hours after inoculation 17.5% of infection sites exhibited dead cells, we choosed the times 24 and 48 hours to construct a coffee cDNA library enriched in sequences specifically expressed during the HR. Total RNAs were extracted from coffee leaves undergoing incompatible or compatible interactions at 24 h and 48 h after inoculation. Total RNAs extracted at 24 h and 48 h after inoculation were pooled (500 ng each) to obtain 1µg total RNA of resistant and susceptible samples. cDNAs were synthesized using the SMART-PCR cDNA synthesis kit (Clontech, USA). The subtractive cDNA library was obtained using the suppression subtractive hybridization (SSH) method (Diatchenko et al., 1996) developped in the PCR-Select cDNA Subtraction kit (Clontech). The tester (resistant) cDNA sample was subtracted twice by the driver (susceptible) cDNAsample following the manufacturer's recommendations. Subtracted cDNA sequences were further ligated into a plasmid vector and

used to transform *Escherischia coli* competent cells (pGEMt-easy kit, Promega, France and TOPO cloning kit, InVitrogen, France).

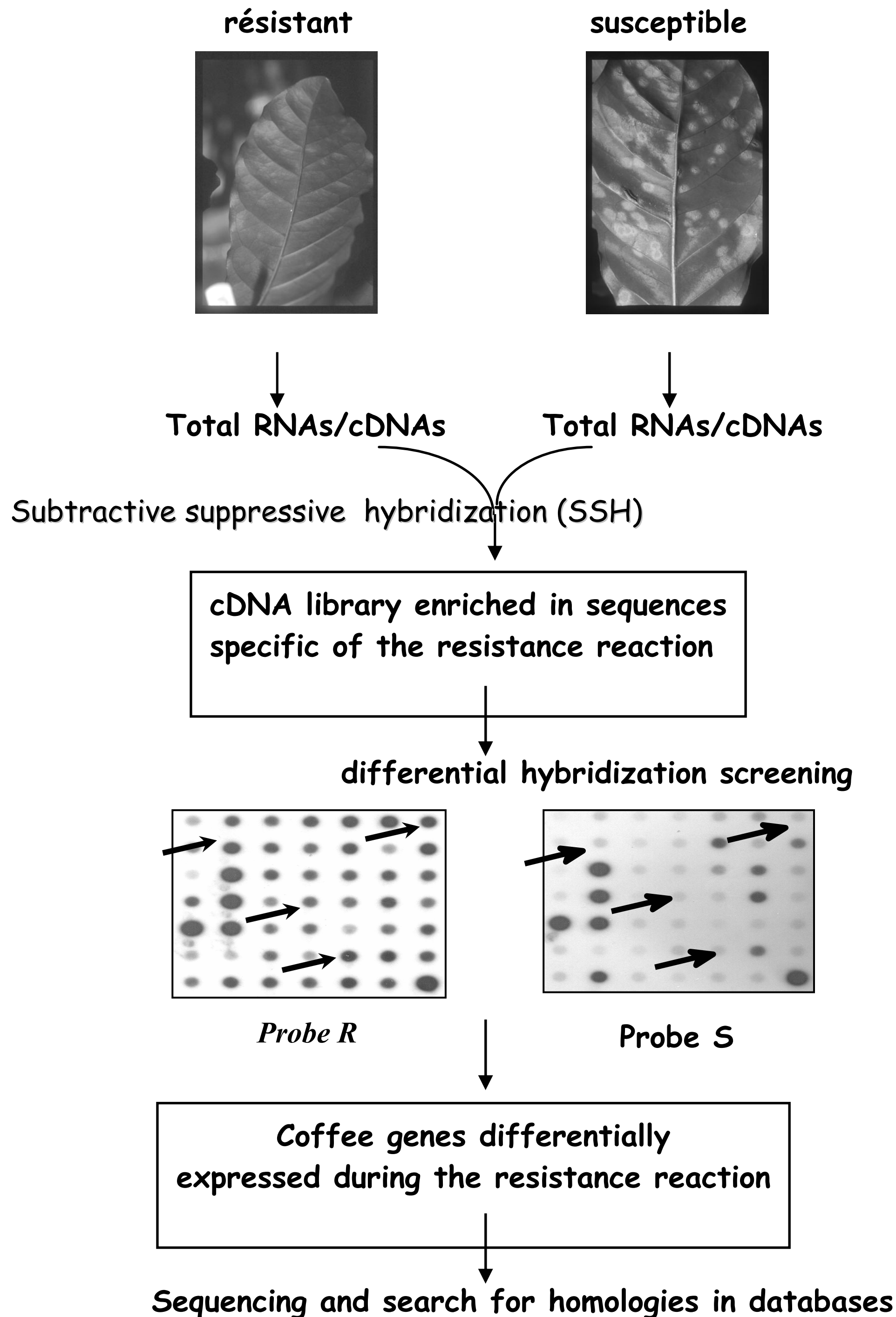

Figure 2. Scheme showing the strategy developped to isolate genes early expressed in the resistance reaction of coffee to rust disease. Arrows indicate some examples of clones that were selected based on the intense hybridization signal with probe R and the weak signal obtained with probe S

Differential screening of subtracted cDNA clones

Selection of cDNA clones specifically expressed in the resistant samples was improved by differential screening of the cDNA clones presents in the subtractive library. After purification, recombinant bacterial colonies were arrayed on Nylon membranes (Hybond NX, Amersham, France) and hybridized with the tester (R) and driver (S) radioactively-labelled probes as indicated in the PCR-Select Differential Screening kit User Manual (Clontech). Clones showing an intense hybridization signal with probe R and a weak signal with probe S were selected. Confirmation of screening was made by a secound round of differential hybridization on purified plasmids extracted from selected clones (50 and 100 ng) arrayed on Nylon membranes (Hybond N+, Amersham).

Sequencing and bioinformatic analysis of cDNA clones

Selected cDNA clones were sequenced (Centre de séquençage de l'INRA Nancy, France) and homologies with sequences present in international databases were searched using algorithms developped at the National Center for Biotechnology Information (NCBI) web site (www.ncbi.org).

RESULTS AND DISCUSSION

Using the suppression subtractive hybridization method (Diatchenko et al., 1996), a coffee cDNA library enriched in sequences specifically expressed during the HR was obtained. In addition, recombinant clones were screened by differential hybridization against complex cDNA probes generated from resistant and susceptible mRNA pools (Figure 2). Thirty cDNA clones selected based on their hybridization signals were sequenced. Most of them (80%) showed significant homologies with plant sequences in databases. More than a third of the sequenced clones showed high similarities (P value $>10^{-20}$) with proteins which role in plant defence reactions have been suggested or demonstrated, such as chitinases, heat shock proteins, cytochrome P450, metallothioneins and ionic channels (Choi et al., 1996; Clough et al., 2000; Guy and Li, 1998; Van Loon and Van Strien, 1999; Whitbred and Schuler, 2000). Other clones either belonged to the category of house-keeping genes or had homologies with putative proteins for which no function had been assessed. These latest cDNA sequences might be coffee-specific.

Future work will aim at understanding the role of selected clones and sequences showing no similarities with known proteins in the mechanism of coffee resistance to parasites. Genes isolated in coffee plants will be studied for their *in vivo* function and the transcriptional regulation of their expression.

REFERENCES

Bertrand B., Anthony F., Lashermes P. (2001) Breeding for resistance to *Meloidogyne exigua* of *Coffea arabica* by introgression of resistance genes of *Coffea canephora.* Plant Pathology, in press.

Choi D., Kim H.M., Yun H.K., Park J.A., Kim W.T. and Bok S.H. (1996) Molecular cloning of a metallothioneine-like gene from *Nicotiana glutinosa* L. and its induction by wounding and tobacco mosaic virus infection, Plant Physiol. **112**: 353-359.

Clough S.J, Fengler K.A., Yu I.C., Lippok B., Smith R.K., Bent A.F. (2000) The Arabidopsis dnd1 "defense, no death" gene encodes a mutated cyclic nucleotide-gated ion channel, Proc. Natl. Acad. Sci. USA **16**: 9323-8.

D'Oliveira B. and Rodrigues Jr, C. J. (1961) O problema das ferrugens do cafeeiro. Rev. Café Port. **8**: 5-50.

Diatchenko L., Lau Y. F. C., Campbell A. P., Chenchik A., Moqadam F., Huang B., Lukyanov S., Lukyanov K., Gurskaya N., Sverdlov E. D. and Siebert P. D. (1996) Suppression subtractive hybridization : A method for generating differentially regulated or tissue-specific cDNA probes and libraries. Proc. Natl. Acad. Sci. USA **93**: 6025-6030.

Ellis J., Dodds P., Pryor T. (2000) Structure, function and evolution of plant disease resistance genes. Curr. Opin. in Plant Biol. **3**: 278-284.

Guy C.L. and Li Q.B. (1998) The organization and evolution of the spinach stress 70 molecular chaperone gene family, Plant Cell **10**: 539-556.

Hatanaka T., Choi Y.E., Kusano T., Sano H. (1999) Transgenic Plants of Coffee Coffea Canephora From Embryogenic Callus Via Agrobacterium Tumefaciens-Mediated Transformation, Plant Cell Reports **19**: 106-110

Lashermes P., Combes M.C., Topart P., Graziosi G., Bertrand B. and F. Anthony (2000a). Molecular breeding in coffee (*Coffea arabica* L.) In "Coffee Biotechnology and Quality" Sera, Soccol, Pandey and Roussos Eds., Kluwer Academic Publisher, Dordrecht, pp 134-146.

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S., Grasiozi G., Trouslot, P., Anthony F. (2000b) Molecular analysis of introgressive breeding in coffee (*Coffea arabica*). Theor. Appl. Genet. **100**: 139-146.

Leroy T., Philippe R., Royer M., Frutos R., Duris D., Dufour M., Jourdan I., Lacombe C. and Fenouillet C. (1999) Genetically modified coffee plants expressing the *Bacillus thuringiensis cry1Ac* gene for resistance to leaf miner, Proceedings of the XVIII Scientific Colloquium on Coffee, ASIC, Helsinki, Finland, Asic (eds.), Paris, 332-338

Noir S., Combes M.C., Anthony F., Lashermes P. (2001) Origin, diversity and evolution of NBS disease-resistance gene homologues in coffee trees (*Coffea* L .). Mol Gen Genomics **265**: 654-662.

Rodrigues C.J.Jr., Bettencourt A.J. and Rijo L. (1975) Races of the pathogen and resistance to coffee rust, Annu. Rev. Phytopathol. **13**: 49-70

Silva M. C., Nicole M., Rijo L., Geiger J. P. and Rodrigues Jr, C. J. (1999) Cytochemical aspects of the plant-rust fungus interface during the compatible interaction *Coffea arabica* (cv. Caturra)-*Hemileia vastatrix* (race III), Int. J. Plant Sci. **160**: 79-91.

Spiral J., Leroy T., Paillard M. and Pétiard V. (1999) Transgenic coffee (*Coffea* species), in Y.P.S. Bajaj (ed.), Biotechnology in Agriculture and Forestry, Vol. **44**, transgenic Trees, Springer-Verlag, Berlin, Heidelberg, pp. 55-76

Van Loon L.C. and Van Strien E.A. (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins, Physiol. Mol Plant Pathol. **55**: 85-97.

Vos P., Hogers R., Bleeker M., Reijans M., Van der Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper, M., Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res **23**: 4407-4414.

Whitbred, J.M., Schuler, M.A. (2000) Molecular Characterization of Cyp73a9 and Cyp82a1 P450 Genes Involved in Plant Defense in Pea, Plant Physiology **124**: 47-58

Coffee Breeding and Selection: Review of Achievements and Challenges

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SUMMARY

Much of the world coffee is still produced by traditional cultivars of *Coffea arabica* (66%) and *Coffea canephora* (34%), released some 50-80 years ago from relatively simple selection and breeding programmes and generally multiplied by seed.

New arabica cultivars with higher yield potential and resistance to Coffee Leaf Rust (CLR) and/or Coffee Berry Disease (CBD) have started to replace traditional varieties on a large scale in several countries, e.g. Catimor and similar compact types in Central & South America and India, Icatu in Brazil, F1 hybrids in Kenya and Ethiopia. Examples of modern cultivars in robusta (*C. canephora*) coffee are the BR (seed) series of India, SA and BP selections in Indonesia, IF clones in the Ivory Coast and Apoata (seed) in Brazil. The Arabusta interspecific hybrid in Ivory Coast was not a success, in contrast to the encouraging performance of the "CxR" variety in India (from a cross *C.congensis* x *C.canephora*).

The repeated appearance of new virulent races of CLR and break-down of resistance in cv. Cauvery (Catimor lines) in India, the sudden re-appearance of a wilt disease (tracheomycosis) in robusta coffee in DR Congo and Uganda, increasing nematode problems in arabica coffee in Central America and the arrival of the Coffee Berry Borer in Colombia (1988) and India (1990) are just a few examples of new challenges to coffee breeding.

Plant biotechnology has evolved, particularly during the past decade, into an applied science providing powerful additional tools for plant breeding with the potential of increasing selection efficiency and creating new approaches to hitherto unattainable objectives. In coffee, molecular marker technology has already been implemented in germplasm characterization and management, detecting genetically divergent breeding sub-populations (e.g. to predict hybrid vigour), establishing gene introgression from related species and molecular marker-assisted selection Generally, successful genetic transformation is still limited to characters controlled by major genes for which gene isolation and transfer are relatively easy. Techniques of regenerating plants from *in vitro* micro-propagation and somatic embryogenesis are by now well established for various coffee species and transgenic coffee plants have been produced already, e.g. with insect resistance and with caffeine-free beans.

Coffee producing countries are once more faced with reduced revenues as a result of structural surpluses on the world coffee market. However, there is a growing market with premium prices for biologically grown speciality coffees, to which "smaller" producers in particular should be able to respond. This requires adaptation in agronomic and socio-economic aspects of coffee production, processing and marketing. It will be a challenge to plant breeding, by classical as well as molecular methods, to make substantial contributions to:

- *lowering production costs*: higher yields, easier harvesting (compact growth), reduced disease and pest control by host resistance (pest control also by biological means in IPM);
- *high bean and cup quality*: first priority in arabica; superior robustas by e.g. CXR cvs; a market niche also for caffeine-free coffee;
- *biological coffee*: no or minimal pesticide use in disease and pest resistant cultivars.

INTRODUCTION

This paper is largely based on a recently published review on coffee breeding and selection (Van der Vossen, 2001), which has been an attempt to update earlier reviews by Van der Vossen (1985), Carvalho (1988), Charrier and Berthaud (1988), Bettencourt and Rodrigues (1988) and Cambrony (1988). Reference has been made also to new papers published during the last 12 months.

WORLD PRODUCTION

Coffee producing countries are once more faced with reduced revenues as a result of low prices due to structural surpluses on the world coffee market. Production increased by about 14% over the past 15 years to 6.41 million t in 1999-2000 (USDA/FAS, 2000), including 66% Arabica and 34% Robusta coffees (in 1985: 75% Arabica and 26% Robusta). About 59% of the world coffee was produced in Latin America, 19% in Africa and 22% in Asia. The 10 most important coffee producing countries accounted for 75% of world coffee production in 1999-2000: Brazil (25.3%), Colombia (9.4%), Vietnam (7.5%), Indonesia (6.7%), Côte d'Ivoire (5.0%), Mexico (4.9%), India (4.6%), Guatemala (4.1%), Uganda (3.7%) and Ethiopia (3.6%). Of particular interest is the accelerated expansion of Robusta coffee production in Vietnam (480,000 t) and Indonesia (430,000 t). Brazil produced some 300,000 t Robusta coffee in addition to its 1.3 million t Arabica crop and is rapidly over-taking the traditionally leading Robusta producers Ivory Coast and Uganda. India has almost doubled its annual production during the last decade to almost 300,000 t (40% Arabica and 60% Robusta coffees).

PROGRESS IN COFFEE BREEDING AND SELECTION

Coffee research centres

Names and locations of 11 major national research centres with important coffee breeding and selection programmes are presented in Table 1. Besides, there are two research centres outside the coffee producing countries, notably the CIFC (Coffee Rust Research Centre) at Oeiras, Portugal, and CIRAD (Centre for International Agricultural Research) at Montpellier, France, which contribute considerably to regionally oriented coffee research (e.g. coffee leaf rust; coffee berry disease) and facilitate international colaboration between research centres.

The ICCRI in Indonesia (1900), IAC in Brazil (1924) and CCRI in India (1925) represent the oldest coffee research centres. They are famous for their pioneering work in genetics and cultivar development, both in Arabica and Robusta, in interspecific hybridization and the CCRI in particular also in the early search for host resistance to coffee leaf rust. Breeding programmes in the other coffee research centres were initiated more recently, most of them after 1960. CATIE in Costa Rica coordinates coffee research in Central America, which includes an important programme on host resistance to nematodes. CENICAFE in Colombia is well known for its breeding programme for resistance to coffee leaf rust, which culminated in the release of the (multiline) cultivar Colombia. The JARC in Ethiopia, CRF in Kenya and

TARO in Tanzania have focused on host resistance to the devastating coffee berry disease, with the Kenyan hybrid cultivar Ruiru II as the most successful result so far. FOFIFA in Madagascar is well known for its extensive studies on the *Mascarocoffea* species. The IRAD in Cameroon has interesting breeding programmes in Arabica as well as Robusta coffees. The CNRA in Côte d'Ivoire has made considerable contributions to Robusta coffee breeding practices and has the largest programme in progress at the moment on reciprocal recurrent selection with impressive results already forthcoming.

Table 1. Major coffee research centres

Acronym	Full name	Place	Country	Founded
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza	Turrialba	**Costa Rica**	1953
CENICAFE	Centro Nacional de Investigaciones de Café	Chinchina	**Colombia**	1938
IAC	Instituto Agronômico de Campinas	Campinas	**Brazil**	1924
CNRA	Centre National de Recherche Agronomique	Divo	**Côte d'Ivoire**	1950
IRAD	Institut de Recherche Agronomique et Développement	Foumbot	**Cameroun**	1964
JARC	Jimma Agricultural Research Centre	Jimma	**Ethiopia**	1970
CRF	Coffee Research Foundation	Ruiru	**Kenya**	1946
TARO	Tanzanian Agricultural Research Organization	Lyamungu	**Tanzania**	1934
FOFIFA	Centre National de Recherche Agronomique Appliquée au Développement	Ilaka	**Madagascar**	1960
CCRI	Central Coffee Research Institute	Balehonnur	**India**	1925
ICCRI	Indonesian Coffee and Cocoa Research Institute	Jember	**Indonesia**	1900

Breeding and selection methods applied in coffee

Arabica and Robusta coffee breeding programmes have the same main objective of developing new cultivars with the potential of yielding optimum economic returns to coffee growers. Yield, plant vigour and quality have been the main selection criteria in both coffee types, but in Arabica coffee resistance to disease and pests is a breeding objective of the highest priority. Variation in the circumstances of climate, soil, biotic and abiotic stresses, cropping systems, socio-economic factors, market dynamics and consumer preferences further defines priorities of selection criteria applied in specific programmes.

Methods applied in breeding and variety propagation depend primarily on the mating systems of Arabica (self-pollinating) and Robusta (cross-pollinating) coffee. Table 2 presents a summary of methods implemented in various coffee research centres, together with examples of released cultivars. Four basic methods of breeding and selection can be distinguished in each of the two species. These are listed in order of increasing complexity from line or mass selection to intra- and interspecific hybridization, the application depending on breeding objectives and intended output (Van der Vossen, 1985, 2001; Charrier and Berthaud, 1988).

Cultivars of the self-pollinating Arabica coffee are usually true-breeding lines from single-plant selections in growers' fields or from progenies of simple crosses and backcrosses, while

those of the outbreeding Robusta are open-pollinated cultivars produced from selected seedling and bi- or poly-clonal gardens. Clonal Robusta cultivars have found limited application so far, except in plantation coffee in Indonesia and Côte d'Ivoire, largely because the logistics of mass propagation and distribution are too complex and expensive for smallholder production systems, which dominate coffee production. Hybrid vigour for yield noticed in crosses between parents of genetically diverse sub-populations has led to the adoption of methods of reciprocal recurrent selection with distinct sub-populations in Côte d'Ivoire to increase chances of producing genotypes superior in yield, quality and other important traits (Leroy et al., 1993, 1994, 1997).

Table 2. Selection and breeding methods; results

Methods	Output	
	Cultivar	Country
A. Arabica Coffee (self-pollinating)		
1 Pure line selection (seeds)	Kents SL28 Caturra	India Kenya Brazil
2 Pedigree selection after hybridization (seeds)	S795 Catimor, Tupi Sarchimor Colombia	India Brazil Costa Rica Colombia
3 Intraspecific F1 hybrids (seeds; clones by embryogenesis ?)	Ruiru II Ababuna Catimor x Et	Kenya Ethiopia Costa Rica
4 Interspecific hybridization & BC's: (Rob. x Ar.) x Ar.; pedigree selection (seeds)	Icatu S2828	Brazil India
B. Robusta Coffee (cross-pollinating)		
1 Mass selection (seeds)	S274 Apoata	India Brazil
2 Family and clonal selection (seeds or clones)	BR series SA & BP sel. IF clones	India Indonesia Côte d'Ivoire
3 Reciprocal recurrent selection (clonal hybrid seed or clones)	in progress	Côte d'Ivoire
4 Interspecific hybridization Arabica x Robusta (clones) C. congensis x Robusta (seeds)	 Arabusta C x R variety	 Côte d'Ivoire India

Efforts to obtain durable resistance to coffee leaf rust (CLR) have had a long history of initial successes followed by disappointments because of repeated appearance of new virulent races of the rust fungus, but some lines of the cultivar Catimor (selected from crosses between Caturra and Hibrido de Timor) have shown complete resistance in most countries. These results were obtained by breeding plans normally applied to self-pollinating crops, including recombination crosses followed by back crossing, inbreeding and pedigree selection. (Carvalho, 1988; Bettencourt and Rodrigues, 1988). A similar plan was initially applied also in a breeding programme in Kenya to obtain resistance to coffee berry disease (CBD), which turned out to be controlled by a few major genes but nevertheless also durable. The change of breeding strategies to produce F1 hybrid (seed) cultivars instead of clones or true breeding lines was partly inspired by the confirmation of transgressive hybrid vigour in genetically divergent crosses also in Arabica coffee. Other advantages were chances of earlier

introduction of cultivars with resistance to both CBD and CLR, as well as several other desirable agronomic characters (Van der Vossen, 1985; Walyaro, 1997).

Interspecific hybridization has played a significant role in coffee, such as crosses between Arabica and Robusta coffee with the objective of introgressing disease resistance into Arabica (e.g. the cultivar Icatu in Brazil) or improved liquor quality into Robusta coffees (the variety Arabusta in the Ivory Coast). Other examples of interspecific hybridization leading to successful cultivars in Arabica and in Robusta coffee can be found in India: the Arabica-like S2828 and the Robusta-like C x R (Srinivasan, 1996).

Table 3. Yield and quality of some Arabica and Robusta cultivars

Cultivar	Yield	Bean size	Cup quality
	t / ha	% A	score 1 - 7
Arabica India (shade)			
Kents	1.2	75	6.5
S795	1.2	75	6.0
S2828	1.1	70	5.0
Cauvery (= Catimor)	1.5	65	5.5
Robusta India (shade)			
S274	2.0	45	4.0
BR series	2.0	50	4.0
C x R	2.0	55	5.0
Arabica Kenya (no shade)			
SL28	2.0	75	7.0
Catimor	1.5	65	5.5
Ruiru II	2.5	70	6.5

Note: bean size A = retained by a No.17 screen; cup quality score 1 = poor; 7 = excellent

New Arabica cultivars with higher yield potential and resistance to important diseases (CLR and CBD) have started to replace traditional varieties on a large scale in several countries: e.g. Catimor and Sarchimor type of cultivars in Colombia, Brazil, Central American countries and India, Icatu in Brazil, Java in Cameroon, Ruiru II in Kenya and Ababuna in Ethiopia (the latter two being F1 hybrids). In Robusta coffee the release of cultivars from advanced selection programmes is taking place more gradually, such as the BP and SA clones in Indonesia, the BR (seed) cultivars in India, the IF clones in the Ivory Coast and the cultivar Apoata in Brazil.

The high expectations of the Arabusta programme in the Ivory Coast have not been fulfilled, because of persistent problems of genetic instability and low fertility. On the other hand, the CxR variety of India has proved to be a success as a productive and stable Robusta coffee with superior bean and liquor characteristics. The performance in yield and quality of modern Arabica and Robusta cultivars in India and Kenya against the traditional cultivars is presented in Table 3. Cup quality is often superior in the older, disease susceptible Arabica cultivars Kents and S795 in India and SL 28 in Kenya, but high yields can only be maintained by costly disease control measures. Cauvery and Catimor have somewhat smaller beans and lower cup quality, while the high yielding Ruiru II (hybrid of Catimor x tall breeding lines) has bean size and cup quality close to the best standard cultivar SL 28. The Indian C x R cultivar is a remarkable improvement in bean size and cup quality on traditional Indian Robustas.

Plant biotechnology has evolved, particularly during the past decade, into an applied science providing powerful additional tools for plant breeding with the potential of increasing selection efficiency and creating new approaches to hitherto unattainable objectives. In coffee, molecular marker technology has already been implemented in germplasm characterization and management, detecting genetically divergent breeding sub-populations (e.g. to predict hybrid vigour), establishing gene introgression from related species and molecular marker-assisted selection (Lashermes et al., 1997, 2000). Generally, successful genetic transformation is still limited to characters controlled by major genes for which gene isolation and transfer are relatively easy. Techniques of regenerating plants from *in vitro* micro-propagation and somatic embryogenesis are by now well established for various coffee species (Etienne et al., 1997a, b; Berthouly and Michaux-Ferrière, 1996) and transgenic coffee plants have been produced already, e.g. with insect resistance and with caffeine-free beans (Leroy et al., 2000; Moysiadi et al., 1999).

PROSPECTS OF FURTHER ADVANCES IN COFFEE PLANT IMPROVEMENT

Genetic resources

Altogether about 100 species (taxa) of the genus *Coffea* have been identified so far (Bridson and Verdcourt, 1988). They are without exception indigenous to the forests of tropical Africa and all are diploid (2n=22) species except the allotetraploid (2n=44) *C.arabica*. Several collecting expeditions for *Coffea* germplasm have been made between 1960 and 1985 to important centres of genetic diversity in Africa and most of these accessions are maintained in field collections in the 11 coffee research centres mentioned in Table 1.

Progressive crop improvement requires easy access to intra- and interspecific genetic variation. The monophyletic origin of all *Coffea* species and general absence of strong interspecific crossing barriers (Charrier and Berthaud, 1985; Charrier and Eskes, 1997) provide opportunities of exploiting also the genetic variation of several other species for the purpose of introgressing agronomically and biochemically interesting characters into the two species of commercial value, *C. arabica* and *C. canephora*. This coffee germplasm should receive adequate support for maintenance and further systematic exploration. An internationally recognized network organization for the conservation and study of coffee genetic resources with participation of all major coffee producing countries is being established to promote unrestricted exchange of information and materials. IPGRI in conjunction with IRD, CIRAD and the African Coffee Research Network (ACRN) has taken initiatives into that direction (Guarino et al., 1995; Ngategize, 1997). Molecular marker technology offers considerable prospects of improving management and characterization of coffee genetic resources (Anthony et al., 2001).

In coffee there have been no alternatives so far to *ex situ* field collections for long-term germplasm conservation, because coffee seeds are recalcitrant and conventional methods of seed storage cannot extend viability beyond 2-3 years (Van der Vossen, 1985). Field collections require expensive resources of land, qualified staff and upkeep, while there is also a risk of losing valuable germplasm due to diseases and pests, as well as to poor adaptation of certain species to the local environment.

By applying slow-growth conditions to *in vitro* cultured explants (zygotic embryos, apical meristem or nodal cuttings) and repeated subculturing, Dussert et al. (1997a) were able to conserve a core collection of coffee germplasm for about three years. Such methods of *in vitro* conservation have great advantages for germplasm distribution (less volume during shipping and simple quarantine procedures), but appear unsuitable for long-term germplasm

conservation. Encouraging results of cryo-preservation techniques (storage under liquid nitrogen at –196°C) applied to coffee seeds, excised embryos and somatic embryos have been reported recently (Dussert et al., 1997b). There is little doubt that cryo-preservation opens interesting perspectives for long-term conservation of coffee germplasm in seed genebanks.

Breeding and selection

Vigour and yield

There are still considerable opportunities for higher yields per plant by exploiting interpopulation crosses, both in Arabica and Robusta coffees. The identification of compact plant types in Robusta and C x R breeding populations in India (Kumar et al., 1994; Srinivasan, 1996) opens the possibility of developing cultivars suitable to intensive Robusta coffee production, similar to the Caturra/Catimor cultivars in Arabica coffee. Molecular markers in combination with high-density molecular linkage maps will soon be standard tools of the breeders to select more efficiently for QTL's (quantitative trait loci) for components of vigour and yield

Quality

Bean size and cup quality continue to receive priority attention in Arabica coffee. The Indian C x R breeding populations in particular offer great opportunities for improving bean size an cup quality in Robusta coffee. Molecular marker technology may help to select for important components of coffee quality, but the final test of cup quality will continue to be made by experienced coffee liqorers. Caffeine-free coffee types produced by genetic transformation will soon be available for further development into practical cultivars. On the other hand, coffee is drunk mainly for its stimulating properties derived from the caffeine it contains and moderate coffee drinking does not pose health hazards to most people. Caffeine-free cultivars may, therefore, attain only limited prominence in coffee cultivation considering the demand for decaffeinated coffees, which is small (10% of total coffee consumption) and unlikely to increase much in the foreseeable future.

Resistance to diseases and pests

Molecular markers linked to SH genes may soon be developed, enabling accumulation (pyramiding) of effective genes in breeding lines to develop new cultivars (Arabica in particular) with expected durable resistance to CLR (Sreenath and Naidu, 1999). The first of such markers have already been identified for genes controlling resistance to CBD (Agwanda et al., 1998) and may also be available soon for the selection of resistance to nematodes (Lashermes et al., 1999). The successful regeneration of transgenic coffee plants expressing resistance to leaf miners based on Bt genes (Leroy et al., 2000) could be the start of molecular breeding for resistance to important coffee pests, especially the endocarpic insects such as the coffee berry borer (Guerreiro et al., 1998). Host resistance to insect pests should be implemented in combination with effective methods of IPM, which may include specific parasitoids and entomopathogens, or a male sex pheromone as in the case of the white stem borer in Arabica coffee in India (Hall et al., 1998; Jayarama et al., 1998). In Central America considerable progress has been made with host resistance to root-knot nemtatodes in Arabica coffee (Anzueto et al., 2001) and in Robusta coffee to be used as rootstock for Arabica cultivars (Bertrand et al., 2000).

Drought tolerance

Arabica coffee is generally more tolerant to water stress than Robusta, at least partly as the result of a more extensive and deeper root system. However, there are also large differences in drought tolerance between genotypes of the same species. Some of the East-African cultivars (e.g. SL28) appear to be the best genotypes available within Arabica germplasm, because of an exceptionally well developed root system, outstanding plant vigour and an ability to retain their leaves under water stress (Van der Vossen and Browning, 1978).

Selection for more drought tolerant Robusta coffee should emphasize depth and extent of root system, as well as leaf retention under stress conditions. The Indian C x R cultivar appears to have better drought tolerance, but it is unlikely that Robusta-like genotypes would be found with better drought tolerance than Arabicas.

Propagation

Seeds

Propagation by seeds continues to be the preferred practice for new coffee cultivars in most countries (Table 2). Seed multiplication of F1 hybrid Arabica cultivars is a logistically complex operation involving hand-pollination of previously emasculated and bagged flowers (Opile and Agwanda, 1993). Experience with the Kenyan hybrid cultivar Ruiru II shows that large-scale seed multiplication is technically feasible and cheaper than clonal propagation. Male sterility conditioned by one recessive gene has been detected in Arabica accessions of Ethiopian origin (Dufour et al., 1997), which provides opportunities of reducing costs of seed production. Male-sterile female parents for seed production may be obtained by genetic transformation within a much shorter period of time, than would be required with conventional methods of introgressive breeding.

Clones

Conventional methods of clonal propagation are about ten times more expensive than multiplication by seed (Montagnon et al., 1998). The induction of high frequency somatic embryogenesis in a liquid medium (Berthouli and Michaux-Ferrière, 1996) appears to be very promising for efficient mass propagation. It is being implemented in Arabica coffee in Central America to multiply Catimor x Et hybrids as an alternative to hybrid seed production (Etienne et al., 1997a and b) and in Uganda to multiply elite Robusta clones (Berthouly et al., 1995). A major advantage of plants raised from somatic embryogenesis is their similarity to seedlings with respect to the root system. Deshayes et al. (1999) claim that costs of producing Robusta coffee plants through somatic embryogenesis are comparable to conventional rooted cuttings, in other words, still considerably more expensive than hybrid seed production. However, Etienne-Barry et al. (1999) have already been able to reduce costs by more efficient regeneration of plants following somatic embryogenesis in Arabica coffee.

CONCLUSIONS

The market outlook for the coming years appears rather grim. Structural surpluses on the world coffee market will keep prices low, except during occasional years of low coffee output in Brazil due to frosts or droughts. This will negatively affect the economic viability of coffee production in many countries. On the other hand, there are opportunities in a small but gradually expanding market with premium prices for high quality (speciality) and organically grown coffees. This will require considerable adaptation in agronomic and socio-economic

aspects of coffee production, processing and marketing. Conventional and molecular plant breeding can make substantial contributions to lowering production costs, increasing bean and liquor quality and suitability of new cultivars to organic coffee production. The challenges to coffee plant breeding to achieve these objectives have been summarized in Table 4.

Table 4. Summary of challenges to coffee plant improvement

A	
Market outlook:	**Structural surpluses on the world market resulting in low prices and reduced revenues for producers**
Opportunities:	**Small but expanding market with premium prices for speciality and/or organically grown coffees**
Required:	**Adaptation in agronomic and socio-economic aspects Of coffee production, processing and marketing**
B	
Contribution by plant breeding, conventional & Molecular methods:	**1. Lowering production costs** *** higher yields / ha** *** easier harvesting (compact growth)** *** reduced disease control by host resistance** *** reduced pest control by host resistance** **(in combination with biological means)** **2. High bean and cup quality** *** first priority in Arabica** *** superior Robustas in C x R cvs.** *** niche market for caffeine-free coffees** **3. Organically grown coffee** *** adaptation to Agro-forestry systems** *** no or minimal pesticide use (host resistance)**

REFERENCES

Agwanda C.O., Lashermes P., Trouslot P., Combes M.C. and Charrier A. (1998) Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee. Euphytica*,* **97**, 241-248.

Anthony F., Bertrand B., Quiros O., Wilches A., Lashermes P., Berthaud J. and Charrier A. (2001) Genetic diversity of wild coffee (*Coffea arabica* L.) using molecular markers. Euphytica **118**: 53-65.

Anzueto F., Bertrand B., Sarah J.L., Eskes, A.B. and Decazy, B. (2001) Resistance to *Meloidogyne incognita* in Ethiopian *Coffea arabica* accessions. Euphytica **118**: 1-8.

Bertrand B., Peña Durán M.X., Anzueto F., Cilas C., Anthony F. and Eskes A.B. (2000) Genetic study of *Coffea canephora* coffee tree reistance to *Meloidogyne incognita* nematodes in Guatemala and *Meloidogyne* sp. nematodes in El Salvador for selection of rootstock varieties in Central America. Euphytica **113**: 79-86.

Berthouly M., Alvaro D., Carasco C. and Duris D. (1995) A technology transfer operation: a commercial *Coffea canephora* micro-propagation laboratory in Uganda. Proceedings

16th International Scientific Colloquium on Coffee, Kyoto, Japan. ASIC, Paris, France, pp. 743-744.

Berthouly M. and Michaux-Ferrière N. (1996) High frequency somatic embryogenesis in *Coffea canephora*. Plant Cell Tissue and Organ Culture, **44** (2), 169-176.

Bettencourt A.J. and Rodrigues C.J. (1988) Principles and practice of coffee breeding for resistance to rust and other diseases. In: Coffee, vol. 4 Agronomy (Ed. by Clarke R.J. and Macrae R.), pp. 199-234. Elsevier Applied Science, London and New York.

Bridson D.M. and Verdcourt B. (1988) Rubiaceae (Part 2). In: Flora of tropical East Africa (Ed. by R.M. Polhill), pp. 703-723. Balkema, Rotterdam and Brookfield.

Carvalho A. (1988) Principles and practice of coffee plant breeding for productivity and quality factors: *Coffea arabica*. In: Coffee, vol. 4 Agronomy (Ed. by Clarke R.J. and Macrae R.), pp. 129-165. Elsevier Applied Science, London and New York.

Charrier A. and Berthaud J. (1985) Botanical classification of coffee. In: Coffee: botany, biochemistry and production of beans and beverage (Ed. by Clifford M.N. and Willson K.C.), pp. 13-47. Croom Helm, London, New York and Sydney.

Charrier A. and Berthaud J. (1988) Principles and methods of coffee plant breeding: *Coffea canephora* Pierre. In: Coffee, vol. 4 Agronomy (Ed. by Clarke R.J. and Macrae R.), pp. 167-197. Elsevier Applied Science, London and New York.

Charrier A. and Eskes A.B. (1997) Les caféiers. In: L'amélioration des plantes tropicales (Ed. by Charrier A., Jackot M., Hamon S. and Nicolas D.), pp.171-196. CIRAD/ORSTOM, Montpellier, France.

Deshayes A., Ducos J.P., Gianforcaro M., Florin B. and Pétiard V. (1999) A technically and economically attractive way to propagate elite coffee cultivars: *in vitro* somatic embryogenesis. Proceedings 18th International Scientific Colloquium on Coffee, Helsinki, Finland. ASIC, Paris, France, pp. 295-304..

Dufour M., Anthony F., Bertrand B. and Eskes A.B. (1997) Identification de caféiers mâle-stériles de *Coffea arabica* au CATIE, Coata Rica. Plantations, recherche, développement, **4** (6), 401-407.

Dussert S., Chabrillange N., Engelmann F., Anthony F., Récalt C. and Hamon S. (1997a) Variability in storage response within a coffee (*Coffea* spp.) core collection. Plant Cell Reports **16**, 344-348.

Dussert S., Chabrillange N., Engelmann F., Anthony F. and Hamon S. (1997b) Cryopreservation of coffee (*Coffea arabica* L.) seeds: importance of the precooling temperature. Cryo-Letters **18**, 269-276.

Etienne H., Bertrand B., Anthony F., Côte F. and Berthouly M. (1997a) L'embryogenèse somatique: un outil pour l'amélioration génétique du caféier. Proceedings 17th International Scientific Colloquium on Coffee, Nairobi, Kenya. ASIC, Paris, France, pp. 457-465.

Etienne H., Solano W., Pereira A., Bertrand B. and Berthouly M. (1997b) Protocole d'acclimatation de plantules de caféiers produits in vitro. Plantations, recherche, développement, **4** (5), 304-309.

Etienne-Barry D., Bertrand B., Vasquez N. and Etienne H. (1999) Direct sowing of *Coffea arabica* somatic embryos mass-produced in a bioreactor and regeneration of plants. Plant Cell Reports **19**: 111-117.

Guarino L., Charrier A. and Opile W.R. (1995) A plan of action for the ACRN programme on plant genetic resources. Proceedings 16th International Scientific Colloquium on Coffee, Kyoto, Japan. ASIC, Paris, France, pp. 626 - 629.

Guerreiro Filho O., Denoit P., Pefercen M., Decazy B., Eskes A.B. and Frutos R. (1998). Susceptibility to the coffee leaf miner (*Perileucoptera* spp.) to *Bacillus thuringiensis d*-endotoxins: a model for transgenic perennial crops resistant to endocarpic insects. Current Microbiology, **36**, 175-179.

Hall D.R., Cork A., Phythian S., Sumathi C., Jayarama M.G., Venkatesha M., D'Souza V. and Naidu R. (1998) Studies on the male sex pheromone of the coffee white stemborer, *Xylotrechus quadripes* Chevrolat (Coleoptera: Cerambycidae). 2nd International Symposium on Insect Pheromones. Book of Abstracts, pp. 50-52. WICC-IAC, Wageningen, the Netherlands.

Jayarama M.G., Venkatesh M., D"Souza V., Naidu R., Hall D.R. and Cork A. (1998) Sex pheromone of coffee white stem borer for monitoring and control is on the anvil. Indian Coffee, **62** (9), 15-16.

Kumar A., Srinivasan C.S. and Nataraj T. (1994) A preliminary note on the occurrence of dwarf mutants in robusta coffee (*Coffea canephora*). Journal of Coffee Research, **24** (1), 41-45.

Lashermes P., Agwanda C. O., Anthony F., Combes M.C., Trouslot P. and Charrier A. (1997) Molecular marker-assisted selection: a powerful approach for coffee improvement. Proceedings 17th International Scientific Colloquium on Coffee, Nairobi, Kenya. ASIC, Paris, France, pp. 474-480.

Lashermes P., Anzueto F., Bertrand B., Graziosi G. and Anthony F. (1999) Sustainable improvement of nematode resistance in coffee cultivars (*Coffea arabica* L.) of Central America: enhanced use of genetic resources by the development of marker-facilitated selection programmes. Proceedings 18th International Scientific Coffee Conference, Helsinki, Finland. ASIC, Paris, France.

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S., Graziosi G. Trouslot P. and Anthony F. (2000) Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.). Theoretical and Applied Genetics, **100**, 139-146.

Leroy T., Montagnon C., Charrier A. and Eskes A.B. (1993) Reciprocal recurrent selection applied to *Coffea canephora* Pierre. I. Characterization and evaluation of breeding populations and value of intergroup hybrids. Euphytica **67**, 113-125.

Leroy T., Montagnon C., Cilas C., Charrier A. and Eskes A.B. (1994) Reciprocal recurrent selection applied to *Coffea canephora* Pierre. II. Estimation of genetic parameters. Euphytica **74**, 121-128.

Leroy T., Montagnon C., Cilas C., Yapo A., Charrier A. and Eskes A.B. (1997) Reciprocal recurrent selection applied to *Coffea canephora* Pierre. III. Genetic gains and results of first cycle intergroup crosses. Euphytica **95**, 347-354.

Leroy T., Henry A-M., Royer M., Altosaar I., Frutos R., Duris D. and Philippe R. (2000) Genetically modified coffee plants expressing the *Bacillus thuringiensis cry* 1Ac gene for resistance to leaf miner. Plant Cell Reports **19**: 382-389.

Moisyadi S., Neupane K.R. and Stiles J.I. (1999) Cloning and characterization of xanthosine-N7-methyltransferase, the first enzyme of the caffeine biosynthesic pathway. Proceedings 18th International Scientific Coffee Conference, Helsinki, Finland. ASIC, Paris, France, pp. 327-331.

Montagnon C., Leroy T. and Eskes A.B. (1998a) Amélioration variétale de *Coffea canephora*. I.Critères et méthodes de sélection. Plantations, recherche, développement, **5** (1), 18-33.

Ngategize P.K. (1997) The African Coffee Research Network: prospects and challenges into the next millennium. Proceedings 17th International Scientific Coffee Conference, Nairobi, Kenya. ASIC, Paris, France, pp. 361-365.

Opile W.R. and Agwanda C.O. (1993) Propagation and distribution of cultivar Ruiru II: a review. Kenya Coffee, **58** (677), 1496-1508.

Sreenath H.L. & Naidu R. (1999) Coffee biotechnology research in India - Potential progress and future thrust areas. Proceedings 18th International Scientific Colloquium on Coffee, Helsinki, Finland. ASIC, Paris, France, pp. 281-294

Srinivasan C.S. (1996). Review: current status and future thrust areas of research on varietal improvement and horticultural aspects of coffee. Journal of Coffee Research **26**, 1-16.

USDA/FAS (2000). Coffee production statistics. Horticultural and Tropical Products Division.

Van der Vossen H.A.M. (1985) Coffee selection and breeding. In: Coffee: botany, biochemistry and production of beans and beverage (Ed. by M.N.Clifford & K.C.Willson), pp. 48-96. Croom Helm, London, New York and Sydney.

Van der Vossen H.A.M. (2001). Agronomy I: Coffee breeding practices. In: Coffee, recent developments (Ed. by R.J.Clarke & O.G.Vitzthum), pp. 184-201. Blackwell Science, Oxford, UK.

Van der Vossen H.A.M. and Browning G. (1978) Prospects of selecting genotypes of *Coffea arabica* which do not require tonic sprays of fungicide for increased leaf retention and yield. Journal of Horticultural Science, **53**, 225-233.

Walyaro D.J. (1997) Breeding for disease and pest resistance and improved quality in coffee. Proceedings 17th International Scientific Colloquium on Coffee, Nairobi, Kenya. ASIC, Paris, France, pp. 391-405.

Conservation of Coffee Genetic Resources: Constraints and Opportunities

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SUMMARY

Coffee is the world's most important beverage and upon which the economies of many developing countries depends. Although the coffee beverage comes from mainly two species namely *Coffea arabica* and *C. canephora*, there are more than 100 wild species, all endemic to the continent of Africa and the West Indian Ocean islands. This diversity represents a store of genetic materials on which the future of coffee improvement both in terms of yield and quality depends. The FAO, IBPGR and ORSTOM have collected much of this diversity and tried to secure them in field genebanks. However the management of these collections has proved to be difficult to maintain and these resources have not been adequately characterized, evaluated and used in coffee breeding programmes. In this paper we critically discuss the methodologies for both *ex situ* and *in situ* conservation of the coffee genetic resources with reference to case studies in Costa Rica, Ethiopia Madagascar and Kenya, discuss the value of these collection for end users and describe the new advances made in the development of the conservation technologies for coffee germplasm.

RÉSUMÉ

Le café est la boisson stimulante la plus importante pour le commerce mondial et pour l'économie (rentrée de devises) de nombreux pays en développement. Le café est produit principalement par 2 espèces de caféiers du genre *Coffea – C.arabica* et *C.canephora* – mais il y existe plus de 100 espèces de caféiers sauvages endémiques du continent africain et des îles de la zone occidentale de l'Océan indien. Ils représentent une source de matériel génétique pour les travaux actuels et futurs d'amélioration génétique des variétés de caféiers cultivés du point de vue de la production et de la qualité du café. La FAO, l'ORSTOM et l'IBPGR ont entrepris la collecte de cette diversité et la conservation sous forme d'arbres cultivés en champ ou en parcelle forestière aménagée, dans quelques grandes collections d'importance régionale. La maintenance et la gestion de telles collections rencontrent nombre de difficultés et n'a pas permis leur caractérisation et leur évaluation approfondie en vue de leur exploitation dans les programmes d'amélioration génétique des caféiers. Dans cette

communication, nous ferons une présentation critique des méthodes de conservation *ex situ* et *in situ* des ressources génétiques des caféiers en référant au cas des collections du Costa Rica, Ethiopie, Madagascar et Kenya, nous discuterons de leur intérêt pour les producteurs, les industriels et les consommateurs de café, et nous présenterons les progrès récents dans les méthodologies de conservation des ressources génétiques caféières.

INTRODUCTION

Throughout the 20th century, coffee has been one of the most widely drunk beverages in the world. The coffee beverage is consumed by more than a third of the world's population and comes from two main species: *C. arabica* L., *C. canephora* Pierre ex Froehner. For the many tropical countries in Africa, Central and South America, Asia and Oceania, coffee production is a very important source of foreign exchange earnings. The genus *Coffea* is endemic to the old world tropics of Africa and Asia and over 100 wild species are found in the Afrotropical-Madagascar region including the Comoros and the Mascarene Islands (Bridson 1982). *C. arabica* originated from the highlands of Ethiopia, and was distributed from Yemen to Ceylon, and Java in the late 17th century and to the New World in the early part of the 18th century (Berthaud and Charrier, 1988). Yemen is thus considered as the centre of dispersal of *C. arabica*. The other African *Coffea* are distributed across the continent, the greatest concentration of species being found along the tributaries of the Congo river (Krug and Carvalho, 1951). This area is a particularly rich centre of genetic diversity for *C. canephora*, as well as for many diploid *Coffea* species, such as *C. congensis* Froehner and *C. liberica* Bull. ex. Hiern. An ecogeographic study on the wild *Coffea* species of East Africa, points to the Eastern Arc Mountain of Tanzania as being a centre of wild coffee species diversity (Cockram, 1997). Another centre of diversity is in the Malagasy-Mascarene region where a large group of 60 species are known to be native (Charrier, 1978). This area is the home of relatively isolated Mascarocoffea section of the genus characterized by low levels or absence of caffeine.

It is recognised that the cultivated varieties, in particular *C. arabica*, have a very narrow genetic base (Lashermes et al., 1996). The centres of diversity mentioned above, together with farmers fields growing old and traditional coffee varieties, are the ultimate sources of coffee genetic diversity. This diversity represents a store of genetic materials on which the future of coffee improvement both in terms of yield and quality depends. Unfortunately, only a small fraction of the unknown genetic variability existing in the wild is represented in various genebanks in the region and very little of this variability has been exploited in coffee improvement programmes. The breeding value of these accessions is unknown, the bulk of the material within the collections having been neither sufficiently characterized nor evaluated and documented. Deforestation and encroachment by agricultural activities, population pressures and economic hardships, are threatening all these reservoirs of great genetic diversity and, with them, comes the danger of significant erosion of the *Coffea* genepool. It is most urgent to secure threatened wild germplasm in *ex situ* collections and/or *in situ*/on-farm, before it is lost to humankind and its value to the coffee industry demonstrated and exploited.

The conservation of coffee genetic resources has not received much attention in the past decades, particularly at the international level. Various techniques for the conservation of coffee germplasm have been previously described (Dulloo et al., 1998). Coffee species have traditionally been conserved *ex situ* as living plants in field genebanks because it has proved difficult to conserve their seeds for long period of time in seed banks. Indeed, seeds of all coffee species studied until now do not exhibit orthodox storage behaviour: although they are

partially desiccation tolerant, they are cold sensitive and desiccation does not increase their longevity (Hong and Ellis, 1995).

Recent works in biotechnology indicate significant progress in the conservation of *Coffea* germplasm including cryopreservation (storage at liquid nitrogen temperature, –196°C) of coffee seeds (Dussert et al., 2001), particularly for *C. arabica*, and use of *in vitro* slow growth and cryopreservation for medium to long-term conservation using zygotic or somatic embryos, apices and buds (Dussert et al., 1997). Cryopreservation is the only available technique for long-term conservation of coffee germplasm. Other options of *ex situ* conservation include pollen storage under vacuum (Walyaro and Van der Vossen, 1977), although the techniques and their utilization, need further investigation, and, DNA banking which is potentially interesting technique but regeneration of whole units or use of particular genes is still quite difficult (Adams and Adams, 1991). Each of these methods have their respective advantages and disadvantages. Coffee pollen is known to conserve well and has major advantages for germplasm exchange as disease free material. Though *in situ* protection of *Coffea*, both in the wild and on farm, is a potentially important conservation approach, it has not received sufficient attention for a long time. There is still much to be done in optimizing the conservation methods for coffee germplasm; the variation in response to different conservation techniques of different *Coffea* species demonstrates the importance of complementarity in effective *ex situ* conservation strategies.

In this paper we review the current status on the management of coffee genetic resources in field genebanks, and describe the new advances made in the development of the conservation technologies for coffee germplasm.

FIELD COLLECTION OF COFFEE GERMPLASM

Field genebank has been the preferred mode of conservation of coffee genetic resources for reasons expressed above. The first coffee collecting missions were made in the 1960's and 1970's by ORSTOM (now IRD), FAO and IBPGR (now IPGRI) and established coffee field genebanks in national institutions in several African countries. A review of the world coffee collections showed that the major coffee field genebanks (with more than 1000 accessions) are located in Cameroon, Colombia, Costa Rica, Cote D'Ivoire, Ethiopia, and Madagascar (Dulloo et al., 1998). The state of the world report on plant genetic resources (FAO, 1998) reported 21,087 accessions of coffee genetic resources world wide. Many of the collection dates back over 30 years. We present below four case studies from Costa Rica, Madagascar, Ethiopia and Kenya that show how field coffee collection were set up and managed and the difficulties experienced in maintaining them.

Case study of CATIE, Costa Rica

CATIE manages one of the largest field genebanks of *C. arabica* in the world. A total of 8,590 coffee trees representing 1,997 accessions are conserved under the shade of the leguminous tree species *Erythrina poeppigiana* which is pruned twice annually. The conserved genetic resources are composed of: i) wild and semi-wild accessions collected in the primary centre of diversity of *C. arabica* (Fernie, 1968; Guillaumet and Hallé, 1978); ii) cultivars grown locally in Ethiopia and Yemen (Eskes, 1989); iii) cultivars and mutants derived from the base populations commonly called Typica and Bourbon; iv) lines introgressed from *C. canephora*; and v) hybrids between cultivars. The other *Coffea* species are not well represented: only *C. canephora* and *C. liberica* are represented by more than 20 genotypes (240 and 70 respectively).

The conserved material is suffering from three main problems: the age of the trees, the climatic conditions and the cultivation method. Most of the accessions (57.5%) were introduced before 1970 and are now at least 30 years old. The localisation of the field genebank at 602 m above sea level in a humid zone of Costa Rica does not supply optimal conditions of cultivation neither for *C. arabica* nor for the other coffee species. The cultivation method has been similar to the method of commercial plantations and the same for wild and cultivated accessions. It is obvious that the lack of knowledge about the value of the conserved genetic resources for breeding has played a crucial role and limited the funds allocated to the maintenance. A detailed analysis of the remaining coffee trees collected in Ethiopia by FAO (Fernie, 1968) reveals that the genetic loses have not been serious despite the problems described previously. A total of 2,523 plants representing 442 collection numbers were introduced in 1965. Four to eight individuals were planted per accession. Thirty-five years later, only eight accessions have been lost and 1,533 trees (60.8%) are still living in the genebank. However 10.1% and 20.3% of the remaining accessions are only represented by one or two individuals, respectively. As *C. arabica* is self-fertile at approximately 90%, the remaining plants can be considered as representative of the mother trees that were harvested in Ethiopia.

In the year 2000, CATIE started a project for rejuvenating the conserved genetic resources and ensuring their preservation. The genetic resources are being rejuvenated by grafting on vigorous rootstocks *C. canephora*, resistant to several root knot nematode species (*Meloidogyne* spp.). The wild material of *C. arabica* and other species will be conserved separately from the cultivars, mutants and introgression lines, which will constitute the working collection. This will allow for giving specific and adapted care to the wild material, such as permanent shade. The wild genotypes will be duplicated and each genotype will be represented by two clonal plants. The number of individuals will be reduced in the working collection to four individuals per accession in case of heterozygotic material (e.g. cv. Mundo Novo, introgression lines) and eight individuals chosen in all remaining accessions in case of homozygotic material (e.g. Typica- and Bourbon-derived cultivars, mutants). Such strategy of rationalisation based on passport and evaluation data will reduce by half the area of conservation and thus the cost of maintenance.

Case study Coffee Research Centre, Madagascar

In Madagascar, two field collections of about 7000 genotypes of Mascarocoffea species were originally established at the Coffee Research Center of Kianjavato and Ilaka Est, between the years 1960 to 1973 and since 1976 is under the management of CENRADERU. The main collection was located on a mountain slope at Kianjavato, where 236 accessions including 56 botanical species and 57 undescribed populations were kept under a converted natural forest. A duplicate collection of 51 accessions obtained by open pollinated seeds was kept at Ilaka Est, under the shade of *Inga dulcis*. The number of trees kept per accession varied from one to as many as 500, but most of the accessions were represented by 10 trees.

Unlike the CATIE case study, the Madagascar collections have suffered tremendously from the lack of resources for maintenance, inadaptability of species and unfavourable climate. The latter has been particularly disastrous at Ilaka Est which is more prone to cyclone and flooding, with the resultant loss of most of the collection. The loss was accelerated during the period 1988 to 1992 when no funds were available for its maintenance. The collection is now composed of only 50 old trees of some resistant species such as *C. perrieri*, *C. resinosa* and C. *tetragona*. At Kianjavato, there has been a loss of 25% and 50% in the number of accessions and genotypes respectively, over a period of 20 years. These losses can again be attributed to the chronic lack of resources for weeding and application of fertilisers. It is

reported that the field collections have not been fertilised for more than 25 years due to lack of funds. In addition some species such as *C. humblotiana*, *C. mauritiana* and *C. myrtifolia*, which are not well adapted to the environment are also threatened to disappear. The procedure used for replacing missing accessions by the use of open pollinated seeds of remaining plants within a population and by the use of cuttings is also seen as a potential source of genetic erosion in the collection.

Case study of Ethiopian Agriculture Research Organisation

The first field collection in Ethiopia was established from wild coffee plants collected in the forest in the 1950's (JATS, 1957), although many botanists have been collecting accessions of *C. arabica* coffee from Ethiopia since 1830's (Sylvain, 1955). More accessions were added from the FAO and French coffee collection mission to Ethiopia and other arabica varieties introduced from different countries. These were established in the field genebank at Jima (Bayetta, 1997). However these collections have not been properly maintained and well documented by JATS, probably because of institutional problems. Later on, after the establishment of JARC in 1967, more collection of coffee germplasm from various potential coffee growing agro-ecological zones of the country to capture maximum genetic variability for breeding (selection and hybridization) and conservation purposes were organised. With the out-break of coffee berry disease (CBD) in 1971, the major research topic became selection of resistant plants from large number of populations in the forest ecosystem in the south western part of the country. Nearly half of the indigenous accessions were identified and collected mainly for CBD resistance, while the remaining half from different parts of the country for various desirable agronomic characteristics (Bayetta, 1997).

So far, a total of 4216 indigenous and 190 exotic materials have been collected (between 1966 and 1998) and 3357 indigenous and 128 exotic accessions are currently available in the field genebank of JARC while the rest died because of mainly root diseases and poor adaptation (IAR, 1998). Some more accessions have also been collected recently from the eastern and western parts of the country. The collections have been planted and field established for breeding and conservation purposes at the national coffee research center in Jima and at its sub-centers (Gera, Tepi, Awada & Haru) located in different coffee growing agro-ecologies, representing the areas where the accessions came from. Besides, an equal number of accessions have been collected by the PGRC/E (now IBRC) from the different parts of the country and planted in the field genebank at Choche in 1980's. However, some of the collections failed to survive due to various problems. Both JARC and IBRC field coffee collections are maintained with all the necessary management inputs applied as recommended for modern coffee farms in the country. But, due to several problems (mainly change of environment) some of the accessions could not adapt in areas different from their original habitat.

Case Study of Coffee Research Foundation, Kenya

In Kenya, available coffee genetic resources are composed of indigenous *Coffea* species, improved varieties, variety collections as well as the introductions, which started in 1893 when the first exotic *Coffea arabica* L. was introduced and planted at Bura in the Coastal province. This was followed by the introduction of seventeen varieties of *C. arabica* between 1893 and 1943 to establish coffee as a commercial crop in Kenya. Most of the introductions later proved to be poorly adapted to Kenyan growing conditions and did not meet the criteria of yield and quality; and had to be discarded, leaving only the best selections. Due to the increase in diseases and pests, the need to conserve was felt as from 1966, when an attempt was made to make crosses between selected genotypes to produce disease resistant lines.

International variety collections, originating from various sources, composed of 226 different accessions, were planted in museum plots in the late 60's. In 1971, a breeding programme was started leading to the need to make more collections especially to look for resistance to CBD. A collection of about 1409 plants representing 188 collections made by the 1964 FAO Coffee Mission to Ethiopia, and some from Brazil was planted at the Oaklands Breeding Station in 1972. These were later followed in June 1982 by another 29 accessions again from the same FAO Mission. The 1966 ORSTOM Mission to Ethiopia later brought in another 473 accessions. Other germplasm exchange programmes led to additional introductions from Brazil, Colombia, Tanzania and Uganda (Diploid and tetraploid Robusta). The 1977 ORSTOM Mission was the last introduction mission which was meant to further enrich the variety collection with coffee indigenous to Kenya by collecting and conserving *C. arabica* ex-marsabit, *C. eugenioides* S. Moore and *C. zanguebariae* Lour.

Case Study Cote D'Ivoire

The field collection has been established at the CNRA in collaboration of French institutions in 1960's from old collections of cultivated varieties and prospections of wild species in 8 African countries. These have been established at 2 stations. At Divo for species adapted in low altitude (*C. canephora, C. congenesis* etc.) and at Mont Tonkoui (Man) for high altitude species (*C. arabica*, *C. eugenioides* etc.). The material is composed of 7000 trees representing more than 300 accessions of 20 wild *Coffea* species. *C. arabica* and *C. canephora* are represented by 800 and 700 wild genotypes (60 and 56 accessions) respectively. There is also a collection of 1000 clones of *C. canephora*. At Man, the collection is under natural forest cover. The major problems experienced in Cote D'Ivoire are unfavourable climate (drought), labour costs to maintain the collection. There is a high risk of damge by fire. The collection has been well characterised for morphologic and biochemical aspects and the wild accessions have also been used to make interspecific hybrids.

It is evident from the case studies described above that field collections are prone to a number of potential sources of erosion, although some collections have been less affected than others, depending on how well they have been located and availability of resources for appropriate management practices. It is also clear that field collection is posing a heavy burden on the national institutions and there is therefore an urgent need for implementation of other effective measures to conserve coffee genetic resources.

INNOVATIVE METHODS FOR COFFEE CONSERVATION

Numerous *in vitro* techniques have been developed for medium-term storage of coffee germplasm (Dussert et al., 1997). The establishment of an *in vitro* coffee core collection was initiated in 1991 at IRD. However, the limits of this technique soon became apparent with the occurrence of some genotypic selection and intraspecific genetic drift. This stressed the importance of developing cryopreservation protocols for cost-effective long-term conservation of coffee germplasm. Research for the development of cryopreservation techniques was performed with seeds, zygotic embryos, apices and somatic embryos (Dussert et al., 1997a). However, at the moment, only seed cryopreservation has been given sufficient attention to allow routine use in coffee genebanks.

Tolerance of seeds of a given coffee species to liquid nitrogen exposure depends on their level of tolerance to desiccation, which is extremely variable between coffee species (Dussert et al., 1999), the cooling procedure (Dussert et al., 2001) and the post-thawing seed rehydration procedure (Dussert et al., 2000). Coffee seeds do not withstand the presence of freezable water in their tissues during liquid nitrogen exposure (Dussert et al., 2001). The unfreezable

water content of seeds is highly variable between coffee species and is negatively correlated to seed lipid content. A strong association between the level of unsaturation of seed lipids and the sensitivity to liquid nitrogen exposure was also found among coffee species. The understanding of the mechanisms involved in seed tolerance to LN exposure facilitated the development of simple and efficient cryopreservation protocols for the coffee species studied. For example, in the case of *C. arabica*, with which seed cryopreservation can be used for conservation of genotypes, very high survival was achieved with using the following protocol (Dussert et al., 2000): desiccation to a seed water content of 17% (fresh weight basis), precooling to –50°C at 1°C.min^{-1} prior immersion into liquid nitrogen, rapid thawing followed by a 6-weeks osmoconditioning treatment prior to culture under germination conditions.

In the framework of an IPGRI-funded project, the possibility of using the protocol developed at IRD as a standard protocol for cryopreservation of *C. arabica* seeds at CATIE (Costa Rica) was tested with 67 accessions of the CATIE's *C. arabica* germplasm collection (Vasquez et al., 2001). The proportion of accessions successfully cryopreserved was of 92% and 43 % with high- and low-vigour seed-lots, respectively. This result clearly indicates that the use of seed cryopreservation for cost-effective long-term conservation of coffee genetic resources can now be routinely applied.

IN SITU CONSERVATION

In situ conservation though previously overlooked, still remains an important component of the overall strategy for long-term conservation of the coffee genepool. It is dynamic in the sense that it allows intimate interaction between species and biotic as well as abiotic factors thus creating conditions ideal for the evolutionary process for various traits including pest/disease resistance and general adaptation. Although *in situ* conservation is the best system for undisturbed ecosystem preservation, it appears to be very difficult to effectively protect most representative samples, because of resource limitation and many uncontrollable social and natural factors. Further, tropical forests, the habitat for most of wild coffee species in Africa, are under tremendous pressure and are disappearing at an alarming rate. Protected area systems (Nature reserves, National parks, Biosphere reserves etc.) may be effective in conserving many species in the wild, but the choice of sites targets either important ecoregions or charismatic animal species. In many countries wild populations of coffee species are passively conserved in protected areas, but the amount of the diversity conserved therein remain largely unknown.

Over the recent years, strategies for more effective *in situ* conservation have been developed and demonstrated for Mascarene *Coffea* in Mauritius (Dulloo et al., 1998.). Maxted et al. (1997) recommend that ecogeographic surveys are important to document the genetic diversity and its distribution in the existing wild populations for the development of appropriate strategies for *in situ* conservation. This was carried out for three wild *Coffea* species in Mascarene islands: *C. mauritiana* Lam., *C. macrocarpa* A.Rich. and *C. myrtifolia* (A. Rich. ex DC) Leroy (Dulloo et al., 1999).The concept of gap analysis was then used as a conservation evaluation technique that identified areas in which selected elements of coffee genetic diversity are represented inside and outside the present system of protected areas, which may also contain high quality native vegetation. Cluster analysis of RAPD data showed that while protected areas have been effective in conserving the wild coffee populations, they did not capture some genetically unique populations, representing valuable coffee genetic resources. Competing alien species were identified as specific threats to wild populations and were managed in some areas of the National Park, termed conservation management areas. These areas offered the best possibilities for long term safe protection of wild populations.

The study identified priority populations for *in situ* conservation of wild coffee species. The case study in Mauritius shows the importance of understanding the distribution of genetic diversity for establishing a strategy for *in situ* conservation.

There are no documented examples of *in situ* conservation of coffee genetic resources, except for some efforts being made in Ethiopia (Worede, 1997), where wild *C. arabica* populations are managed *in situ*. Farmers clear the natural forest, leaving only the plants of *C. arabica* trees and cultivate the land in between the trees. This is an interesting *in situ* management technique that help to protect wild coffee trees, but where the natural ecosystem processes are disturbed and manipulated by man. On the other hand these practices may endanger coffee resources due to dominance of disease susceptible populations. In Ethiopia, forest coffee conservation project has been proposed to preserve the diversity in the ecosystem (biosphere reserve), sustainable utilize the genetic resources to develop varieties with desirable characters, and ultimately improve local living conditions and contribute to foreign exchange earnings of the country. Accordingly, three sites were selected as potential sites for biosphere reserve in the south-western parts of the country. The criteria used to select these sites were the presence of naturally regenerating /wild coffee trees, shrubs and/ or seedlings in the forest, minimum interference by human beings, and accessibility. However, the execution or implementation phase of the project was delayed as yet, mainly because of lack or shortage of funding.

So far no systematic effort has been made to preserve, document or further develop coffee genetic resources. For effective nature conservation in a sustainable way, the active involvement of all stakeholders or dedication of concerned bodies both within and outside the country and financial support from donor organizations are very essential.

CONCLUSION

Coffee field genebanks have been created and maintained by the main coffee-producing countries. They have been enriched by surveys carried out throughout the coffee distribution range in the second part of the twentieth century. These collections are to-day facing many technical, financial and political challenges, which are difficult to resolve. We here propose several actions that are required to ensure the sustainability of the *ex situ* collections for use by coffee breeders and other end users.

- Urgent rejuvenation of ageing trees within collections.
- Development of alternative methods of conservation such as cryopreservation of *C. arabica* seeds.
- Designation and support of a regional duplicate collection under the care of an international network (perhaps ACRN or IPGRI) in accord with the Convention of Biological diversity and International Undertaking.
- Mechanism to provide access to germplasm within core collections representative of the coffee genetic diversity by bona fide end users.
- Evaluation of coffee germplasm contained in collections for disease resistance and coffee quality for use in coffee improvement programmes. This objective should be done using advanced molecular technique (molecular markers, genetic map etc.).

Further, the conservation and use of coffee species in the wild has received little attention and yet represent a large reservoir of germplasm and are threatened by tropical deforestation. Efforts for the *in situ* conservation need to be deployed in collaboration with nature conservation programmes, particularly in region of high diversity such as Congo Basin, Eastern Arc Mountains of Tanzania and Malagasy-Mascarene region and South Western

Ethiopia. Finally it is recognized that much diversity also exist in plantations created by traditional coffee farmers from wild coffee trees, especially in SW Ethiopia and Yemen. Other similar situations may also exist for *C. canephora* in Uganda and Central and West Africa and need to be identified. These should be conserved on farm using a participatory approach.

LIST OF ABBREVIATIONS

CATIE: Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica;
CENRADERU: Centre National de Recherche Appliquée au Développement Rural, Madagascar;
CNRA: Centre National de Recherche Agronomique, Cote D'Ivoire;
FAO: Food and Agriculture Organisation of the United Nations, Rome;
JARC: Jima Agricultural Research Center, Ethiopia;
JATS: Jima Agricultural Technical School, Ethiopia;
IBRC: Institute of Biodiversity Research and Conservation, Ethiopia;
IBPGR: International Board for Plant Genetic Resources, Italy;
IPGRI: International Plant Genetic Resources Institute, Italy;
IRD: Institut de Recherche pour le Développement, France;
ORSTOM: Organisation de Recherche Scientifique et Technique Outre Mers, France.

REFERENCES

Adams R.P. and Adams J.E. (Eds), 1991. Conservation of plant genes: DNA banking and in vitro biotechnology. Academic Press, New York.

Bayetta Belachew, 1997. Arabica Coffee Breeding in Ethiopia: a review. 17th International Scientific Colloquium on coffee. 20-25 July, 1997. Nairobi, Kenya. pp. 406-414.

Berthaud J. and Charrier A., 1988. Genetic resources of *Coffea*. In Clarke R.J. and Macrae R. (eds.) Coffee Volume 4: Agronomy. Chapter 1. Pp1-42.

Bridson D.M. (1982). Studies in *Coffea* and *Psilanthus* (*Rubiaceae* subfam. *Cinchonoideae*) for part 2 of the Flora of tropical East Africa: *Rubiaceae*. Kew Bulletin **36**: 817-859.

Charrier A. (1978). La structure génétiques des caféiers spontanés de la région malgache *(Mascarocoffea)*. Leur relations avec les caféiers d'origine africaine *(Eucoffea)*. Mémoire ORSTOM No. **87**. Paris.

Cockram J. (1997). A herbarium-based ecogeographic survey of wild taxa of the genus *Coffea* of tropical East Africa. Msc. Thesis , Univeristy of Birmingham 106pp.

Dulloo M.E., Guarino L., Engelmann F., Maxted N., Newbury H.J., Attere F. and Ford Lloyd B.V. (1998). Complementary conservation strategies for the genus *Coffea*: a case study of Mascarene *Coffea* species. Genetic Resources and Crop Evolution **45**: 565-579.

Dulloo M.E., Maxted N., Newbury H., Florens D. and Ford Lloyd B.V. (1999). Ecogeographic survey of the genus *Coffea* in the Mascarene Islands. Botanical Journal of the Linnean Society **131** (3): 263-284.

Dussert S., Chabrillange N., Engelmann F., Anthony F., Noirot M., Hamon S. (1997a) *In vitro* conservation of coffee (*Coffea* spp.) germplasm. In: Razdan MK, Cocking EC (eds) Conservation of plant genetic resources in vitro. M/s Science Publishers, Enfield, USA, pp 287-305

Dussert S., Chabrillange N., Engelmann F., Hamon S. (1999) Quantitative estimation of seed desiccation sensitivity using a quantal response model: application to nine species of the genus *Coffea* L. Seed Sci Res **9**: 135-144

Dussert S., Chabrillange N., Vasquez N., Engelmann F., Anthony F., Guyot A., Hamon S. (2000) Beneficial effect of post-thawing osmoconditioning on the recovery of cryopreserved coffee (*Coffea arabica* L.) seeds. Cryo Lett **21**:47-52

Dussert S., Chabrillange N., Rocquelin G., Engelmann F., Lopez M., Hamon S. (2001) Tolerance of coffee (*Coffea* spp.) seeds to ultra-low temperature exposure in relation to calorimetric properties of tissue water, lipid composition and cooling procedure. Physiol Plant **112**: in press.

Eskes A.B. (1989) Identification, description and collection of coffee types in P.D.R. Yemen. IPGRI, Rome.

Fernie L.M., 1968. FAO Coffee Mission to Ethiopia 1964-65, Food and Agricultural Organization of United Nations, Rome. 200 pp.

FAO (1998): State of the world report on plant genetic resources for food and agriculture. FAO Rome 510 pp..

Guillaumet J.L., Hallé F. (1978) Echantillonnage du matériel récolté en Ethiopie. *Bull IFCC* **14**: 13-18

Hong T.S. and Ellis R.H. (1995). Interspecific variation in seed storage behaviour within two genera - *Coffea* and *Citrus*. Seed Science and Technology **23**: 165-181.

Institute of Agricultural Research (IAR). 1998. Progress report for the period 1996/97. Coffee Research Center, Jimma-Melko.

Jima Agricultural Technical School (JATS). 1957. The Agriculture of Ethiopia, U.S.A. Operation Mission to Ethiopia-Point 4. January, 1957. Vol. **IV**: 55-56.

Krug C.A. and Carvalho A. (1951). The genetics of coffee. Advances in Genetics **4**: 127-168.

Lashermes P., Trouslot P., Anthony F., Combes M.C. and Charrier A. (1996). Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. Euphytica **87**: 59-64.

Maxted N., Ford-Lloyd B.V. and Hawkes J.G. (1997). Plant genetic conservation: the in situ approach. Chapman and Hall. Pp. 15-39.

Sylvain P.G. 1955. Some observations on *Coffea arabica* L. Ethiopia. Turrialba **5** (1-2): 37-53.

Vasquez N., Dussert S., Salazar K., Anthony F. and Engelmann F. (2001) Variability in response of seeds to cryopreservation within a coffee core collection. Final Report. IPGRI, Rome Italy.

Walyaro D.J. and Van der Vossen, 1977. Pollen longevity and artificial cross – pollination in *Coffea arabica* L. Euphytica **26**: 225-231.

Worede M. (1997). Ethiopian *in situ* conservation. In Maxted N., Ford -Lloyd B.V. and Hawkes J.G. Plant genetic conservation: the *in situ* approach. Chapman and Hall. Pp. 290-301.

Coffee Breeding and Selection in Hawaii

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SUMMARY

A cooperative coffee breeding and selection program between Hawaii Agriculture Research Center and University of Hawaii supported by the Hawaii Coffee Growers' Association and the State of Hawaii was established in 1997. The primary objective of the program is to develop high-yielding, excellent bean and cupping quality cultivars with distinctive flavors adapted to specific growing conditions in Hawaii. Disease resistance and mechanical harvestability are also important criteria for breeding and selection of Hawaii cultivars. As the area under coffee cultivation expands and as airline and ship arrivals increase, we must anticipate the eventual arrival of serious diseases and pests. Cultivars currently grown in Hawaii are not resistant to coffee rust (*Hemileia vastatrix*) and newly found nematodes (*Meliodegyne konaensis*) in Kona.

A common orchard was established at a Kunia field on the Island of Oahu for the breeding program and includes 37 potentially elite trees selected from five coffee growing areas in Hawaii. The trees in the orchard were evaluated for tree morphology and cherry/bean characteristics. Characters included tree height, cherry weight and size. We made 160 crosses among these trees and rust resistant cultivars in spring 1999. About 1,500 progeny of this first generation (F1) of the progeny resulting from these crosses were planted in fields in 2000. These progeny will be evaluated in summer.

INTRODUCTION

Coffee is a major agricultural commodity in Hawaii. With production of 4,086 mt (9 million lbs) (green bean basis) in 1999-2000, coffee ranked 6th in value (value of sales = \$21M) statewide among crop commodities (Statistics of Hawaii Agriculture, 2,000). The total area under cultivation is 3,200 ha in 2000-01, of which 58% is outside the traditional coffee region of Kona.

Hawaii's coffee production began in the early 1800s. It was grown throughout the Hawaii islands (Ukers, 1922) but was gradually localized in the Kona district of the Island of Hawaii. Coffee production in Kona reached over 9 million pounds (4,086 mt) in the 1960s, but both the area under production and yield decreased during the '70s to 2-3 million pounds (900-1,350 mt) (Statistics of Hawaii Agriculture 1965-1975).

Coffee production in Hawaii has increased substantially during the last 15 years, from 731 mt ('85) to 4,086 mt ('00). Four new coffee growing areas have emerged outside the traditional Kona district since the mid-80s while the area under cultivation in Kona has also increased. Most new coffee growing areas represent a conversion from former sugarcane (Kauai, Maui and Oahu islands) and pineapple (Molokai). In Kona, many new coffee growers have emerged

in response to the burgeoning specialty gourmet coffee market established in the 1990s (Essoyan, 1998). In Kona, land formerly in coffee is being put back into production.

Hawaii is the only coffee-growing state in the United States. Located in the middle of the Pacific Ocean at 21°N, Hawaii's environment is unique compared to most coffee growing areas in the world. In Kona, most farms are located on the leeward slope of Mauna Loa at an altitude of 400 to 1,000 m. The larger growers on the other islands are located at elevations between 90-300 m. By comparison, other world-renowned, high-quality arabica coffee are grown at high elevation (1000-2000 m) and within 15° of the equator (Feria-Morales, 1996).

Hawaii farmers grow only arabica coffee. Coffee production methods, however, have diversified from traditional hand-picking of a single variety ('guatemalan', 'typica') in Kona to mechanical harvesting of other cultivars, such as 'yellow catuai', 'red catuai' and 'mokka' on the other islands except for Oahu where 'typica' is grown. All new large scale growers use drip-irrigation; most farmers in Kona grow coffee without irrigation.

The earliest record of importation of coffee cultivars to Hawaii was in 1813; however, these plants either did not survive or never achieved significant cultivation (Hawaii Tribune-Herald 1985). In 1825, 30 coffee plants were successfully imported. Coffee cultivation in Kona can be traced to these plants. The variety became known as "Hawaiian coffee" or "Old Hawaiian" in the Kona area. The cultivar known to be "Guatemalan" among coffee growers in Hawaii was first introduced into Hawaii in 1892. This cultivar, "Guatemalan" – typica, is the genotype grown in Kona today (Goto, 1982).

Between 1954 and 1969, the University of Hawaii imported coffee cultivars and established a germplasm collection at Kainaliu Experiment Station, Island of Hawaii. Over 40 cultivars were imported from coffee growing regions around the world (Hamilton, personal communication, 1998). In the mid-1980s, new large scale coffee growers began importing high-yielding, semi-dwarf catuai cultivars, hybrids of 'caturra' and 'mundo novo' cultivars from Brazil (Bittenbender et al., 1990).

Coffee breeding to produce cultivars with desirable characteristics such as enhanced flavor, increased yield, disease resistance was not initiated in Hawaii until 1997. In that year, a coooperative coffee breeding and selection program was established at Hawaii Agriculture Research Center (HARC) with the support of the Hawaii Coffee Growers' Association (HCGA). In cooperation with the University of Hawai, State of Hawaii matching funds were applied. This program focuses on developing high-yielding cultivars with excellent bean and cupping quality adapted to specific growing conditions in Hawaii (Osgood, 1997). Disease resistance and mechanical harvestability have also been established as important criteria for breeding and selection. Breeding for disease resistance is considered important but is not a top priority, since coffee rust (*Hemileia vastatrix*) and CBD (*Colletorichum coffeanum*) are not current problems in Hawaii. However, even with the vigilant quarantine, the eventual arrival of serious diseases and pests in Hawaii's coffee fields is inevitable. Impacts from the Kona root-knot nematode (*Meliodegyne konaensis*) have been noted in Kona since 1994 (Eisenback et al., 1994). This report describes the breeding and selection approaches in Hawaii established as a cooperative project by HARC, University of Hawaii, and Hawaii Coffee Growers' Association. During the last four years, individual, potentially elite trees were selected from five coffee growing areas in Hawaii, and a field was established from seed and cuttings (clones) in a common field at Kunia, Oahu, for a breeding orchard for variety improvement for the Hawaiian coffee industry. Additional germplasm was added from Central and South American germplasm. Progeny from 1999 crosses were planted at the same area, and are being evaluated this year.

SELECTION OF INDIVIDUAL TREES AND ESTABLISHMENT OF A COMMON FIELD PLANTING AT KUNIA

A total of 37 potentially elite individual trees were selected from five Hawaii coffee growing regions during September to October 1997 (Table 1). A team of plant breeders and physiologists visited selected fields at each location following recommendations of coffee growers. The team walked through fields to get acquainted with overall phenotypes of trees, and visually selected trees based on tree vigor, good balance of fruit bearing and vegetative growth, bean size and timing of flowering (Nagai et al., 1998).

Tree phenotypes were less variable in red catuai, yellow catuai, red caturra and 'guatemalan' typica than in mokka and 'mokka hybrids' in which a large range of morphological variation was observed. The most distinctive types of trees were selected in mokka (Maui) and mokka hybrids (Oahu) sites. Both cultivars had been propagated from open pollinated seed over several generations of trees originating at the University of Hawaii, Kona Experiment Station (Kainaliu) for commercial fields. Morphological segregation of these trees could be attributable to natural hybridization (out crosses) at the Kona Station or perhaps before importation to Kona.

Trees were cut back to 1 to 3 ft from the ground to obtain vertical shoots for vegetative propagation by cuttings (Sun et al., 1998). New vertical shoots were cut from the selected trees three months after cut back. The cut shoots were wrapped in wet newspaper and transferred to the HARC Maunawili Station for vegetative propagation on the day of collection, or the next day. Seeds from selected trees were germinated in the greenhouse.

The number of selected trees was limited to five per cultivar. Tree height and crown width were measured. Other tree characteristics recorded included vertical stem numbers, leaf and fruit (cherry) characteristics, and uniformity of ripening. Mature fruits (cherry) were collected for determination of cherry and bean weight and size, and bean/pulp ratios. Average weight, length and width per cherry were determined from the total weight and cumulative length and width of ten cherries. Cherry volume (units cm^3) was estimated from the formula $4/3 \times \pi \times (W/2)^2 \times (L/2)/1000$, where W = average cherry width and L = average cherry length. Bean/pulp ratios were obtained from the dry weight of green beans without parchment, divided by the original fresh weight of the cherries.

Seedlings from open pollinated seeds collected from these trees and rooted vegetative cuttings were field planted at the HARC Experiment Station, Kunia, Island of Oahu, in April 1998. The field was prepared 4.0 m x 0.6 m spacing with bana grass as wind breaks. Self progenies of the rust-resistant Catimor cultivars 'promecafe 1' (T5175) and 'promecafe 2' (T8667) from Guatemala (Osorto, 1991) were also planted with these selected trees. Twenty selfs were planted when selected trees were expected to segregate, while 10 selfs were planted from the original trees expected to be uniform inbred lines. Ten clones (cuttings) of the original trees were planted at the same field, designated as MA1C, KA17C, etc.

EVALUATION OF SELFED PROGENY OF SELECTED LINES

The trees in the orchard were evaluated for morphological traits and uniformity in 1999 (12-15 months from planting). Traits included tree height, cherry weight and size.

Table 1. Selection of individual elite trees at five coffee growing areas in Hawaii

Coffee growing area/Farm	Island	Cultivars	No. of Trees Selected	Genotype Number
Kaanapali Coffee	Maui	mokka, red catuai	5 5	MA1-MA5 MA6-MA10
Waialua Coffee/Dole Fresh Fruit	Oahu	typica/mokka hybrids	5	OA11-OA15
Kauai/Kauai Coffee	Kauai	yellow catuai red catuai	3 2	KA16-KA18 KA19-KA20
Molokai/Coffees of Hawaii	Molokai	red catuai red caturra	7 2	MO21-MO27 MO29-MO30
Kona/Greenwell Coffee and Kona Mountain Farms	Hawaii	guatemalan typica others [1]	3 1	KO31,32,34, KO33
HARC Maunawili Experimental Station	Oahu	catimor [2]	2 2	5175-6,5175-7 8667-5,8667-6
Total			37	

[1] *Coffee cultivar "Old Hawaiian" imported to Kona before 1860s*
[2] *Rust disease resistant variety from Promecafe*

Morphological characteristics:

Morphological data including tree height and leaf characteristics of the 36 lines in the Kunia common orchard were taken (Table 2). Leaf types were segregated in ten lines of 'mokka' and 'mokka hybrid' groups, MA1, 2, 3, 4, 5 and OA11, 12, 13, 14, 15. The old Hawaiian cultivar at Kona, 'KO33', showed characteristic 'rippled' leaves. We selected trees with one to three vertical stems. About 66% of the total selected 444 trees had only one vertical stem at this stage. Tree height and leaf characteristics were uniform among 15 'catuai' lines which were introduced by commercial farms at different times, while 'mokka' and hybrid lines showed large variations between and within lines. Larger variations in typica lines compared to 'catua' should be growth variations since tip color and leaf types were uniform. These results confirmed that phenotypic variations observed in the original fields in the selfed progenies.

Table 2. Morphological characteristics of 36 individually selected coffee trees at 13 months from planting

Group	Number of selected lines	Tree height (cm)	Mean of CV within each line	Tip color	Leaf types
Catuai	15	83.9 ± 1.6[1]	6.3	Green	Catuai type
Caturra	2	84.5 ± 1.5	5.1	Green	Catuai type
Mokka	5	100.0 ± 3.7	17.8	Brown/green segregated	Mokka:intermediate: Seg[2] = 1: 2 :2
Mokka Hybrids	6	91.9 ± 5.6	15.8	Brown	Mokka:Typica: Seg[2] = 3 :2:2
Catimor	4	83.5± 3.5	4.2	Dark Brown	Catimor
Kona Typica	4	78 ± 10.0	19.3	Brown	Typica

[1] *Each selection has 10 - 20 selfed progenies*
[2] *Leaf types segregated as mokka and typica types in 2 lines*

Fruit and bean characteristics

Fruit and bean characteristics were measured when seeds were collected from the original fields (Nagai et al., 1998). Ripe fruits were also collected from the trees at Kunia fields in October and November 1999 (18 months from planting). Fruit characteristics of 37 selected trees including 100-cherry weight, average cherry volume and dimensions, and the ratio of green bean/cherry were determined. The largest variation was found in 100-cherry weight (108 to 272 g) and average 10-cherry volume (9.0-35 cm^3). Variations in 100-cherry weight were small among genotypes in non-segregating varieties (CV = 10-12%), while large variation (CV = 30%) was observed among trees in mokka hybrids (OA11-15, Waialua, Oahu) (Table 3). The distribution of cherry weight and cherry dimensions are shown in Figures 1 and 2. All the trees showing the mokka phenotype had round cherries with length/width ratio of 1.0-1.1, while trees of catuai, caturra and 'guatemalan' typica had more oblong cherries with a length/width ratio of 1.1-1.2 (Table 3).

Table 3. Fruit and bean characteristics of selected trees in coffee groups

Group	No. of selected lines	100 Cherry wt (g)	Cherry shape ratio length/width	Recovery of green bean from cherry (%)[(b)]
Mokka hybrids	5	190 ± 25[(c)] (cv=30)	1.07± 0.02[(c)] (cv=3.5)	17.4 ± 0.6[(c)] (cv=7.7)
Guatemalan	3	178 ± 10 (cv=10)	1.12 ± 0.02 (cv=3.8)	18.7 ± 0.9 (cv=8.2)
Red Caturra	2	172 ± 13 (cv=10.7)	1.22 ± 0	-
Red Catuai	14	152 ± 5 (cv=12.5)	1.20 ± 0.01 (cv=3.7)	18.3 ± 0.7 (cv=6.3)
Yellow Catuai	3	156 ± 10 (cv=10.8)	1.16 ± 0.01 (cv=2.1)	17.0[(a)]
Mokka	5	121 ± 6 (cv=10.5)	1.09 ± 0.03 (cv=6.7)	21.0[(a)]
Promecafe	4	244 ± 13 (cv=10.3)	1.28 ± 0.06 (cv=9.0)	19.0 ± 0.6 (cv=6.1)
Other	1	122	1.10	18.0

'Mokka' (original 'mokka') has been recognized as an arabica cultivar but no information is available concerning its origin as indicated by Krug and Calvalho (1951). In Brazil, where extensive genetic studies were carried out beginning in the 1930s, two genes *lr* (laurina), and *mo* (mokka), were found to control the 'mokka' characteristics. 'Mokka' is characterized by having the smallest fruits and seed size among *C. arabica* cultivars, but the quality of coffee has been considered excellent. All of the 'mokka' plants grown in Hawaii are hybrids with 'typica' according to H. P. Medina (personal communication, 1998). Medina refers to the hybrids as 'Tall Mokka'.

Trees of the "mokka hybrids" group in Hawaii originated from a plot of 'blue mountain' cultivar originating from 'Typica' coffee in Jamaica. Since these trees were spread from a plot of 'blue mountain' trees from HAES 6433 at the UH Kona Kainaliu Station, it is expected that blue mountain typica is one of the original parents. These trees showed large segregation in their phenotypes with mokka and typica characteristics. Selected trees, OA11 and OA15 for example, have strong 'mokka' characteristics in their leaves and fruits. We suspect that the original 'Blue Mountain' trees introduced to the UH Kona Station outcrossed with mokka at the station. The segregating 'blue mountain' trees in Hawaii are a unique population for selection regardless of their original pedigrees.

Four individual trees selected from rust-resistant cultivars, Promecafe 1 and Promecafe 2, showed large fruit size and weight (Table 3). We are planning to cross these trees with other cultivars in the next several flowering seasons to begin the development or creation of rust-resistant Hawaii cultivars.

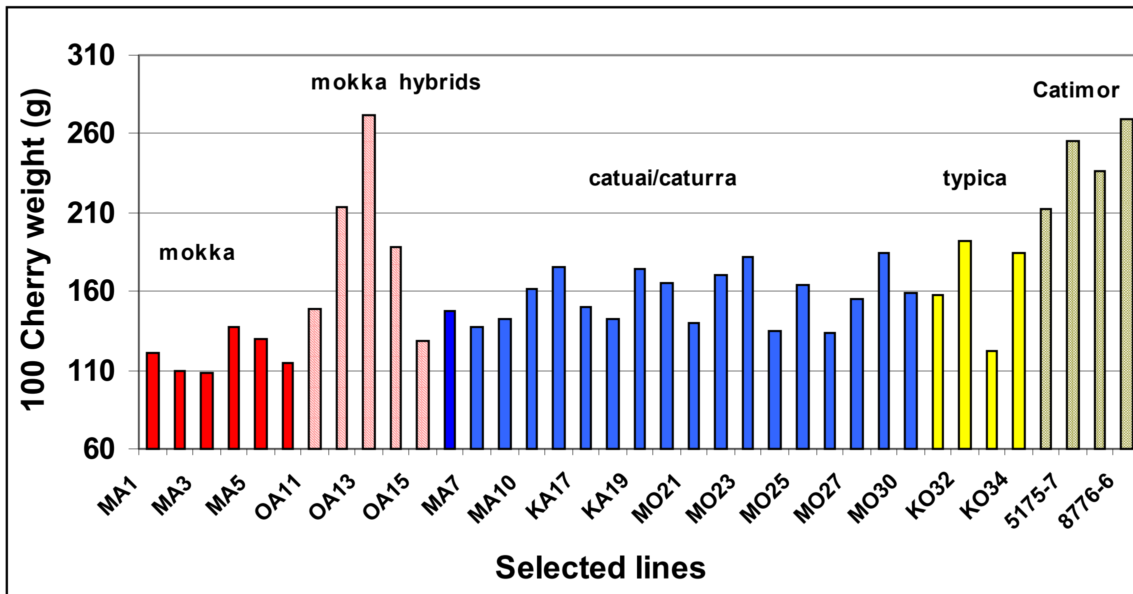

Figure 1. 100-Cherry weight of selected lines

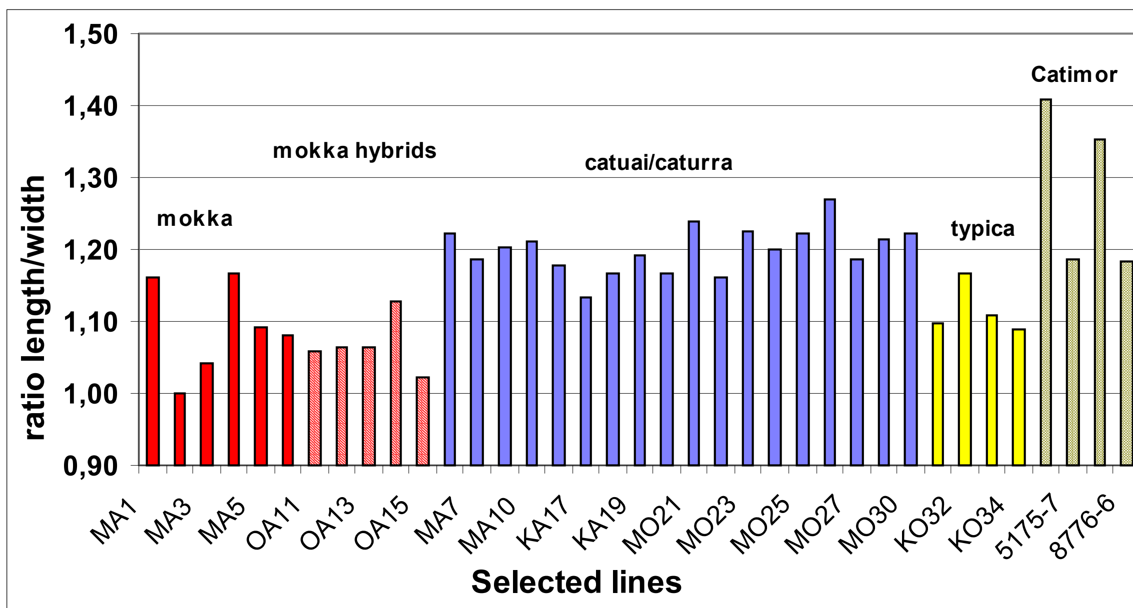

Figure 2. Cherry dimensions of selected lines

PRODUCTION OF PROGENY IN 1999

We made 167 crosses among the trees in the common orchard in spring 1999. Flowers were available for pollination 10 months after planting. About 1,500 progeny of the first generation (F1) of the progeny resulting from these crosses were planted in the field in June 2000. In 10 lines, flowers were initiated and selfs producing F2 populations were made in March 2001. These progeny will be evaluated for morphologial characteristics the summer of 2001, and cherry characteristics in October 2001.

Table 4. Crosses made during spring 1999 at HARC Kunia and Maunawili Experiment Stations

Group	Number of crosses
Arabica x Catimor	53
Arabica x Icatu	8
Selfs of selected arabica	40
Among various arabica	66
Total	167

NEW APPROACHES

Two new research projects were started at HARC using the base germplasm and progeny. Molecular marker studies of *Coffea arabica* were initiated in 1999. An objective of the studies was to investigate the genetic diversity of *Coffea arabica* cultivars using amplified fragment polymorphism (AFLP) markers. An F1 (psudo F2) population was developed for mapping and QTLs study. About 150 progeny were produced from a cross between catimor and mokka (5175-1 x MA2-7). Seedlings are ready to transplant in the field with both parents for phenotypic evaluation.

A second project is investigating sugar and organic acid content of green beans and their relation to flavor. A number of Hawaii-grown coffees are being chemically evaluated. This information will be related to various descriptors in cupping quality assessment, species, variety and environment. In Hawaii, we have the opportunity to evaluate sets of genotypes from various environments. In 1988, the Hawaii State Coffee Trial was initiated to evaluate arabica varieties in 18 locations for yield, bean size and cupping quality (Cavaletto et al., 1991). Coffee grown at five of the same locations in the test fields is being used for this project. Progeny from the parents that are different in sugar and organic acid composition and cupping quality will also be tested in 2002.

ACKNOWLEDGMENT

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REFERENCES

Bittenbender H.C., Kefford N., Rohrbach K.G., 1990. Coffee Industry Annalysis Number 3, HITAHR, College of Tropical Agriculture and Human Resources, University of Hawaii. p 16.

Cavaletto C.G., Nagai N.Y., Bittenbender H.C., 1991. Yield, size and cup cuality of coffees grown in the Hawaii State Coffee Trial. Pp 674-678. Proceedings of 14th ASIC International Conference on Coffee Science, San Francisco.

Goto Y.B., 1982. Ethnic groups and the coffee industry in Hawaii. Hawaiian Journal of History **16**: 111-124.

Esenback J.D., Bernard E.C. and Schmit D.P., 1994. Description of the Kona coffee root-knot nematode, Meloidogynekonaensis n. sp. Journal of Nematology **26**: 363-374.

Feria-Morales A.M., 1996. Overview of coffee world & main factors affecting quality. Pp 4-15. Proceedings of the Hawaii Coffee Association's 1st annual Convention. July 18-19. Kailua-Kona, Hawaii.

Krug C.A. and Carvalho A., 1951. The genetics of Coffea. Advances in Genetics. **4**: 127-158.

Medina-Filho H.P., Carvalho A., Sondahl M.R., Fazuoli L.C. and Costa W.M., 1984. Coffee breeding and related evolutionary aspects. Plant Breeding Review. **2**: 157-193.

Nagai C. and Osgood R.V., 1993. Rust-resistant coffee varieties from Guatemala and Brazil. Annual Report 1992 Experiment Station HSPA. p 47.

Nagai C., Sun W.G., Osgood R.V., Meinzer F.C. and Bittenbender H.C., 1998. Selection of potentially elite individual coffee trees on coffee farms on five Hawaiian Islands. Hawaii Agriculture Research Center. Coffee Report No. 1.

Osgood R.V., 1997. Does Hawaii Need a Coffee Breeding and Section Program? p36-40. Proceedings of the Hawaii Coffee Association's 2nd Annual Convention. July 25-26. Kona, Hawaii.

Osorto J.J., 1991. Coffee variety and selection program in Central American countries. 451-459. Proceedings of 14th ASIC International Conference on Coffee Science, San Francisco.

Sun W., Nagai C. and Osgood R.V., 1998. Vegetative propagation of *Coffea arabica* using softwood cuttings. Hawaii Agriculture Research Center. Coffee Report No. 1.

Ukers W.H., 1922. All about Coffee. The and Coffee Trade Journal Company. NewYork. P. 375.

van der Vossen H.A.M., 1988. Coffee selection and breeding. Pp 48-96. In Coffee botany, biochemistry and production of beans and beverage. M.N. Clifford and K.C. Wilson (ed.). The AVI Pub. Co. West Port CT.

Use of Multivariate Estimators in Genetic Stability of Coffee Lines (*Coffea arabica* L.)

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SUMMARY

The main goal of any plant breeding program is to obtain improved cultivars. In general, a cultivar performs differently under different growing conditions and different cultivars grown in the same environment often yield different responses. Genotype x Environment interaction (GE) occurs when the magnitude of changes differs in cultivars as a response to the distinct environments. GE interaction has important theoretical and practical implications in plant breeding. By means of methods, coffee breeders could obtain resistant cultivars to biotic factors, with better potential yield and adapted to a broad range of environmental conditions; these cultivars will be chosen by farmers to be sown under a wide range of altitude. At present, several methods are used to evaluate stability to measure the effects of Genotype x environment interaction. Eberhart and Russell regression coefficient is an univariate option, while the additive main effects and multiplicative interaction effects model (AMMI) is a very useful multivariate option. This study used the AMMI model to evaluate F6 lines of coffee, short plant and resistant to rust (*Hemileia vastatrix*) at five locations during three consecutive years. Stability of yield by plant as cherry coffee and percentage of empty grains where evaluated under fifteen different environments. The AMMI model was efficient to estimate the GxE interaction. The first principal component explained 94.07% of total variance. This analysis allows to select the best genotypes in the stability range (–0.4 > CP1 < 0.4) per plant yield of cherry coffee increase by more than 25% compared with the check, Catuaí variety. Also, showed 5% less of empty grains percentage than the check, leading to conclude that AMMI model showed close association between the best lines and the best environment.

Key words: Coffee, yield, stability, AMMI, plant breeding.

RESUMEN

La selección de material genético resistente a factores bióticos, con alto potencial de rendimiento, de amplia adaptabilidad a varios pisos altitudinales y condiciones agroecológicas diferentes, que puedan ser sembradas por un gran número de productores; es el principal objetivo de un programa de mejoramiento genético de café. Esto debido al lento proceso que implica el lanzamiento de una nueva variedad. Existen diferentes metodologías para evaluar la estabilidad, expresada como la interacción genotipo x ambiente; la cual se manifiesta cuando las condiciones ambientales repercuten en los efectos diferenciales de los genotipos. Una es la propuesta por Eberhart y Russell de tipo univariada. Otra metodología es el modelo AMMI (Efecto Principal Aditivo e Interacciones Multiplicativas), que usa estimadores multivariados, ya usado en otros cultivos. Ésta última fue usada en líneas F6 de café, de porte bajo y resistentes a roya (*Hemileia vastatrix*) evaluadas en cinco localidades durante tres años consecutivos en función de producción de café cereza por planta, porcentaje de granos vanos; para un total de 15 ambientes se determinó su estabilidad genética. El modelo AMMI fue

eficiente para estimar la interacción, ya que el componente principal 1 explicó el 94,07% de la variabilidad. De esta manera se logró seleccionar los mejores genotipos, ubicados dentro de la franja de estabilidad ($-0.4 > \text{CP1} < 0.4$) y con producción de café cereza por planta 25% superior que la variedad Catuaí, usada como testigo y granos vanos menor al 5%. Permitió además asociar la respuesta de las mejores líneas al mejor ambiente.

Palabras Clave: Café, Mejoramiento Genético, Producción, Estabilidad, AMMI

INTRODUCTION

Coffee is a crop of great worldwide importance. In Venezuela has been producing considerable amount of foreign currencies since the 19th century. It is also, the means of subsistence of a great part of the small farmers. Coffee production is based fundamentally on the specie *Coffea arabica* (2n = 4x = 44 chromosomes). The specie is predominantly self-pollinated, being able to presents cross-pollination up to 9-11% in natural conditions (Carvalho and Monaco, 1964). Genetic variability in this species is scarce (Bustamante and Polanco, 1999). The specie *Coffea arabica* represents around 70% of the world production. Venezuelan production is based almost in its use. *Coffea arabica* is characterized by producing drink of soft aroma and flavor.

Genetic improvement programs in Coffee take a long time. Breeders have to offer as final product varieties of high production, genetically stable and resistant to biotic or abiotic factors. These varieties could be used in different agroecological areas and by a great number of producers.

To study the genetic stability, different methodologies have been proposed, some of them focus on study the genotype x environment interaction. This interaction is manifested when the environmental conditions affect the differential response of genotypes (Falconer, 1989).

In 1966, Eberhart and Russell proposed an univariate method to study stability; based on the use of regression coefficient estimator. It is a measure of response to each one cultivates to an environmental index. This method to study genetic stability uses the magnitude of deviation from lineal regression.

Univariate procedures have great advantages some of them are: being mathematically simple and of easy biologically interpretation. However, their also have some disadvantages such as when collineality exists, procedures lose precision. Univariate procedures depend on the genotypes group and environments included in the analysis and they tend to simplify patterns responses when they are, in fact very complex.

As an answer to these limitations, researchers have intended multivariate methods. In this sense Zobel et al. (1989) outline an alternative, crop response can be divided in two components, one that considers that main effects, genotype and environment have additives effects, and the other component that corresponds to genotype x environment interaction of multiplicative effect. Crossa (1990), present the Additive Main Effect and Multiplicative Interaction Effects Model (AMMI), as a combination of Finlay and Wilkilson (1963) regression model with the multivariate analysis method by Principal Components. In other words, it is the combination of both methodologies.

With the purpose of evaluating the AMMI model in genetic stability determination of promissory lines coming from the Coffee Genetic Improvement Program of the Instituto Nacional de Investigaciones Agrícolas de Venezuela (INIA), we undertook this work with the

main objective of select the best lines of coffee (*Coffea arabica*), lines of high production, resistant to rust, and also genetically stable. It is expected that those lines mixed in given proportions could conform a new variety ready to be liberated to the market.

Table 1. Genotypes evaluated in Five Locates during Three Years

LINE	IDENTIFICATION	LINE	IDENTIFICATION
01	1097/3 Blue Mountain x H.W 26/5	08	705/5 Pacas x 110/5 S.4 Agaro
02	1086/3 S.L 14 x H.W 26/5	09	1637/36 Yellow Caturra x H. 59/4
03	19/1 Caturra x 134/4-79 S.12 Kaffa	10	1637/21 Yellow Caturra x H.59/3
04	1083/9 S.L 28 x H.W 26/5	11	H. 276/2 x H. 275/1
05	10/1 Bourbón (43-7 x R.P.13) x H.W 26/5	12	H. 176/8 x H.276/2
06	830/3 Caturra x H.W 26/11	13-22	2482/20 Yellow Catuaí x H.W 26/13
07	1078/2 S.L. 34 x H.W 26/14	23	Timor Hybrid x Yellow Caturra

MATERIALS AND METHODS

The experimental material consist of introduced germplasm, in its majority in the second segregate generation (F_2), coming from the Center of Research of Rust, in Portugal (CIFC). Germplasm was sowed in “El Trompillo” experimental station, belonging to INIA Táchira state (ex FONAIAP), Venezuela, Which is located 7° 39’ 22 of north latitude and 72° 23’ 40 of west longitude.

Table 2. Analysis of Variance for Yield of Cherry Beans per Plant in Five Localities during Three Years

SOURCE	Degrees of Freedom	Mean Square
Location (A)	4	1525.59**
Rep (Location)	20	16.0053**
Year (B)	2	111.859**
Location x Year	8	250.387**
Year x Rep (Location)	40	6.01160**
Genotype (C)	24	5.15468**
Location x Genotype	96	2.20123**
Year x Genotype	48	2.00887**
Location x Year x Genotype	192	1.47525**
Error	1440	0.95217

***Different at $P \leq 0.01$*

In the sixth segregate generation, (F_6) twenty-three lines were selected (Pedigree Method). Some of them were Sarchimores, Cavimores, Catimores lines and also crosses of Catimores by Bourbón and Blue Mountain varieties; or crosses of Caturra and Pacas by Ethiopian germplasm like “SL 14”, “S.4 Agaro”, “S.795”, “DK1/6”, “Dilla&Alghe”, “Geisha” and “Kaffa”. These selected lines and Caturra and Catuaí, both commercial varieties used as checks were established experimental trials at important coffee towns in Táchira and Mérida states, Venezuela.

A complete randomized blocks design with five repetitions and six plants for experimental unit was used at each location. Plants were sown at 2.0 meters between arrays and 1.0 meters among plants, which allowed us to manage a population of 5.000 plants for hectare.

During the first three years of commercial production, we evaluate the production of coffee cherry beans per plant. Since the production of each tree in every year depends greatly on the climatic conditions, it was assumed for the purposes of this study, that year-location combination was like a different environment. In this sense, we obtain estimates the stability for genotypes for a total of fifteen environments (five locations and three years). The statistical analyses for the AMMI model was carried out using MATMODEL version 1.0, program (Gauch, 1988).

The results were expressed as biplot, where the yield (quintals of green coffee beans per Hectare) is shown in abscissas axis and the first principal component (CP1) in the ordinates axis, for the model AMMI.

Table 3. Analysis of Variance for Principal Components of deviations from the grand mean of the yield, according AMMI model

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Probability
TEST	374	9051.69	24.20	0.0000000**
CP1	39	8515.74	218.35	0.0000000**
CP2	37	164.90	4.46	0.0000000**
RESIDUAL	298	371.05	1.25	0.0010188*
BLOCK	60	562.20	9.37	0.0000000**
ERROR	1440	1371.35	0.95	
TOTAL	1874	10985.24	5.86	

RESULTS

The variance analyses showed a highly significant effect for the genotype by year; genotype by location and genotype by location by year interaction. AMMI model showed a highly significant difference for genotype by environment interaction effect. Being AMMI model an efficient tool to define interaction effect since the first principal component (CP1) explained 94.08% of the total effect of the interaction. Additionally, the AMMI model allowed a higher discrimination of genotypes from the stability point of view. Lines 3, 19, 20 and 10; which showed superiors mean than the general mean. These lines are located above the range of stability, showing values of 0.42; 0.47; 0.48; and 0.50 respectively for CP1.

The AMMI model also allowed us to associate the response of more yielding lines to the best environments. For instance, lines 3 and 10 with an appropriate agronomic handling showed very high potential yield, but those environments with certain agroecological limitations, sensibly affected them. This can be explained when we compare the lowest yield average environments with the highest one. This comparison corresponds to "Environment 13 (E13): Santa Ana – Year1" (11.92 qq[1] of green coffee beans per hectare, –0.45 for CP1) and "Environment 11 (E11): La Mesa de Bolívar – Year 2" (165.7 qq of green coffee beans per

[1] qq = quintal =46 kilograms of green coffee beans

hectare, 1.35 for CP1). The lines 3 and 10 yields in those environments, presented average of 6.74 and 8.98 qq of green coffee beans per hectare, at "Santa Ana" and equal to 177.4 and 186.8 qq of green coffee beans per hectare at "La Mesa de Bolívar". Those same materials were evaluated taking into account other agronomic characteristics such as resistance to rust, seed size, cherry coffee beans/ green coffee beans ratio, and weight of a thousand seeds (data not shown).

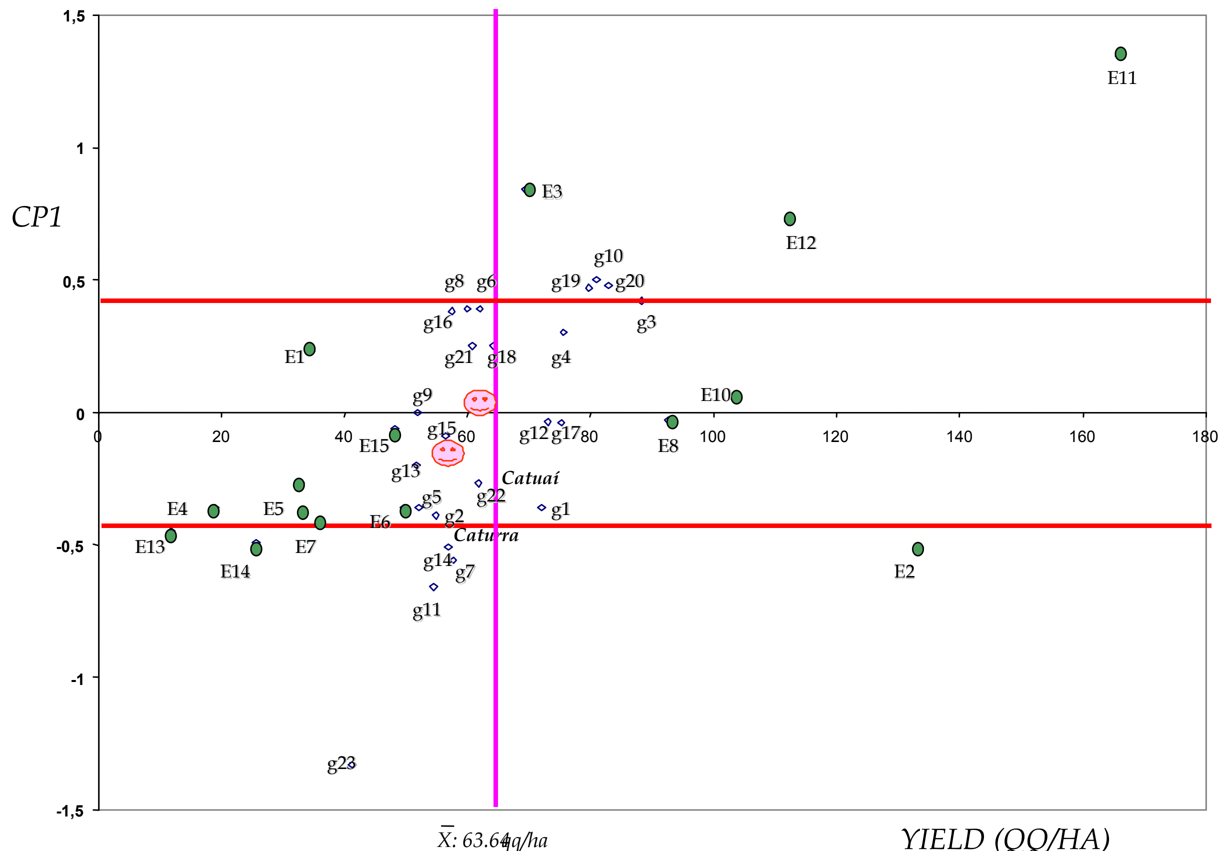

Figure 1. Stability Analysis of 23 lines of coffee evaluated in 15 Environments according AMMI model

The results obtained allowed us to conclude that offspring of lines 1, 3, 4, 6, 10, 12, 17, 19 and 20 should be considered in the conformation of a new variety of coffee with high yield potential. This new variety should be superior to varieties Caturra and Catuaí at the moment used by the farmers and used in this investigation like check.

CROSSES IDENTIFICATION

HW 26/5: 19/1 Red Caturra x 832/1 (Timor Hybrid)
HW 26/11: 19/1 Red Caturra x 832/1 (Timor Hybrid)
HW 26/13: 19/1 Red Caturra x 832/1 (Timor Hybrid)
HW 26/14: 19/1 Red Caturra x 832/1 (Timor Hybrid)
H. 59/3: 110/5 (S. 4 Agaro) x 87/1 (Geisha)
H. 59/4: 110/5 (S. 4 Agaro) x 87/1 (Geisha)
H. 176/8: 19/1 Red Caturra x 128/2 (Dilla & Alghe)
H. 275/1: 19/1 Red Caturra x 1344/ (S. 795)
H. 276/2: 19/1 Red Caturra x 32/1-190 (DK 1/6)

Analysis of Genetically Transformed Coffee Plants (*Coffea canephora* Pierre) for Resistance to Coffee Leaf Miner: Bioassays, Molecular and Immunological Analyses

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SUMMARY

Sixty transgenic coffee plants (*Coffea canephora*) were obtained with genetic constructs containing a *Bacillus thuringiensis* gene efficient against Lepidopterae (*cry*IAc). Their molecular analyses (Southern blots and PCR) have been performed including analysis of T-DNA integration quality, and estimation of copy number. These plants were also submitted to bioassays and to studies of toxin occurrences by immunoassays (Western blotting). For the bioassays, coffee plants were put in presence of adult Tanzania leaf miners (*Leucoptera caffeina*) for 24 hours and checked two weeks later. In order to perform biochemical analysis, proteins were extracted from the same plants, concentrated and checked for protein content. After migration and transfer to nitrocellulose membranes, CryIA(c) protein was looked for with a polyclonal rabbit antiserum.

Most of plants harbour single copy integration of complete T-DNA. Transgene expression varies among bioassays and this variation is not always detected through the Western blots. Hypothesis about the kind of antibodies for future experiments will be discussed, as well as the integration of new genetic constructs, harbouring tissue-specific promoters.

RÉSUMÉ

Soixante plantes transgéniques de caféiers (*Coffea canephora*) ont été obtenues à l'aide de constructions contenant un gène de *Bacillus thuringiensis* efficace contre les Lépidoptères (*cry*IA(c)). Leur analyse moléculaire (Southern blots et PCR) a été réalisée, y compris l'analyse de la qualité de l'intégration du T-DNA et l'estimation du nombre de copies de gènes insérés. Ces plantes ont également fait l'objet de bioessais et d'études de la présence de protéines toxiques par immunodétection ("Westen blotting"). Pour les bioessais, les plantes ont été mises 24 heures en présence de mineuses Tanzaniennes adultes (*Leucoptera caffeina*) et évaluées deux semaines plus tard. Afin de réaliser les analyses biochimiques, les protéines ont été extraites à partir des mêmes plantes, concentrées et dosées. Après migration et transfert sur une membrane de nitrocellulose, la protéine CryIA(c) a été recherchée par réaction avec un anticorps polyclonal développé chez le lapin.

La plupart des plantes ont une seule copie du T-DNA. Les résultats obtenus permettent de faire des commentaires sur l'expression du transgène; celle-ci varie dans les bioessais, mais cette variation n'est pas toujours bien mise en évidence par les tests Western. Une interprétation des résultats sera présentés ainsi que des hypothèses sur les types d'anticorps à utiliser dans les études futures.

INTRODUCTION

Coffee being a perennial crop, its biological cycle is quite long and breeding programs spread over more than 20 years. Non conventional breeding techniques would hasten the process ; among those, genetic transformation is the best choice for combining several desired traits in a single genotype. Coffee genetic engineering has been under study for several years. The first attempts involved protoplast electroporation (Barton et al., 1991) or biolistics (Van Boxtel et al., 1995) but none of those direct transformation techniques succeeded in genetically modified plant regeneration. Other attempts were undertaken using *Agrobacterium* sp. (Feng et al., 1992; Freire et al., 1994). The first genetically modified coffee plants were obtained after co-culturing somatic embryos with an *A. rhizogenes* strain containing marker and selectable genes (Spiral and Petiard, 1993). Later, Leroy et al. (1997) successfully used a disarmed strain of *Agrobacterium tumefaciens* and regenerated *Coffea arabica* and *Coffea canephora* plants expressing a novel agronomic trait, insect resistance (Spiral et al., 1999; Leroy et al., 1999, 2000). In those studies, insect resistance is conferred by a synthetic *cry*IAc gene derived from a *Bacillus thuringiensis* (B.t.) native gene. This gene is specifically toxic for Lepidopterae larval stages (Dandekar et al., 1988). For coffee, it is aimed at conferring resistance to the leaf miner *Perileucoptera* spp .

Coffee leaf miner is an economically important pest for *C. arabica* in East Africa and Brazil (Guerreiro et al., 1990). In order to limit the use of chemical insecticides and therefore implement an environment-friendly strategy, genetically modified plants have been obtained using B.t. genes. This technique is efficient in fighting a number of different insects (Estruch et al., 1997; Schuler et al., 1998). More than 1000 transformed coffee plants harboring the *cry*IAc gene have been planted in the field and are currently under study for agronomic behaviour and insect resistance characteristics (Perthuis et al., 2001).

In this paper we analyze 60 different transgenic coffee plants (independant transgenic events) for several aspects: molecular (number and quality of inserted genes), biochemical (expression of inserted genes: toxin production) and bioassays (effective resistance to leaf miner at the laboratory level) and we study the relationships and the consistency of those three different aspects.

MATERIALS AND METHODS

Plant material

Genetically modified plants were obtained from one *Coffea canephora* genotype of good agronomic value: clone 126. It was selected in Côte d'Ivoire from an intraspecific cross between congolese and guinean types.

Genetic transformation and constructs

The transformation protocole has already been described (Leroy et al., 2000). Two different *Agrobacterium* strains were used: *A. rhizogenes* strain A4 and *A. tumefaciens* disarmed strain LBA 4404. Those strains harbor a *vir*D2 gene.

Three constructs were used (Figure 1): B2, C2 and C3. They all contain the *uidA* gene coding for β-glucuronidase (GUS) modified with an intron for a specific plant expression; this gene is under the control of CaMV 35S promotor. Two different herbicide genes have been used: B2 construct has the *bar* gene for phosphinothricine resistance driven by pE35S; the gene has been constructed antisens compared to *uidA.* C2 and C3 constructs harbor *csr 1-1* gene conferring resistance to chlorsulfuron; that gene is oriented the same way as *uidA.*

B2 and C2 constructs also have native B. t. gene *cry*IAc under the control of pEF1α promotor modified with δ' enhancer. The terminator for this gene is nos (from nopaline synthase).

C3 construct was designed with a synthetic *cry*IAc gene obtained from the University of Ottawa (Sardana et al., 1996). In that special gene, the G+C content is 47.7% whereas it is only 37% in the native gene. That difference has been made in order to facilitate the gene expression in dicots. Promotors and terminators are the same as in B2 and C2. Only gus positive plants were used to carry out the subsequent analyses (Table 1).

Molecular analyses

Total DNA was extracted from in vitro plantlets and from greenhouse grown plants. 59 independant transformation events were analyzed, as well as one non transformed control *C. canephora* plant.

Several pairs of primers were designed to reveal different regions of the plasmids (Figure 1). Procedures for PCR and Southern analyses have already been described (Leroy et al., 2000).

Western blots

Proteins were extracted from about one gram of fresh leaves, and the insecticidal protein was detected by Western blotting (Rogers et al., 1991) using a rabbit polyclonal antiserum raised against the purified CryIAc protein (1/1200 v/v). A secondary goat anti-rabbit antiserum alkaline phosphatase conjugate (Sigma) was used for final detection (1/1000 v/v).

Bioassays

Bioassays were performed with a leaf miner species from Tanzania (*Leucoptera caffeina*). Plants and insects were put in insect-proof cages for 24 hours, the time for the adults to lay eggs on the leaves. Two weeks later, a score was attributed to the plants related to the number of galleries observed per leaf (Leroy et al., 2000). One week later, the number of pupae was recorded. The plants were classified in three categories according to their performances: susceptible plants have an overall score superior to 2 and 5 or more pupae recorded; resistant plants have a score less than 1 and no pupa recorded. Intermediary plants are those with a good score but a high number of pupae, or on the opposite, no observed pupa, but a lot of damages expressed by a high score.

RESULTS AND DISCUSSION

Molecular analyses

Six PCR amplifications were performed on each of the 59 plants from independant events and on the absolute control. The PCR amplifications with primer Chlor, designed to amplify the T-DNA gene *csr 1-1* were positive except on four plants (one C2/A4 and three C3/LBA). However, a correct hybridization was observed by Southern blotting for those plants. The PCR amplifications with primer Bar were positive for two out of three plants harboring the B2 construct. The two amplifications with primers Bt synth and Bt native, designed to amplify the *cry*IAc genes were positive except for two B2/A4 plants, one C3/A4 plant and one C3/LBA plant. No amplification was observed with RK2 primer on any plant, as expected. That specific primer was designed to amplify the plasmid replication origin. Likewise, no amplification was observed with VirD2 primer,designed to amplify sequences

from *vir*D2 gene located on the *Agrobacterium* virulence plasmid. This absence of amplification confirms that there is no residual bacteria in the transformed plants. The untransformed control behaved as expected for all amplifications.

Table 1. Number of gus-positive independant transgenic events used for each combination between *Agrobacterium* strain and genetic construct

Constructs	B2	C2	C3
A. rhizogenes (A4)	B2/A4 3 plants	C2/A4 2 plants	C3/A4 3 plants
A. tumefasciens (LBA4404)	-	-	C3/LBA 51 plants

Table 2. Comparison of western blot (WB) profiles with leaf miner bioassay data for transgenic *Coffea canephora* plants

Number of plants	Bioassay data*	Consistency WB*
10	Resistant	8++/2+
13	Intermediary	10+/3–
5	Susceptible	3–/2+
1 control**	Susceptible	1–

**see text; **non transformed plant; ++ presence of band (very intense), + presence of band (light band); – absence of band*

Southern blotting was used to evaluate the number of T-DNA copies integrated into the plant genome. Among the 59 tansformation events studied, 73% presented one T-DNA copy, 15% two copies, 5% presented 3 and 4 copies and 2% of the plants had 5 copies. This variation in copy number has been encountered in other plants (Zambryski, 1988).

Protein expression

Among the 59 tansformation events studied here, 28 C3/LBA type plants were submitted to Western blots, in order to confirm the expression of the B.t. gene. As stated before, those 28 plants were gus-positive and had integrated one or more copies of *cry*IAc gene as shown by the PCR amplification and Southern blots results. The obtained data were compared to the bioassays scores (Table 2).

No relationship could be established between the level of resistance to the leaf miner and the number of inserted copies: plants carrying 2, 3, 4 or even 5 copies being classified as "intermediary", or slightly susceptible.

As expected, 10 resistant plants (100%) showed the presence of a 65 KDa band on the Western blots.

13 plants expressed intermediary response for the bioassay, with a limitation in the insect activity, but not a complete absence of galleries. For 10 (77%) of those plants, the protein was detected by Western blot. The three other plants were effectively transformed since the *cry*IAc gene was detected by PCR and Southern blotting, but no insecticidal protein was detected by the immunoassays. Using this technique, the detection threshold by the antibody is about 0.1% of the total proteins. It is possible that the amount of produced toxin is below that value, thus not detected and not very efficient, leading to an intermediary response to the bioassay (Dufour et al., 2000). Another possibility is that the *cry*IAc gene is not expressed at

all due to post transcriptional gene silencing as often seen in transgenic plants (Vaucheret et al., 1998); but the likelihood of this hypothesis is quite low since none of these three plants is totally susceptible. Finally, Leroy et al. (2000) made the hypothesis that the *cry*IAc gene structure or expression might have been altered by a recombination event within the T-DNA.

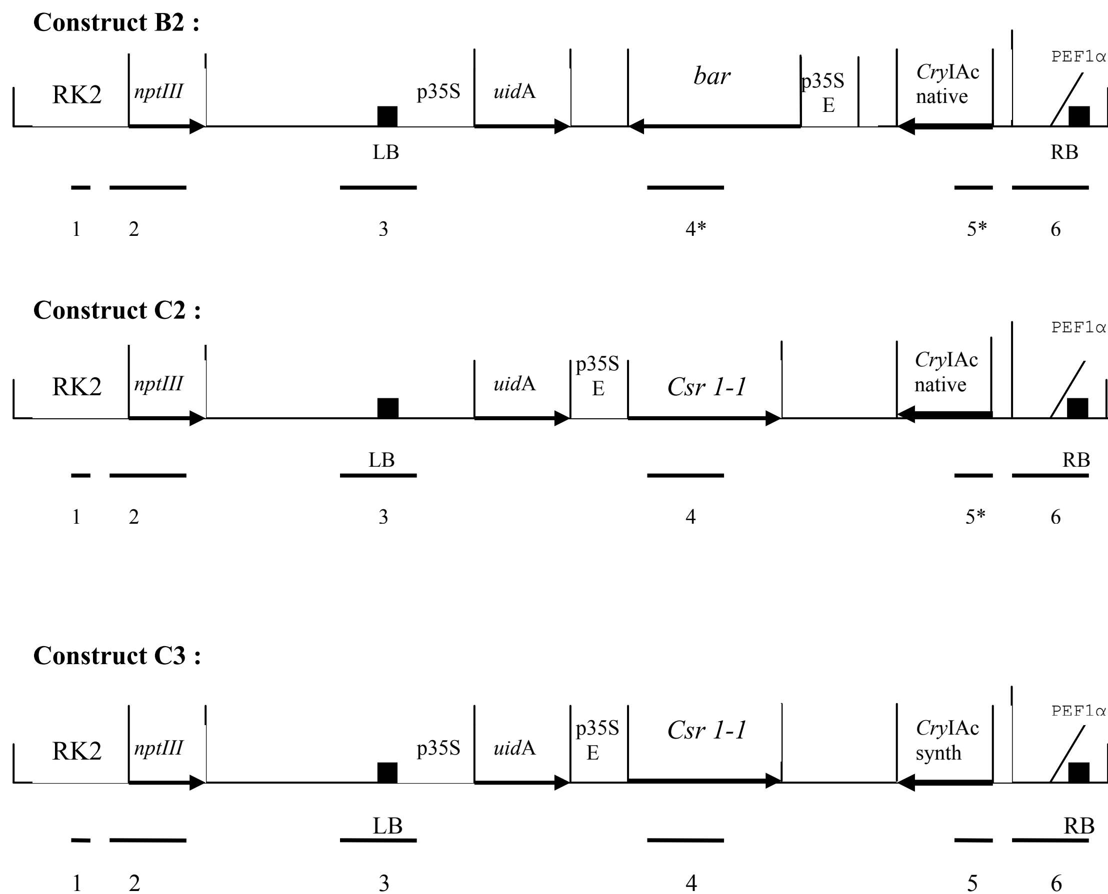

Figure 1. Linear plasmid maps for the three constructs: B2, C2 and C3. The bold lines below the maps indicate the PCR amplification segments: 1: RK2, 2: NPTIII, 3: BG, 4: Chlor, 4*: Bar, 5: Bt Synth, 5*: Bt native, 6: BD

Finally, among five susceptible plants, only 3 (60%) had an expected absence of expressed B.t. gene. Again, the non expression of the B.t. gene might be due to the same reasons as above, leading to a an absence of detected toxin and to a complete susceptibility of the plants. Two plants (40%) had some toxin produced, as revealed by the Western blot, but probably not in a very high amount. Consequently, the insects were not killed nor completely inhibited and the transformed plants behaved as susceptible. Specificity of the chosen CryIAc antibody can also be discussed : we used a rabbit polyclonal antiserum, and this might not be the most optimal antibody. Hen polyclonal antiserum did not give better results (data not shown).

CONCLUSIONS

There is a clear relationship between bioassays and protein expression visualized by Western blots for 93% of the resistant or susceptible plants analyzed. Intermediary or slightly susceptible plants had either a light Western blot pattern (light bands) or no protein expression detected (no band), probably related to the low level of toxin. A more specific antibody could

probably help discriminate low levels of protein expression. In the next future, we plan to use monoclonal antibodies for carrying out another set of Western blots on those plants.

Another prospect is to complete the biochemical analyses of all 59 plants as well as correlate the laboratory bioassays with field data. In the future, work with different promotors and different insect resistance genes would allow a close-up on coffee resistance to leaf miner or other important insect pests like berry or stem borers.

REFERENCES

Barton C.R., Adams T.L., Zarovitz M.A., 1991. Stable transformation of foreign DNA into *Coffea arabica* plants. In: ASIC (eds), 14th colloquium, San Francisco (USA), pp 332-338. ASIC, Paris (France).

Dandekar A.M., McGranahan G.H.; Vail P.V., Uratsu S.L., Leslie C.A., Tebbets J.S., 1988. High levels of expression of full-length *cryIA(c)* gene from *Bacillus thuringiensis* in transgenic somatic walnut embryos. Plant Science **131**: 181-193.

Dufour M., Leroy T., Carasco-Lacombe C., Philippe, R., Fenouillet, C., 2000. Coffee (*Coffea* spp.) genetic transformation for insect resistance. In: Sera T., Soccol C.R., Pandey A., Roussos S. (eds.) Coffee Biotechnology and Quality, pp 209-217. Kluwer Academic Publishers, Dordrecht (The Netherlands).

Estruch J.J., Carozzi N.B., Desai N., Duck N.B., Warren G.W., Koziel M.G., 1997. Transgenic plants: an emerging approach to pest control. Nat. Biotechnol **15**: 137-141

Feng Q., Yang M.Z., Zheng X.Q., Zhen X.S., Pan N.S., Chen Z.L., 1992. *Agrobacterium*-mediated transformation of Coffee (*Coffea arabica* L.). Chin J Biotechnol 8: 255-260.

Freire A.V., Lightfoot D.A., Preece J.E., 1994. Genetic transformation of coffee (*Coffea arabica* L.) by *Agrobacterium* spp. Hortscience **29**: 454.

Guerreiro Filho O., Penna Medina F.H., Gonçalves W., Carvalho A., 1990. Melhoramento do cafeeiro: XLIII. Selecao do cafeeiros resistentes ao bicho-mineiro. Bragantia **49**: 291-304.

Leroy T., Royer M., Paillard M., Berthouly M., Spiral J., Tessereau S., Legavre T., Altosaar I., 1997. Introduction de gènes d'intérêt agronomique dans l'espèce *Coffea canephora* Pierre par transformation avec *Agrobacterium* sp. In: ASIC (eds.), 17th colloquium, Nairobi (Ken), 20-25 July 1997 pp 439-446. ASIC, Paris (France).

Leroy T., Philippe R., Royer M., Frutos R., Duris D., Dufour M., Jourdan I., Lacombe C., Fenouillet C, 1999. Genetically modified coffee trees for resistance to coffee leaf miner. Analysis of gene expression, resistance to insects and agronomic value. In: ASIC (eds), 18th colloquium, Helsinki (Fin), pp 332-338. ASIC, Paris (France).

Leroy T., Henry A.M., Royer M., Altosaar I., Frutos R., Duris D., Philippe R., 2000. Genetically modified coffee plants expressing the *Bacillus thuringiensis* cry1Ac gene for resistance to leaf miner. Plant Cell Reports, **19**: 382-389

Perthuis B., Philippe R., Pradon J.L., Dufour M., Leroy T., 2001. Premières observations sur la résistance au champ de plantes de *Coffea canephora* génétiquement modifiées contre la mineuse des feuilles *Perileucoptera coffeella* Guérin-Méneville. In: ASIC (eds), 19th colloquium, Trieste (Italie). ASIC, Paris (France).

Rogers W.J., Jordan, B.R., Rawsthorne S., Tobin A.K., 1991. Changes to the stoichiometry of glycinedecarboxylase subunits during wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) leaf development. Plant Physiol. **96**: 952-956.

Sardana R., Dukiandjiev S., Giband M., Cheng Y.X., Cowan K., Sauder C., Altosaar I. , 1996. Construction and rapid testing of synthetic and modified toxin gene sequences CryIA (b&c) by expression in maize endosperm culture. Plant Cell Rep **15**: 677-681.

Schuler T.H., Poppy G.M., Kerry B.R., Denholm I., 1998. Insect-resistant transgenic plants. Tib Technol **16**: 168-175.

Spiral J., Pétiard V., 1993. Obtention de plantules *de Coffea canephora* Pierre (Robusta) transformées par *Agrobacterium rhizogenes*. C.R. Acad Sci Paris (Ser 3), **316**: 1-6.

Spiral J., Leroy T., Paillard M., Pétiard V., 1999. Transgenic Coffee (*Coffea* species). In: Y.P.S. Bajaj (scientific ed.), Biotechnology in Agriculture and Forestry, Vol. **44**, pp. 55-76. Springer-Verlag, Berlin.

Van Boxtel J., Berthouly M., Carasco C., Dufour M., Eskes A.B., 1995. Transient expression of beta-glucuronidase following biolistic delivery of foreign DNA into coffee tissues. Plant Cell Reports **14** (12): 748-752.

Vaucheret H., Beclin C., Elmayan T., Feuerbach F., Godon C., Morel J.-B., Mourrain P., Palauqui J.-C. and Vernhettes S., 1998. Transgene-induced gene silencing in plants. Plant J. **16**: 651-659.

Zambryski P., 1988. Basic processes underlying *Agrobacterium*-mediated DNA transfer to plant cells. Annu Rev Genet **22**: 1-30.

Robusta Coffee Improvement in Ghana: Achievements and Prospects

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SUMMARY

Several factors have contributed to low coffee production in Ghana but poor planting material has been the major contributing factor. Farmers in the past have relied on local planting material, which is generally low yielding. To ameliorate the situation, a Robusta coffee improvement programme was initiated in 1977. Seed-lot of five half-sib families introduced that year was tested at CRIG against two locally produced half-sib families. Evaluation of individual trees has resulted in the selection of nine clones with average yields of 2-3 tons per hectare over two 5-year cycles of production without fertiliser application. Compatibility and rooting test show that these clones are generally cross compatible and a rooting success of 75-85% achieved by seven clones within 4 months. Subsequently these seven clones have been used tentatively in 1992 to establish wood gardens for mass production of planting material for growers. In this presentation, the breeding strategies adopted to produce new improved planting material in Ghana are discussed. In particular, accounts is given of how parents are chosen with emphasis on yield, yield stability, genotype adaptability, disease and drought resistance and improved coffee quality attributes.

INTRODUCTION

Robusta coffee cultivation in Ghana started around the same time as cocoa in the high forest zone. As coffee was regarded as financially less attractive and more labour intensive, it became a minor crop and was therefore planted to the poorer soils. Small holdings were scattered throughout the cocoa areas. As at 1985, total area under cultivation was 3000 hectares (Anon, 1996). Among the major factors identified for the low production of the crop were poor yields ranging between 100 and 200 kg/ha. clean coffee which was attributed to unselected planting material of unknown origin. A selection programme was started at CRIG in 1977 with the introduction of open pollinated seed from Cote D`Ivoire and subsequently clones from Togo resulting in the selection and distribution of nine clones to growers by 1992. These clones were however not tested for general adaptability and other important agronomic characteristics.

Various breeding and selection programmes initiated from 1990 to produce new planting material to replace or augment what is in use are in progress. Major considerations are; adaptability, productivity, tolerance to major pest and diseases and quality. The breeding technique, and the main results achieved and the directions along which current research is being conducted are outlined in this report.

EARLY BREEDING EFFORTS IN GHANA

Introduction of Robusta coffee genotypes and selection of clones

Two separate introductions were made from Cote D`Ivoire in 1977 and Togo in 1990. Five seed-lots from elite clones in Cote D`Ivoire coded A to E, together with seed from two local clones also coded F & G, were planted in an observational trial at the Afosu sub-station of the Cocoa Research Institute of Ghana (CRIG) in 1978. Eighteen to thirty-five stands of each half-sib family were used. Yield recordings were over two cycles of production (1980/81-1984/85 and 1986/87-1991/92) after which data was summarised to assess the performance of the seven families. Since data recordings were on individual plant basis, individual selection was undertaken over the two production cycles. Selected stands were later tested for rooting ability.

The best yield were recorded by the E-family followed by B and A. The local selections, F and G, however gave the lowest yields (Table 1). The C, F and E families have high average bean weight. Clonal trials from these selections were presented in earlier reports (Adu-Ampomah et al., 1993).

In the second trial, six clones introduced from Togo in 1990 were planted in 1991 at Tafo in a randomised trial together with two clones from the first trial. 144 stands were planted of each clone in a randomised complete block design. Yield data were recorded for three years after which the trial was terminated as a result of unexpected damage to most trees. Table 3 shows early yield of the clones. The termination of the trial could not allow for selection. Clones 197,149,126 and 181 however gave better scores.

Table 2 shows selected stands with average yields of at least two and a half times the plot average over a ten-year period without fertiliser application (most farmers in Ghana cultivate coffee with no fertiliser application). Nine of these with higher yields were selected tentatively as planting material, but two clones with rooting succes at below 75%, one of which having very small beans, were subsequently discarded.

The first and second progeny trials

High yielding plants selected based on individual yields from some selected families of the observational plot described above were used in two separate diallele crosses. Due to poor weather conditions at the time, most crosses failed and therefore variable number of stands were planted from the successful crosses. The aim was therefore to select plants with good agronomic characteristics as clonal planting material.

Table 4 shows the performance of the individual families after the second year yield for the first progeny trial. Early selection was made for individual stands with yields three times the average of the plot. Early selection was also made for the second progeny trial based on first year berry load and plant architecture for further trials.

CURRENT BREEDING AND SELECTION PROGRAMMES

Development of an index for early selection of Robusta coffee genotypes

The long breeding cycle of 7-8 years necessitates the development of fast methods of selection during a breeding cycle. A trial has been set up which aims primarily at providing an index of selection to shorten the breeding cycle. It also aims at estimating the heritability of

certain plant characters that could aid future breeding programmes. Twelve stands of each of 33 selected clones from the above mentioned progeny trials were planted in a completely randomised design.

Table 1. Yield (clean coffee) and bean weight of seven half-sib families of robusta coffee

Family	A	B	C	D	E	F	G	Mean
N° of stands	35	35	33	18	33	25	23	29
1st cycle yield (kg/ha)	889	1037	554	735	1270	153	223	695
2nd cycle yield (kg/ha)	806	817	878	828	1001	397	305	733
Mean 10 year yield (kg/ha)	848	927	716	782	1186	275	264	714
Yield range	117 - 2504	53 – 2588	116 - 1347	55 – 1657	69 - 2372	44 - 689	54 - 719	73 - 1696
N° of plants 2,5 times plot average	3	4	0	0	6	0	0	2
Mean weight of 100 beans (g)	13,4	13,0	15,5	13,6	14,0	14,3	13,1	13,8
Range of bean weight (g)	9,75 -20,0	9,7 -15,8	12,1 -25,0	11,6 -18,0	9,8 - 18,5	11,3 - 19,0	10,3 -17,0	10,6 -19,0

Table 2. Mean 10-year yield (clean coffee) and bean weight of selected stands

Family	Stands 2.5 times plot average	First cycle yield (kg/ha.)	Second cycle yield (kg/ha.)	Mean 10-year yield (kg/ha.)	Mean 100-bean weight (g)	Selected top 5% of plot
A	**A129**	3067	1940	2504	13.5	✓
	A115	2400	1696	2048	13.2	✓*
	A101	2502	1579	2041	14.4	✓
B	**B170**	2660	2517	2588	10.8	✓*
	B96	2866	1208	2037	14.9	✓
	B36	2069	1868	1969	14.5	
	B190	1487	2276	1882	15.2	
E	**E186**	2287	2456	2372	17.3	✓
	E174	1653	2770	2212	15.0	✓
	E138	2478	1809	2143	14.5	✓
	E139	2895	1199	2047	16.6	✓
	E164	1462	2580	2021	13.9	
	E63	2117	1725	1921	14.2	

**Poor rooting clones*

Table 3. Yield of introduced coffee (in kg/ha) for first three (3) years

Clone	Year			Mean
	1992/93	1993/94	1994/95	
197	348	1,903	2,017	1,423
149	258	1,744	1,997	1,333
126	219	1,539	2,017	1,258
181	262	1,491	1,676	1,143
E	183	1,668	1,527	1,126
375	43	877	1,732	884
107	71	443	1,981	832
D46	125	838	1,073	679
Mean	189	1,313	1,753	1,085

Table 4. Mean 2-year yield of clean coffee for families in the first progeny trial

Cross/family	No.of Stands	First yield (kg/ha)	Second yield (kg/ha)	Mean yield (kg/ha)	No. of stands 3 times plot average	Mean yield range of selected stands
B191XF74	103	74	678	376	4	1010 – 1797
B191XE152	105	71	799	435	6	1091 – 2193
B170XA129	104	63	991	527	6	1531 – 3660
E152XC180	103	82	795	439	6	1085 - 2455
A129XC180	6	0	1527	764		
B191XD46	6	0	799	400		
B191XB170	6	0	1077	539		
B191XC180	6	0	14	14		
B191XC138	6	0	0	0		
B170XF74	6	0	137	137		
E152XA129	6	0	251	126		
E152XE138	6	0	102	51		
E152XE139	6	0	0	0		
E152XE74	6	0	55	28		
Mean		21	527	274		

Data currently being collected on individual plant basis for the 33 clones are on a number of parameters. These include height and girth of the main stem; length, diameter and number of primary branches; number of nodes and length of inter-nodes of primary branches; number of secondary branches and leaf area. The rest mainly reproductive parameters are: number of flowers and fruits per node, percent fruit-set, number of fruiting nodes, bean weight and yield. At the end of the first cycle (after the fifth-year harvest), data collected will be used in a multivariate analysis to develop the index.

Testing of clones for adaptability

Micro-climatic conditions have varying effects on different genotypes (Kempton and Talbot, 1988). Hence, the outstanding performance of a new variety on a trial field of an experimental station by no means implies that its behaviour will be similar under other climatic or edaphic conditions. In Ghana, coffee is cultivated under varying climatic conditions. Thus, trials have been set up with the aim of assessing the yield performance of the coffee cultivars under different geographical locations with the view to obtaining genotypes with a wider range of

adaptability. It is also the aim to study plant characters associated with adaptability that could be used in future breeding and selection programmes.

Twelve genotypes among the top 7% of the coffee observational plot planted 1978 were selected at the end of two cycles of production and together with six clones introduced from the Togo in 1990 planted in three locations. Planting was in June 1996 with 32 plants per clone in a completely randomised design in each of the three environments. Data recordings on vegetative and reproductive parameters are as described above. Data is also taken on, fruit and bean characteristics, pest and disease incidence and drought response. Records of factors such as soil physical and chemical properties and climatic factors during the experimental period are also under consideration.

Data collection started in 1996 and two more yield records are required for the data to be analysed. It was envisaged that cultivars with high mean performance and lower environmental sensitivity shall be selected as planting material for farmers.

Selection and breeding of robusta coffee genotypes for high density planting

Investigation into high density planting has been undertaken in various crops (David et al., 1991; Kahar et al., 1991) but there is limited information in this direction for robusta coffee (Perez 1983; Carvalho 1988). The spreading growth form of most Robusta clones does not allow close spacing to increase productivity. A programme has therefore been initiated with the objective to produce high yielding genotypes with compact growth habit amenable to high density planting. Yield data analysis of robusta coffee genotypes from experimental plots planted in 1978 has resulted in the selection of high yielding plants. Eleven moderately to high yielding clones selected as parents were used in this programme (Table 5). Eight female parents of canopy radius of 0.91-1.28m were crossed with three male parents with canopy radius of 0.83-0.89m by the North Carolina Design 2 crossing model. Twenty-four progeny families resulting from the cross were planted together with two standard clones in May 1998. Planting was in a randomised complete block design with 4 replicates, 8 plants per family per replicate at a spacing of 2 x 3 m.

Data recordings are on-going and are as described in 2.1. above. Analyses of data will be as by Kearsey 1965.

Development of Seed Planting Material of Robusta Coffee

The high cost and cumbersome procedure of clonal planting material production and its low multiplication rate necessitates the use of hybrid varieties along side clonal ones. As in the Cameroon, Cote d'Ivoire, Madagascar, Indonesia and most Robusta producing countries, the planting material being recommended to farmers consists of clonal and hybrid varieties (Bonharmont et al., 1986; Capot, 1977; Ravohitrarivo, 1980). In Ghana, a programme has been initiated with the aims of; (i) reducing the cost of production and transportation of planting material, (ii) to supplying the farmer with planting material which will be available in large quantities and (iii) to supplementing the existing clonal material with hybrid varieties of similar yields.

Parents were selected based on average yields in two production cycles, except clone 181 which was selected from introductions from Togo after yield evaluations for 3 years. Six female parents and five male parents (Table 6) crossed using the North Carolina 2 crossing model resulted in thirty progeny families. These were planted together with two standard

clones in May 1998. The planting design and data being collected are as in 2.1.above. Analyses of data will be as by Kearsey 1965.

Table 5. Parents used for crosses for trial on high density planting

Female Parents			Male Parents		
Clone	Yield (kg/ha.)	Canopy radius(m)	Clone	Yield (kg/ha.)	Canopy radius(m)
A129	2504	0,93	**B191**	1638	0,83
A115	2048	0,98	**197**	1423	0,89
A101	2041	0,99	**C180**	915	0,84
B170	2588	0,91			
B96	2037	1,28			
E174	2212	1,08			
E138	2143	1,09			
E139	2047	1,06			

Table 6. Parents used for crosses for seed planting material development

Female Parents		Male Parents	
Clone	Yield (kg/ha.)	Clone	Yield (kg/ha.)
A129	2504	**A115**	2048
A213	1463	**A101**	2041
B170	2588	**181**	1143
B96	2037	**E186**	2372
E138	2143	**E174**	2212
E139	2047		

Quality Improvement of Robusta Coffee

Characteristics determining the quality of green coffee namely; the size of beans, proportion of defective beans and chemical composition which are considered for the improvement of green coffee are of major importance in the breeding activities of Ghana. Bean weight of the selected clones distributed to growers' range from 11.4-15.6 g/100 beans. Analyses of the coffee germplasm at CRIG have given a range of 9.7-25.0 g/100 beans. This observed variability is being exploited for selection and breeding to combine the good characteristics of the genotypes. A programme with the objective of producing coffee genotypes with good bean characteristics among other factors, for commercial cultivation in Ghana, was initiated last year (2000).

Nine (9) clones, some currently being supplied to farmers (with bean weights of 11.4-15.6 g/100 beans) and four (4) high to moderately high yielding clones (with bean weights of 17.3-25 g/100 beans) are being used for this trial (Table 7). Crossing was by the North Carolina Design 2. Thirty-six progeny families produced by crossing each of the 4 clones with large beans (17.3-25 g/100beans) as male parents to the remaining 9 clones as female parents are ready for field planting. Data collection will be on yield, berry and bean physical characteristics, caffeine content and chemical composition. Data analysis will also be as by Kearsey 1965.

Table 7. Parents used for crosses for quality improvement of green coffee

Female Parents			Male Parents		
Clone	Yield (kg/ha.)	100 bean weight (g)	Clone	Yield (kg/ha.)	100 bean weight (g)
A129	2504	13,5	**C193**	961	25,0
B96	2037	14,9	**C134**	1181	19,7
B191	1638	15,2	**D47**	1488	18,3
B170	2588	10,8	**E186**	2372	17,3
E138	2143	14,9			
E139	2047	16,6			
E152	1733	15,4			
197	2147	11,4			
149	1272	15,0			

THE WAY FOWARD

The breeding and selection programme started 1977 was rather too slow due to lack of funds for coffee research by CRIG. The initial selection trials were all at the Afosu sub-station with annual rainfall range of 1034-1753 mm from 1981 to 1992, rather too low for optimum production. Three clone evaluation trials at the same station with selections from the observational trial gave conflicting results on the performance of the genotypes. Selection of the interim planting material was, hence, based on yield of single stands in the observational plot over a 10-year period. The inclusion of these clones in location trials will make selection more efficient.

The hybridisation and selection programmes currently on-going are part of a broad programme of recurrent selection. The estimation of heritability and general combining ability values is necessary for the selection of parents, which within them have additive effect of genes controlling the characters under consideration. Genotypes (clonal and seed varieties) that combine high yields, resistance to disease and pests, desirable growth habit, good berry and bean attributes, and adaptability among other desirable agronomic characteristics is an ultimate goal. With the breeding cycle shortened, the selection process could be fast enough to make this overall goal achievable in not too long a time.

REFERENCES

Adu-Ampomah Y., Martinson V.A., Dasi, E. and Amonoo R.S. (1993). Breeding efforts to improve coffee production in Ghana. Asic, 15 Colloque, Montpellier, 1993, 253-261.

Anon, 1996. Ghana coffee study. Cargill Technical Services Ltd., Knowle Hill Park, Surrey, U. K. Consultant Report for the Government of Ghana.

Bouharmont P., Lotodè R., Awemo, J. and Castaing X., 1986. Generating breeding of Robusta coffee tree in Cameroon. Analysis of results of partial diallel hybrids Trial planted in 1973. Cafe` Cacao The` **30**: 2, 93-112.

Capot J. (1977) L'ame'lioration du cafe'ier robusta en cote d'Ivoire. Cafe', Cacao, The' 21:233-44

Carvalho A., 1988. Principles and Practice of Coffee Plant Breeding for Productivity and Quality Factors of Arabica. In: R.J. Clarke and R. Macrae (Eds.), Coffee, Vol. **4**: Agronomy, pp. 129-166, , Elsevier Appl. Sci., London, U.K.

David H. Klim, Joe Pang Than Yiri, Stephen Yow and Lockwood G. (1991). Selection of cocoa Genotypes for High Density Cocoa Planting and Guidelines to its Management. In Abstracts; International Cocoa Conference: Challenges in the 90s, Kuala Lumpor, Malaysia 13.

Kahar S. A.B., Abdullah O., Denamany G. and Jelani M.B. (1991). Early Performance of High Density Cocoa Plantings in Different Locations in Peninsular Malaysia. In Abstracts, International Cocoa Conference Challenges in the 90s, Kuala Lumpor, Malaysia, 12.

Kearsey M.J. (1965). Biometrical analysis of a random mating population: A comparison of five experimental designs. Heredity, **20**, 205-235.

Kempton R. and Talbot M. (1988). The development of new crop varieties. J.R. statist. Soc. A. 151, part 2, pp.327-341.

Peres V. M1 (1983). Treinta e dos anos de investigacion sistemataca y. transferencia tecnologica de cultivo de cafe' en Costa Rica, 1950-1982. Oficina de Café San José, Costa Rica. 31pp.

Ravohitrarivo C.P. (1980). Elude de la variabilité des descendances et des problemes lies a l'ame'lioration des cafeiers cultive's diploides' These de Doctorat de zeme cycle,

Outcome of Two Decades of Reciprocal Recurrent Selection Applied to *C. canephora* in Côte d'Ivoire: New Outstanding Hybrids Available for Growers

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SUMMARY

Since the beginning of the 80s, a reciprocal recurrent selection programme has been applied to *C. canephora* in Côte d'Ivoire, under the collaboration between the Centre National de Recherche de Côte d'Ivoire (CNRA) and the Centre International de Recherche Agronomique pour le Développement (CIRAD).

The study of *C. canephora* genetic diversity lies at root of this programme. Work by the ORSTOM (now the Institut de Recherche pour le Développement – IRD) revealed the existence of two genetic pools: the Guinean pool and the Congolese pool. The structure of that diversity was then specified and four groups were identified within the Congolese pool.

Hybrid vigour, or heterosis, was then observed in progenies between Guinean and Congolese parents: this hybrid vigour is exploited through reciprocal recurrent selection (RRS). The genetic groups were characterized and assessed prior to defining basic populations for RRS. Between-pool tests were then carried out using twot or three testors from the reciprocal population (indirect RRS on testers). An overview of the first selection cycle is now possible. Concerning the Guinean pool, genetic gains on test value reached +38% for yield, +3% on rust resistance and +7% on bean size. Concerning the Congolese pool, SG1 proved to be superior to all other groups for yield test values but less resistant to rust. There was no significant difference between Congolese groups for bean size.

The major outcome of these two decades and of the first cycle of RRS is the identification of 10 between-pool hybrids (BPH), disseminated in seed forms, producing as much or even more than current clones, distributed as cuttings. The best BPH showed a potential yield of 3,5 tons of green coffee per hectare and per year, which is 40% more than clone 461 (one of the best currently distributed clone).

Future prospects for the programme are discussed.

RÉSUMÉ

Depuis le début des années 80, un programme de sélection récurrente réciproque est appliqué à *C. canephora* en Côte d'Ivoire dans le cadre d'une collaboration entre le Centre National de

Recherche Agronomique de Côte d'Ivoire (CNRA) et le Centre International de Recherche Agronomique pour le Développement (CIRAD).

A la base de ce programme, il y a l'étude de la diversité génétique de *C. canephora*. Les travaux de l'ORSTOM (devenu l'Institut de Recherche pour le Développement – IRD) ont permis de mettre en évidence l'existence de deux pools génétiques : le pool Guinéen et le pool Congolais. La structure de cette diversité a par la suite été précisée et quatre groupes génétiques différents ont été identifiés dans le pool Congolais.

La vigueur hybride ou hétérosis observée au niveau des descendances entre parents Guinéens et Congolais a été exploitée par la sélection récurrente réciproque (SRR). Les groupes génétiques ont été caractérisés et évalués avant de définir les populations de base de la SRR. Les tests intergroupes ont ensuite été réalisés en utilisant deux ou trois testeurs de la population réciproque (SRR indirecte sur testeurs). Aujourd'hui, un bilan du premier cycle de sélection peut être dressé. Pour le groupe Guinéen, les gains génétiques sur les valeurs en test atteignent 38%, 7% et 3%, respectivement pour le rendement, la taille des grains et la sensibilité à la rouille orangée. Pour le groupe SG2 du groupe Congolais, les gains sont de 31%, –3% et 25% pour les mêmes caractères.

L'aboutissement majeur de ces deux décennies de sélection est l'identification de 10 hybrides interpools, diffusés sous forme de semences, produisant autant sinon plus que les clones actuels, distribués sous forme de boutures. Le meilleur hybride interpool a un potentiel de production de 3,5 tonnes de café marchand par hectare et par an, soit 40% en plus que le clone 461, l'un des clones les plus producteurs actuellement vulgarisés. Ceci constitue une évolution décisive pour l'utilisation du matériel végétal sélectionné par les planteurs.

INTRODUCTION

Breeding of *Coffea canephora* started some 100 years ago in Java. In Côte d'Ivoire, the first large programme was set up in the 60s (Montagnon et al., 1998). Varietal improvement was mainly based on clonal or hybrids selection from parents chosen for their good combining ability, either general (GCA) alone or together with specific (SCA). In the 80s, the study of *C. canephora* genetic diversity opened the way for a new programme implemented in Côte d'Ivoire: the Reciprocal Recurrent Selection or RRS. After highlighting one of the main constraint of *C. canephora* varietal improvement, this paper traces back the different steps of this programme and makes an overview of its achievements.

PREAMBLE: DILEMMA BETWEEN CLONES AND HYBRIDS VARIETIES

One of the main constraint for *C. canephora* cultivation in Africa is the low share of selected varieties grown by planters. In Côte d'Ivoire, they represent not more than about ten per cent of coffee plantations (Roux and Duris, 1995). In general, three kinds of coffee trees are used (Table 1):

- **Unselected trees:** issued from seeds picked on heterogeneous local populations.
- **Selected clones:** mix of at least five clones distributed as cuttings; this mix is made necessary by *C. canephora* strict incompatibility (Berthaud, 1980).
- **Selected hybrids:** are distributed by seeds from bi- or tri-clonal seed gardens (Charmetant et al., 1990).

These three kinds of material potentially yield 400, 2400 and 1600 Kg of green coffee per hectare and per year (Kg gc.ha^{-1}. y^{-1}) (Capot, 1977; Charmetant et al., 1900). Thus, in several

countries, Research & Development (R&D) services have made the choice to distribute only selected clones, regarding their superior yield potential (+50% compared to selected hybrids).

However, cuttings are much more difficult to produce and distribute than seeds. As a result, the cost of a cutting is at least three fold that of a seed (Roux and Duris, 1995). In addition, coffee growers have a much better know-how for raising seedlings rather than cuttings, leading to a low and high mortality rate, repectively, in nursery (see also Rakotamalala et al., 1997).

Table 1. Different considerations from Research & Development and growers on *C. canephora* vegetal material and breeders goal

Vegetal material	*Support*	*Yield potential (Kg gc.ha^{-1}. y^{-1})*	*Research & development choice*	*Growers conclusion*
Selected clones	Cuttings	2400	YES	NO
Selected hybrids	Seeds	1600	NO	Unavailable
Unselected	Seeds	400	NO	YES
Breeders Goal: Hybrids	**Seeds**	**2400**	**Agreement**	

Coffee growers were hence finally led to use the only available seeds, that is from unselected material.

The identification of hybrids varieties yielding as much as clones has thus been one major goal of *C. canephora* breeding in order to find a agreement between R&D services and growers. Here is the story of how this goal has been achieved thanks to a collaboration involving firstly IRD, and then mostly CIRAD and CNRA.

FROM 70'S ONWARD: GERMPLASM SURVEYS AND CONSERVATION

From the 70's onward, the Institut de Recherche du Café Cacao (IRCC now CIRAD-CP) and the ORSTOM (now IRD) have made a great effort on collecting wild populations in a major part of the *C. canephora* distribution area (see Montagnon, 2000 for a review). Those surveyed populations were implanted in field collections, specially in Côte d'Ivoire. At the CNRA research station of Divo, are conserved:

- 17 wild populations from Côte d'Ivoire,
- 5 from the Centrafrican Republic,
- 9 from Cameroon,
- 7 from Congo,
- and 3 from Guinea.

Together with those wild populations, cultivated populations are conserved:

- 1 from Côte d'Ivoire,
- 4 from Gabon,
- and 4 from Democratic Republic of Congo.

STUDY OF GERMPLASM GENETIC DIVERSITY

After having gathered wild and cultivated populations in field collections, their genetic diversity was studied using first isozymes (Berthaud, 1986; Montagnon et al., 1992) and then RFLPs (Dussert et al., 1999). Two pools were identified (Figure 1): the Guinean pool and the Congolese pool, corresponding to the main forest refuges during the Last Maximum Glaciation period, 18000 years ago. The Guinean pool is composed of one single Guinean group. On the other hand, the Congolese pool firstly identified by Berthaud (1986) was shown to be composed of two groups (SG1 and SG2) by Montagnon et al. (1992) and then four groups (SG1, SG2, B and C) by Dussert et al. (1999).

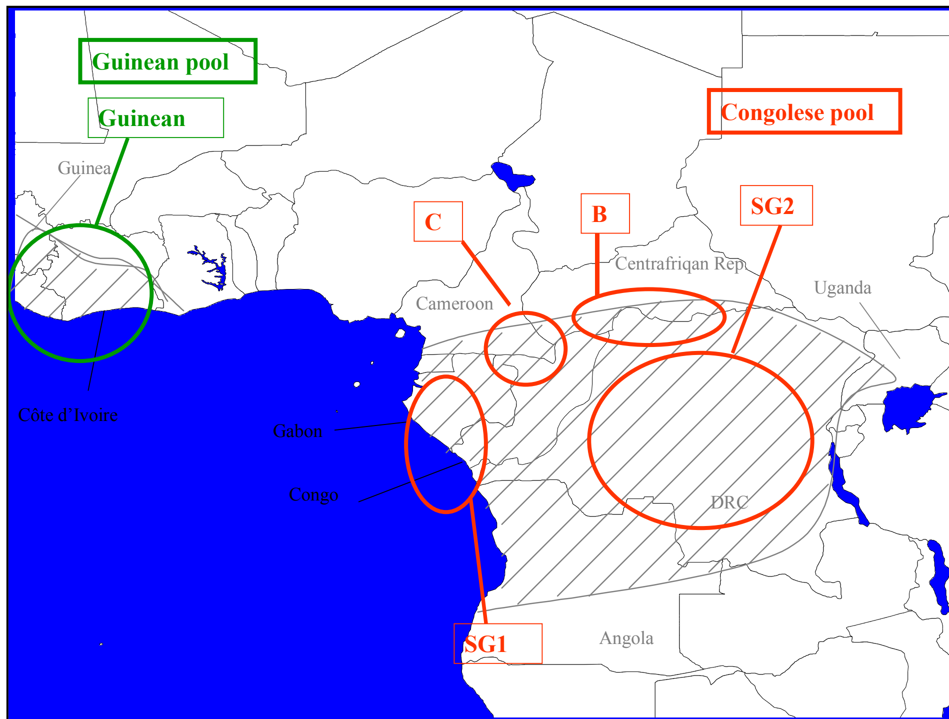

Figure 1. Genetic diversity of *C. canephora.* (Berthaud, 1986; Montagnon et al., 1992; Dussert et al., 1999)

Towards a Reciprocal Recurrent Selection (RRS)

Using the isozymes patterns, Berthaud (1986) observed that:

- the best clones selected during the 70's were issued from natural hybridisation between a Guinean and a Congolese parent,
- the best hybrids selected during the 70's were corresponding to combinations between a Guinean and a Congolese parent.

The author thus suggested the existence of an hybrid vigour, or heterosis, between the Guinean and the Congolese pools. He argued that, in such a situation, a reciprocal recurrent selection (RRS) should be applied to *C. canephora* in order to achieve high genetic gains.

Leroy et al. (1993) confirmed the hybrid vigour between both pools, comparing on a large scale controlled intra and interpool crosses and started an indirect RRS, RRSi, using two or three constant testors (Figure 2).

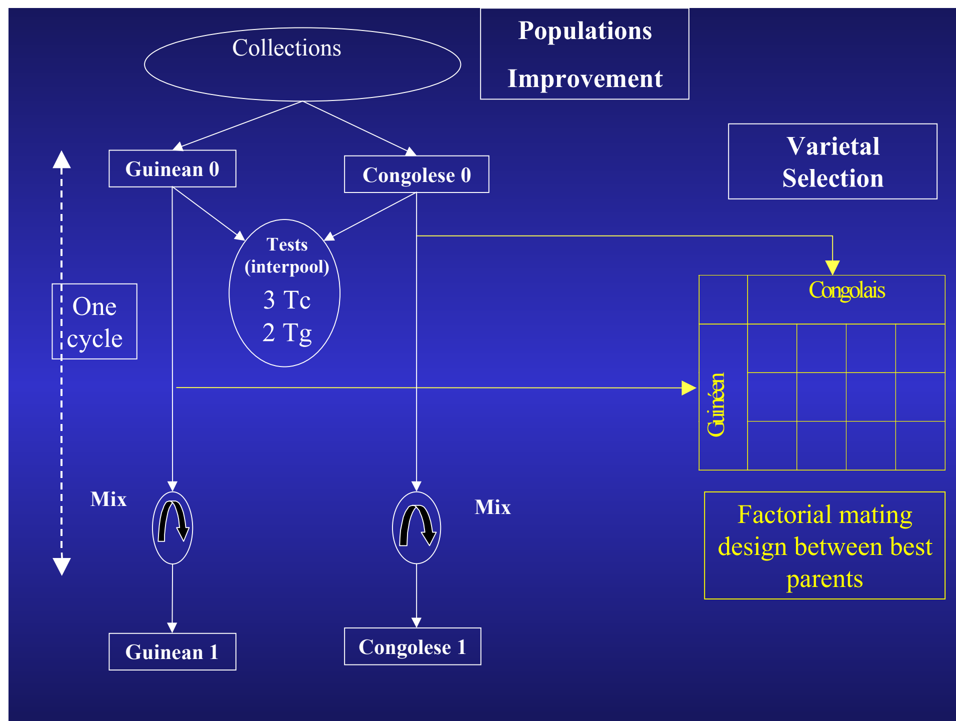

Figure 2. RRS applied to *C. canephora* in Côte d'Ivoire (after Leroy et al., 1993)

Two base populations, Guinean and Congolese, are formed from available genotypes in collections. Each individual from both base populations is tested by crossing it with tow or three reciprocal testers. The test value of each tested genotype is the mean of the progeny issued from the cross between this genotype and the considered tester. Each tested genotypes has as much test values as reciprocal testers.

The best genotypes, judged on their test values, are:

1. intra-crossed to obtain the improved populations of the next cycles and,
2. inter-crossed following a factorial mating design in order to optimise varietal selection.

The RRS started in 1985 (when the Congolese pool was thought to be composed of one single group) and the first cycle was ended in 1998.

REALISED GENETIC GAINS ON POPULATIONS TEST VALUES

Montagnon (2000) explored the quantitative genetic aspects of the first cycle of RRS. Among other conclusions, he found that, thanks to the great additive part of genetic variability observed for most characters, one tester could be enough to assess the genotypes test value. Thus, only one test value (on Guinean tester 410 and Congolese 464) is further considered. Results are presented here for the following characters: yield expressed as the percentage of the constant control (clone 461) T%, rust resistance expressed as the percentage of resistant plants per progeny (see Montagnon et al., 1994 for the detailed method) and bean size expressed in grammes for 100 beans at 12% humidity.

Table 2. Genetic gains on test values during the first cycle of RRS – Guinean pool

Character	Overall base population (96 genotypes)	Selected genotypes (16)	Genetic gain
Yield *(T%)*	49	72	+ 38%
Rust resistance *(% of resistant plants per progeny)*	47	49	+ 3%
Bean size (g per 100 beans at 12 % humidity)	13,63	14,74	+ 7 %

Table 3. Congolese groups mean test values from the first cycle of RRS[1]

Character	SG1 (12)	SG2 (31)	B (19)	C (7)	MEAN
Yield *(T%)*	85 a	61 b	34 c	51 b	58
Rust resistance *(% of resistant plants per progeny)*	65 b	87 a	79 a	88 a	80
Bean size (g per 100 beans at 12 % humidity)	13,55	12,98	13,56	12,22	13,08

[1]Means corresponding to different letters are different according to Newman and Keuls test at 5 % level

Concerning the Guinean pool (Table 2), genetic gain on test value is very high for yield (+38%), satisfying for bean size (+7%) and less important for rust resistance (+3%).

Results are presented differently for the Congolese pool (Table 3). Indeed, the different Congolese groups were identified after the Congolese base population was formed. Hence, the size of the groups did not allow to realise a selection in each of them. However, groups could be compared for their mean test values. SG1 group was significantly better combining for yield with the Guinean tester than all other Congolese groups. Groups SG2 and C did not differ significantly whereas group B was significantly lower than all other groups. The overall level of rust resistance is good even if SG1 test value was significantly lower. No significant differences between groups were found for bean size.

It must be outlined that SG1 alone brings a genetic gain of 46% for yield compared with the overall mean of the Congolese pool.

REALISED GENETIC GAINS ON HYBRID VARIETIES

Hybrids created during the first cycle of RRS are issued from the factorial mating designed with the best genotypes of both Congolese and Guinean base populations (Figure 2). Their distribution for yield (four harvests) is illustrated Figure 3.

The clone 461 is one of the highest yielding clone currently distributed: between 2 and 2.2 tons of green coffee per hectare and per year. Ten interpool hybrids are superior or equivalent to this clone. Futhermore, the very best hybrid yields some 3.5 tons $gc.ha^{-1}.y^{-1}$, that is close to 40% more than clone 461.

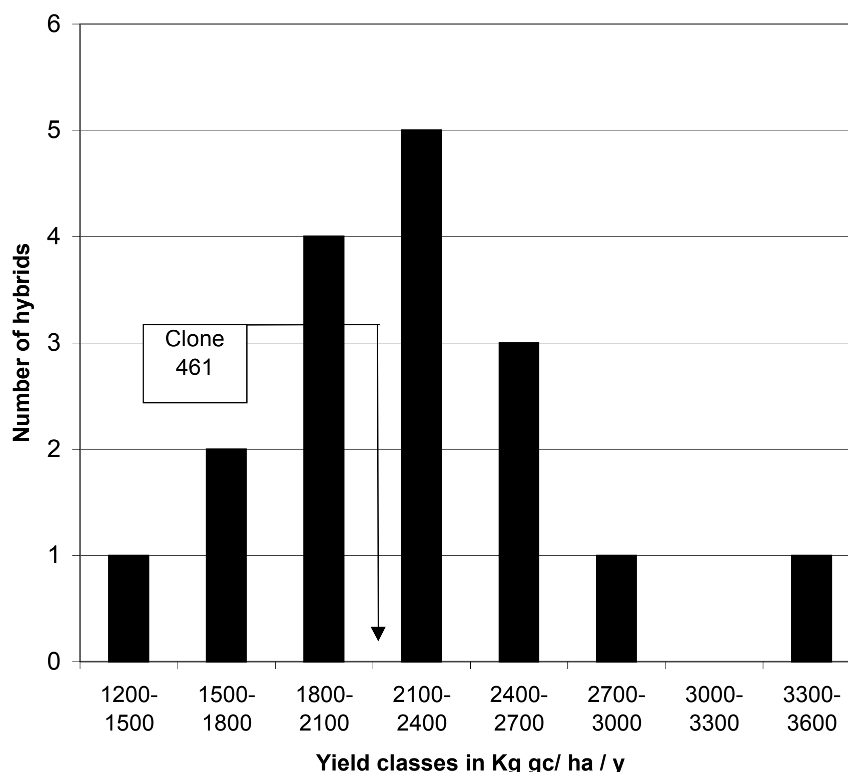

Figure 3. Yield distribution of RRS hybrids issued from the factorial mating design of the first cycle

The genetic gain for hybrid varieties can be replaced in an historical perspective (Figure 4). Attributing base 100 to currently distributed clones, it can be observed that:

1. former hybrids (Hybrids '1970' = before RRS) yield 60 or 40% less than clones,
2. RRS hybrids, as a mean, yield 85, which represents a genetic gain of 42% as compared to former hybrids,
3. the best RRS hybrids (candidates for distribution) yield 115, which is more than clones and represents a genetic gain of 92% as compared to former hybrids.

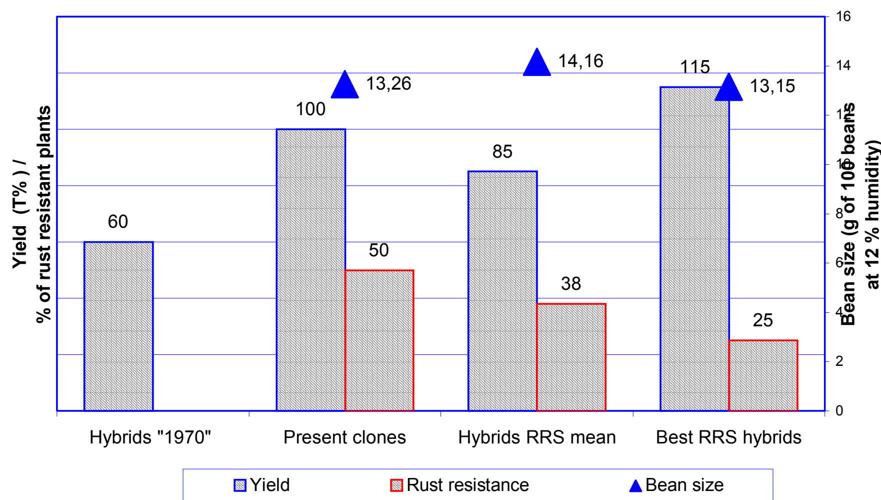

Figure 4. Comparaison between clones, former hybrids, mean and best RRS hybrids for yield, rust suscptibility and bean size

The percentage of plants susceptible to leaf rust has been lowered from 50 (clones) to 25% (best RRS hybrids). Bean size hardly changed.

Furthermore, RRS hybrids are genetically homogeneous. They have been selected for their bushy growth habit that allows a higher planting density and a better soil cover. Their quality characteristics are systematically checked (every harvest year).

CONCLUSIONS AND PERSPECTIVES

Toward an enhanced use of selected varieties:

Two decades of RRS have led to the creation of hybrids which yield twice as much as former ones. This is a decisive qualitative steps for *C. canephora* breeding. Using seeds, rather than cuttings, as the support of genetic gain should enhance the use of selected material by growers.

Clones may nevertheless stay useful in order to rapidly fix genetic gains for bean size, some disease resistance (tracheomycosis for instance) or some characters linked to cup quality.

A good example of a comprehensive breeding system:

The different steps of *C. canephora* breeding tallies well with a comprehensive breeding system.

1. Germplasm has been surveyd and conserved,
2. Germplasm has been characterised (genetic structure) and evaluated (agronomic performances),
3. It has led to the proposition and the implementation of RRS,
4. It has already brought some substantial and decisive progress in population imporvement and hybrid selection.

In the near future:

1. we should keep on surveying *C. canephora,* specially in Gabon, Uganda, RDC and Angola as few genotypes from these countries are avalaible,

2. we also should focus on quality. However, two questions need to find a clear answer from roasters : What is quality ? Which quality (ies) is (are) needed by the market ?

3. a great amount of knowledge has been accumulated on *C. canephora* genetic diversity (Berthaud, 1986; Montagnon et al., 1992; Dussert et al., 1999) and quantitative genetics (Leroy et al., 1994; Montagnon, 2000). Time has come now to integrate in a comprehensive way the molecular markers in the *C. canephora* breeding program. For this, we first need a genetic map, saturated enough in order to locate QTL's. Such works are on the way in the CIRAD-IRD team (Noirot et al., 2001).

The main interest of molecular markers, in a perennial plant such as coffee tree, should stand in an improved genetic gain per time. This will be the challenge for the next years.

REFERENCES

Berthaud J. (1980). L'incompatibilité chez *Coffea canephora*: méthode de test et déterminisme génétique. Café Cacao Thé **24**: 267-274.

Berthaud J. (1986). Les ressources génétiques pour l'amélioration des caféiers africains diploïdes . Evaluation de la richesse génétique des populations sylvestres et de ses mécanismes organisateurs. Conséquences pour l'application, Paris (FRA), ORSTOM, 379 pp.

Capot J. (1977). L'amélioration du caféier Robusta en Côte d'Ivoire. Café Cacao Thé **21**: 233-244.

Charmetant P., Leroy T., Bontems S. et Delsol E. (1990). Evaluation d'hybrides de *Coffea canephora* produits en champs semenciers en Côte d'Ivoire. Café Cacao Thé **34**: 257-264.

Dussert D., Lashermes P., Anthony F., Montagnon C., Trouslot P., Combes M. C., Berthaud J., Noirot M. et Hamon S. (1999). Le caféier, *Coffea canephora*. in*:* P. Hamon, M. Seguin, X. Perrier et J. C. Glaszmann.(Eds): Diversité génétique des plantes tropicales cultivées. CIRAD, Montpellier**:** 175-194.

Leroy T., Montagnon C., Charrier A. et Eskes A. B. (1993). Reciprocal recurrent selection applied to *Coffea canephora* Pierre. I . Characterization and evaluation of breeding populations and value of intergroup hybrids. Euphytica **67**: 113-125.

Leroy T., Montagnon C., Cilas C., Charrier A. et Eskes A. B. (1994). Reciprocal recurrent selection applied to *Coffea canephora* Pierre. II. Estimation of genetic parameters. Euphytica **74**: 121-128.

Montagnon C. (2000). Optimisation des gains génétiques dans le schéma de sélection récurrente réciproque de *Coffea canephora* Pierre. Thèse de Doctorat, Ecole Nationale Supérieure Agronomique de Montpellier: 133 pp.

Montagnon C., Leroy T., Kebe I. et Eskes A. B. (1994). Importance de la rouille orangée et facteurs impliqués dans l'évaluation de la résistance au champ de *Coffea canephora* en Côte d'Ivoire. Café Cacao Thé **38**: 103-112.

Montagnon C., Leroy T. et Yapo A. B. (1992b). Etude complémentaire de la diversité génotypique et phénotypique des caféiers de l'espèce *Coffea canephora* en collection en Côte d'Ivoire. XIVe Colloque Scientifique International sur le Café, San Francisco (USA), Paris (FRA), ASIC: 444-450.

Rakotomalala J. O. E., Rakotomalala J. J. R. et Rabemiafara A. (1997). The dispersal of seeds of canephoroid coffee trees: An evil once again necessary in Madagascar. 17 eme conférence ASIC, 1997/07/20-25, Nairobi (KEN), Paris (FRA), ASIC: 332-337.

Noirot M., de Kochko A., Leroy T., Campa C., Hamon P., Dufour M., Dussert S., Louarn J. and Chrestin H. (2000). From genetic ressources towards genetics of coffee : A new approach for an old problem. 19 eme conférence ASIC, 2001/05/14-19, Trieste (ITA), Paris (FRA), ASIC: sous presse.

Roux G. et Duris D. (1995). Etude de faisabilité de relance caféière en République de Côte d'Ivoire. Première phase: diagnostic, rapport préliminaire, Courbevoie (FRA): SOCA2, 1995/03-04. - 143 p.

Utilisation des caractères architecturaux pour prédire la production chez *Coffea canephora* Pierre

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SUMMARY

For coffee, notably the species *Coffea canephora* Pierre, it takes many years of observations to estimate productivity in genetic trials. Breeders generally use yields over the first four or five years of production, corresponding to the first cycle before cutting back. However, data recorded over fourteen years indicate that first cycle yields are not always well correlated to those in the following cycles.

With a view to the early identification of traits likely to predict the production capacity of trees in trials, architectural observations were carried out in a clone trial. The observation protocols were drawn up in such a way as to constitute databases exploitable with AMAPmod, a software to analyse plant architecture. It has thus been possible to extract a large number of architectural traits and test their ability to predict cumulated yields over eight years. Some of these traits, such as the number of sterile plagiotropic stages at the tops of trees, or the density of fruiting nodes at certain stages, have provided reliable predictions of cumulated yields. Moreover, these traits provide a clearer understanding of how yields are elaborated and make it possible to differentiate between genotypes through traits that are indicative of their production capacity.

RÉSUMÉ

Chez le caféier, et notamment chez l'espèce Coffea canephora Pierre, l'estimation de la productivité dans les essais génétiques nécessite de nombreuses années d'observation. Les sélectionneurs utilisent généralement les productions des quatre ou cinq premières années de récolte, correspondant au premier cycle avant recepage. Cependant des données enregistrées sur quatorze années indiquent que les productions de ce premier cycle ne sont pas toujours bien corrélées à celles des cycles suivants.

Dans l'objectif d'identifier précocement des caractères aptes à prédire les capacités productives des arbres en essai, des observations architecturales ont été réalisés dans un essai clonal. Les protocoles d'observation ont été établis de façon à constituer des bases de données exploitables avec le logiciel AMAPmod. Il a ainsi été possible d'extraire un grand nombre de caractères architecturaux et de tester leur aptitude à prédire les cumuls de production de huit années. Certains de ces caractères, comme le nombre d'étages plagiotropes stériles au sommet des arbres ou la densité des nœuds fructifères à certains étages ont permis d'obtenir une bonne prédiction des cumuls de production. Ces caractères apportent par ailleurs une meilleure compréhension de l'élaboration des rendements et permettent de différencier les génotypes par des caractères rendant compte de leur capacité productive.

INTRODUCTION

Le caféier est une plante pérenne dont la période de production peut s'étaler sur un grand nombre d'années. A l'intérieur de cette longue période de production, qui peut atteindre une quarantaine d'années, des cycles de production de 5 à 6 ans sont rythmés par la fréquence des tailles (recépages ou étêtages). La taille des caféiers est en effet indispensable pour assurer aux arbres un volume fructifère suffisant tout au long de la vie des arbres (Coste, 1989). En effet, que ce soit des semis ou des plants issus de boutures, la production croît au cours des 3 à 4 premières années après la plantation, puis, cette production se stabilise et se met généralement à décroître au fur et à mesure de la croissance des arbres. De plus, cette diminution de la production s'accompagne par l'inaccessibilité des récoltes qui se situent au sommet des arbres. Ce schéma, plus ou moins perturbé par des alternances entre années successives, a conduit les agronomes à préconiser des rythmes de tailles. Ces tailles sont réalisées tous les 5 ans en moyenne, mais leur rythme dépend du matériel végétal utilisé, des conditions édapho-climatiques plus ou moins propices à la croissance des arbres et de l'ensemble des techniques culturales adoptées par les planteurs.

L'un des objectifs de l'amélioration génétique du caféier est d'augmenter la productivité des surfaces exploitées (Bouharmont et al., 1979; Bouharmont et al., 1986). Pour cela, il est nécessaire d'estimer la production des clones ou des hybrides en comparaison dans les essais. La valeur productive du matériel végétal devrait théoriquement être estimée par le cumul des productions obtenues sur toute la durée de vie des arbres en essai. Les sélectionneurs de matériel végétal ne vont cependant pas attendre 30 ou 40 ans avant de proposer des nouveaux clones ou de nouveaux hybrides. En effet, il paraît plus important d'optimiser les gains génétiques par unité de temps, c'est à dire les gains génétiques annuels plutôt que de vouloir connaître avec certitude la production des arbres sur un grand nombre d'années. Pour cela, il est important de connaître la relation entre les premières années de production et les années ultérieures. Par ailleurs, des caractères estimables précocement peuvent être adjoints aux productions des premières années dans l'objectif de mieux prédire la production des arbres. La production des caféiers est liée au développement architectural des arbres (De Reffye, 1979), c'est à dire qu'il existe une relation étroite entre la croissance des arbres et leur capacité productive.

Dans cette présentation, nous allons étudier les productions des cycles successifs de façon à estimer la relation entre les productions des premières années et les productions des années ultérieures. Nous allons ensuite présenter un protocole d'étude de l'architecture des caféiers dans la perspective d'identifier des caractères architecturaux aptes à prédire la productivité des arbres. Ces caractères architecturaux constituent des descripteurs de la capacité productive des arbres.

MATÉRIEL ET MÉTHODES

Relation entre les productions de cycles successifs

Les caféiers observés sont issus d'un plan de croisements de type diallèle triangulaire sans les autofécondations. Avec 6 géniteurs, le nombre de croisements réalisés est donc de 5x6/2 = 15. Les 6 géniteurs sont: les clones B30, B38 et B41, sélectionnés à la station de Boukoko en République centrafricaine, les clones J8, J26 et J32 provenant de sélections de Java. L'espèce *C. canephora* est séparée en deux groupes génétiques: les guinéens, issus de l'Afrique de l'ouest et les congolais, issus de l'Afrique centrale (Charrier et al., 1988); les clones utilisés dans cette étude sont tous issus de la population congolaise.

Les caféiers des 15 croisements ont été plantés en 1974 suivant un dispositif en 6 blocs. A l'intérieur de chacun des blocs, une randomisation des parcelles élémentaires constituées d'une ligne de 10 arbres par croisement a été effectuée. L'écartement entre les arbres est de 3 m par 3 m et les caféiers sont conduits en tiges multiples sans ombrage. Les productions par parcelle élémentaires ont été mesurées durant 14 années consécutives: durant le premier cycle de production qui a duré 6 ans, puis, après recépage, durant le second cycle qui a duré 5 ans et enfin durant les 3 premières années du troisième cycle après écimage des tiges en fin de second cycle. Les productions sont exprimées en kg de café marchand récolté. Afin de comparer les différents cycles entre eux, la production de chaque cycle est divisée par le nombre d'années pris en compte.

Les analyses diallèles sont effectuées selon le modèle de Griffing (1956) à effets fixes pour les différentes variables étudiées. Les corrélations de rangs entre les AGC (Aptitudes Générales à la Combinaison) des différents géniteurs sont également estimées pour étudier la liaison entre les différents caractères.

Protocole d'étude de l'architecture

L'architecture des plantes est une discipline relativement récente dont les premières modélisations quantitatives ont d'ailleurs été réalisées chez le caféier (De Reffye, 1979; De Reffye et al., 1990). L'organisation géométrique et topologique des entités d'une plante définit son architecture (Godin, 2000). Cette architecture se met en place au cours du temps suivant des dynamiques de croissance qui sont fonction du génome et de l'environnement des plantes (De Reffye et al., 1989).

Les observations architecturales réalisées dans cette étude ont été réalisées sur 6 clones de *Coffea canephora* Pierre installés en essai comparatif dans la station de Divo (CNRA, Côte d'Ivoire). Environ 28 arbres par clone ont été observés, soit un total de 167 caféiers. Ces caféiers étant cultivés sur 4 tiges, une tige par arbre a été échantillonnée.

Les observations architecturales des caféiers ont été définies à partir de l'expérience du laboratoire de modélisation de l'architecture des plantes (Godin, 2000) dans la perspective de constituer des bases de données aussi complète que possible.

La description de la tige échantillonnée par arbre et des branches plagiotropes prises à certains niveaux de la tige est effectuée, entre-nœud par entre-nœud. La description des tiges a pour but de détailler leur ramification et leur géométrie. La ramification est décrite par la séquence du nombre de branches par nœud en partant du sommet de la tige, jusqu'à sa base. Le premier nœud identifié au sommet correspond au premier nœud portant des ramifications. Ce repère constitue un critère homogène de synchronisation des observations. La géométrie de chaque tige est décrite par une mesure de son diamètre basal (mesuré au niveau du sol) et apical (mesuré au niveau du premier nœud fructifère) et de sa hauteur depuis le sol jusqu'au repère apical. Le diamètre apical est toujours mesuré dans la plus grande largeur de l'entre-nœud. Les caractéristiques géométriques de la tige au sommet de chaque zone sont déterminées par la mesure de la hauteur et du diamètre des nœuds de la tige aux étages 5, 15, 25 et 35.

Les étages plagiotropes des mêmes niveaux (5, 15, 25 et 35) ou les étages les plus proches, lorsque les branches sont absentes à ces étages, ont été ont été échantillonnées. Pour chaque branche (au plus 2) on note la séquence des nœuds qui constitue cette branche, avec pour chaque nœud:

- le nombre de feuilles présentes sur ce nœud (0,1,2),

- la présence ou l'absence de fleurs ou de fruits (0,1),
- la présence ou l'absence de ramification secondaire (rameaux). Pour chaque rameau, le nombre total de feuilles et de nœuds fructifères qu'il porte est noté,
- l'état de l'apex de la branche (mort ou vivant),
- leur longueur totale (en cm),
- l'azimut des branches.

Les données ont été saisies à un format compatible avec le logiciel AMAPmod, logiciel spécialisé dans l'exploration des bases de données architecturales (Godin et al., 1998, Godin et al., 1999).

RÉSULTATS

Relation entre les productions des cycles successifs

Le principal caractère à améliorer est la production cumulée des 14 années (variable "*Cum14*"); sa moyenne s'élève à 1568 kg de café marchand par hectare et par an. Les deux meilleures familles proviennent des croisements J32 x B30 et B41 x B30 avec des productions respectives de 1940 et 1930 kg/ha/an.

Une forte aptitude générale à la combinaison est détectée pour les différents caractères alors que l'aptitude spécifique est faible et n'est significative que pour la production du second cycle. Le classement des AGC (aptitudes générales à la combinaison) pour les différents cumuls de production est réalisé avec le test de comparaison multiple de Newman et Keuls (Tableau 1).

Tableau 1. Comparaisons des AGC estimées. Test de Newman et Keuls (5%)

Géniteur	Cumul 14	Cumul 1c	Cumul 2c	Cumul 3c
B30	226 a	141 a	235 a	383 a
B38	126 ab	102 a	147 a	141 b
B41	123 ab	–6 b	93 a	433 a
J32	76 b	149 a	113 a	–133 c
J8	–143 c	–177 c	–90 b	–164 c
J26	–409 d	–210 c	–497 c	–661 d

Le classement des géniteurs pour le cumul du premier cycle ne correspond pas à celui obtenu pour les 14 années de production. Les corrélations de rangs entre les effets génétiques additifs complètent ces résultats (Tableau 2). Une bonne prédiction de la production cumulée peut être obtenue par la production du 2ème cycle de production (bonne corrélation de rang). La production du premier cycle ne suffit pas pour prédire la production cumulée des 14 années. Il parait donc important d'adjoindre des variables complémentaires à la production du premier cycle pour prédire la production cumulée sur 14 années.

Constitution d'une base de données architecturale

La base de données est constituée de 167 caféiers. Une partie de la plante 81 est présentée dans le Tableau 3.

Tableau 2. Corrélations de rangs entre les AGC des différents caractères (proba)

	Cumul 14	Cumul 1c	Cumul 2c
Cumul 14	1		
Cumul 1c	0.657 (0.156)	1	
Cumul 2c	0.943 (0.005)	0.829 (0.042)	1
Cumul 3c	0.829 (0.042)	0.486 (0.329)	0.657 (0.156)

Avec: Cumul 14: moyenne annuelle du cumul des 3 cycles de production (14 années); Cumul 1c, Cumul 2c, Cumul 3c: moyennes annuelles des 3 cycles de production

Seul le premier étage orthotrope (étage 6) est présenté dans ce tableau. Dans la base de données les étages 15, 25, 37 et 45 sont également renseignés. Cette base de données représente un total de 28774 lignes; elle permet de visualiser certaines parties des plantes et d'extraire des caractères architecturaux qui pourront ensuite être utilisés comme prédicteurs de la production.

Premiers résultats sur la relation architecture-production

Les premiers résultats obtenus pour les 6 clones de l'essai clonal installé en Côte d'Ivoire indique que certains paramètres architecturaux sont génétiquement corrélés au cumul de production obtenu sur 2 cycles de production (Tableau 4).

Les nombres moyens de nœuds fructifères aux étages 5 et 15 en partant du sommet sont les caractères corrélés aux cumuls de production. Le nombre d'étages de portant pas de branches fructifères est également corrélés au cumul de production.

D'autres caractères doivent être extraits de la base de données afin de tester leurs capacités prédictrices. Il est à noter que la hauteur et le diamètre des tiges sont plutôt liés aux cumuls de production d'un point de vue environnemental (corrélations environnementales, correspondants au niveau arbre à l'intérieur des clones).

CONCLUSION

La production du premier cycle, enregistrée sur 6 ans, ne suffit pas pour obtenir un classement fiable des géniteurs pour la production à plus long terme. Il serait donc nécessaire de suivre d'autres plans de croisements sur des durées assez longues pour comprendre les modifications de classement observées sur des cycles consécutifs de production.

Tableau 3. Echantillon de la plante 81 dans la base de données architecturale

Code		Nb Rameaux	Nb Feuilles	Nb Nœuds Fructifères	Etat	Longueur	Diamètre	Clone	Ligne	Arbre
/P81								119	222	8
	/A1						38			
	^/E46									
	^<E45	1			R					
	^<E44									
	^<E43									
	^<E42									
	^<E41	1			R					
	^<E40									
	^<E39	1								
	^<E38									
	^<E37	2			R	93	22			
	^<E36	1								
	^<E35									
	^<E34	2								
	^<E33	1			R					
	^<E32									
	^<E31	1			R					
	^<E30	1			R					
	^<E29	1			R					
	^<E28									
	^<E27	1								
	^<E26	1			R					
	^<E25	2				122	16			
	^<E24	1			R					
	^<E23	2								
	^<E22	2								
	.									
	.									
	^<E7	2								
	^<E6	2				192	8			
						21	5			
			2	1						
			2	1						
			2	1						
			2							
			6							
						21	4			
			1	1						
			2	1						
			2							
			2							
			6							
	^<E5									
	^<E4	2								
	^<E3	2			S					
	^<E2	1			S					
	^<E1	2			S					
	^<E0		10			197	6			

Tableau 4. Corrélations génétiques (environnementales) entre les caractères architecturaux et les rendements sur différents périodes

	Cum1c	Cum2c	Cum1_8	Cum97
Haut	–0.577 ns (0.203)	–0.009 ns (0.402)	–0.302 ns (0.341)	–0.039 ns (0.200)
Diam	–0.273 ns (0.267)	–0.242 ns (0.360)	–0.301 ns 0.359	–0.283 ns (0.200)
Nben	–0.302 ns (0.224)	0.038 ns (0.384)	–0.130 ns (0.344)	0.025 ns (0.225)
Nbenst	–0.713 ns –0.140	–0.063 ns (–0.113)	–0.405 ns (- 0.145)	–0.178 ns (–0.173)
Nbenet5	–0.833* 0.153	–0.243 ns 0.203	–0.583 ns 0.202	–0.420 ns 0.255
Nbfeui5	–0.141 ns 0.176	0.306 ns (0.208)	0.128 ns 0.218	0.076 ns 0.283
Nbfrui5	**0.998* (0.209)**	0.261 ns (0.186)	0.680 ns 0.226	0.333 ns (0.232)
Long5	–0.213 ns (0.104)	0.701 ns (0.204)	0.349 ns (0.174)	0.606 ns (0.205)
Nbenet15	0.154 ns (0.107)	0.166 ns (0.157)	0.188 ns 0.150	0.017 ns 0.156
Nbfeui15	–0.640 ns (0.098)	–0.184 ns (0.137)	–0.449 ns (0.134)	–0.351 ns (0.152)
Nbfrui15	0.759 ns (0.142)	0.742 ns (0.154)	**0.875 * (0.168)**	0.655 ns (0.201)
Long15	0.265 ns (–0.003)	0.732 ns (0.081)	0.613 ns (0.043)	0.636 ns (0.103)
Nbenet25	–0.278 ns (0.129)	–0.154 ns (0.191)	–0.243 ns (0.181)	–0.301 ns (0.181)
Nbfeui25	–0.723 ns (0.101)	–0.647 ns (0.167)	–0.796 ns (0.152)	–0.792 ns (0.175)
Nbfrui25	0.092 ns (0.145)	0.607 ns (0.255)	0.444 ns (0.226)	0.487 ns (0.227)
Long25	–0.116 ns (0.123)	0.270 ns (0.195)	0.117 ns (0.180)	0.157 ns (0.197)

Avec Haut: hauteur de la tige échantillonné
Diam: diamètre basal de la tige
Nben: nombre d'entre-nœuds (EN) de la tige
Nbenst: nombre d'EN portant des branches stériles
Nbenet5: nombre d'EN moyen à l'étage 5 (moyenne des 2 branches)
Nbfeui5: nombre d'EN moyen portant des feuilles à l'étage 5
Nbfrui5: nombre d'EN moyen portant des fruits à l'étage 5
Long5: longueur moyenne des branches de l'étage 5
Cum1c: Cumul de production par arbre des 4 premières années de récolte (1er cycle)
Cum 2c: Cumul de production du 2ème cycle
Cum1_8: Cumul de production des 8 premières années de récolte
Cum97: Production de l'année 1997 (année au cours de laquelle ont eu lieu les observations architecturales)

Une autre approche a consisté à mesurer le développement architectural de caféiers issus d'un essai clonal. Une base de données exploitable sous le logiciel AMAPmod a été créée. Cette base de données permet d'extraire certains paramètres architecturaux dont la capacité à prédire le cumul de production est testée. Parmi les quelques caractères testés, seuls les nombres moyens de nœuds fructifères aux étages 5 et 15 en partant du sommet de l'arbre présentent des corrélations génétiques significatives avec les cumuls de production. D'autres caractères doivent prochainement être extraits et testés, et des représentations graphiques seront réalisées pour visualiser certains paramètres caractéristiques des clones (Godin and Costes, 1996). D'autres approches consisteront à quantifier le volume fructifère de chaque arbre, à étudier la densité des nœuds fructifères en fruits et à étudier les rapports feuilles/fruits aux différents niveaux des arbres. Les caractères physiques du bois, et notamment le module d'élasticité doit également être inclus dans ces analyses (Cilas et al., 2000).

BIBLIOGRAPHIE

Bouharmont P., Awemo J. (1979) La sélection végétative du caféier Robusta au Cameroun. 1ère Partie : Programme de sélection. Café Cacao Thé*,* **23**, 227-254.

Bouharmont P., Lotodé R., Awemo J., Castaing X. (1986) La sélection générative du caféier Robusta au Cameroun. Analyse des résultats d'un essai d'hybrides diallèle partiel implanté en 1973. Café Cacao Thé, **30**, 93-112.

Charrier A., Berthaud J. (1988) Principles and methods in coffee plant breeding: *Coffea canephora* Pierre. In: Coffee, Ed. R. J. Clarke & R. Macrae, vol. **4**, Agronomy, 167-197.

Cilas C, Montagnon C, Bertrand B, Godin C (2000) Wood elasticity of several *Coffea canephora* Pierre clones. A new trait to included in selection schemes,. Agronomie **20**: 439-444.

Coste R.(1989) Caféiers et cafés. Ed. Maisonneuve et Larose. Paris, France, 373 p.

De Reffye Ph. (1979) Modélisation de l'architecture des arbres par des processus stochastiques. Simulation spatiale des modèles tropicaux sous l'effet de la pesanteur. Application au *Coffea robusta*. Thesis, Université de Paris-Sud, 196 p.

De Reffye Ph., Edelin C., Jaeger M. (1989) Modelli di crescita delle piante. Scienza e Tecnica. Annuario della Estratti: 205-216

De Reffye Ph., Snoek J., Jaeger M. (1990) Modélisation et simulation de la croissance et de l'architecture du caféier. In: 13th International Scientific Colloquium on Coffee, ASIC, Columbia, pp 523-546.

Godin C. (2000) Representing and encoding plant architecture: a review. Annals of Forest Science **57**: 413-438.

Godin C., Costes E. (1996) How to get representations of real plants in computers for exploring their botanical organization. Acta Horticulturae: 45-53.

Godin C., Costes E., Caraglio Y. (1998) Exploring plant topological structure with the AMAPmod software: an outline. Silva Fennica **31**: 355-366.

Godin C., Guédon Y., Costes E. (1999) Exploration d'une base de données architecturales à l'aide du logiciel AMAPmod: application à une famille d'hybrides pommiers. Agronomie **19**: 163-184.

Griffing B. (1956) Concept of general and specific combining ability in relation to diallel crossing systems. Anal. J. Biol. Sci, (9), p. 463-469.

Breeding for Speciality Coffee Markets: What Options Do the Coffee Breeders Have?

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SUMMARY

The dominance of Arabica coffee in the world coffee market is the result of superior cup quality of the *Coffea arabica* L. species when compared to the other species. In deed, the quantity of coffee consumed depend on the pleasure derived from consuming the beverage. Over time, the world coffee market has somewhat been stratified into market segments on the basis of quality; with various producer countries identifying with one of the segments. Recently however, a more specialised segment has been emerging under the buzz name "speciality" or gourmet coffee. The emphasis of the gourmet market is cup quality. The coffee breeders, on the other hand, have put a lot of efforts towards introgressing resistance to major diseases into commercial varieties while maintaining the high yields and good quality of such traditional varieties. The Colombian variety and cultivar Ruiru 11 are examples of results of such efforts.

Over and gain, doubts have been raised from the speciality cycles as to the suitability of newly bred coffees for the gourmet markets. This is despite the fact that such varieties have been developed under close collaboration with professional liquorers within the coffee trade who usually assess the research samples for quality characterisation. The question which arises is whether the time old quality criteria used by the veterans of organoleptic assessment of coffee quality are still relevant under the speciality coffee arena or whether new quality criteria have since emerged. From the breeding point of view, it would also be interesting to know whether the new quality criteria are quantifiable and to understand their sensitivity limits. For example, is the "Black currant taste" quantifiable, does it have a genetic basis or is it easily camouflaged by non genetic factors such as climate, agronomy and processing. In this paper, attempt has been made to delineate the response path which could be adopted by the coffee breeders in attempting to develop, disease resistant, high yielding coffee varieties whose bean and liquor qualities are tailor made to for the speciality coffee markets. The responsibility of the speciality coffee dealers in development of such varieties and the possible yield quality, resistance and environmental trade-offs likely to be incurred are also highlighted and discussed.

INTRODUCTION

Coffee is undoubtedly the most important Agricultural commodity in the world trade, commanding a turn-over of US$ 10 billion annually (Graaf, 1986; Saitoto, 1997 Rice and McLean, 1999). Its production is fundamental to over 50 developing Nations to which it is frequently the main source of foreign currency earnings (Kushalapaa, 1989). Coffee is also a major source of employment to the rural population in the producer Nations, thereby making it an important avenue for the alleviation of poverty in these countries.

World production of coffee relies mainly on three species; *Coffea arabica C. Canephora* and *C. Liberia* (Carvalho and Monaco, 1969). Due to quality considerations however, Arabica coffee has predominated the world trade, accounting for about 70% of the total coffee traded annually (Van der Vossen, 1985; Rice and McLean, 1999). Nevertheless, the exclusive production of

Arabica coffee is limited by a number of factors. For example, Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* is a major constraint to Arabica coffee production in both the high and low altitude coffee producing areas in Africa. Coffee Leaf Rust (*Hemelleia vastatrix*), moisture stress and temperature fluctuations, on the other hand, limits the production of the species, particularly in the low altitude zones. In addition, other considerations such as the low percentage of soluble solids characteristic of the species makes it less suitable as a commodity for certain sectors of the coffee market such as the instant coffee markets.

Typically, the world coffee business involve three main stakeholders, namely, the producers (small or large scale), the exporter/importer and the consumer. The producers main objectives are to maximise profits and to stabilise income. Consequently their interest include increased and stable productivity, high price realisation and cost minimisation. The exporter/importers, on the other, aims at maximising profits and are thus mainly concerned with having access to reliable and consistent supply of a variety of high quality green beans. For the consumer, cup quality, affordability and human and environmental health considerations are the key issues determining their consumption patterns.

In attempt to resolve some of the problems related to disease resistance and in recognition of the requirements of the various stakeholders, a number of breeding programmes have been put in place in various countries world wide. The main objectives of the programmes include yield improvement through compact growth, resistance to the major diseases and improved bean and liquor qualities. Considerable success has been realised (Carvalho, 1985; Van der Vossen, 1985; Agwanda and Owuor, 1989); with disease resistant varieties being released in countries like Kenya and Colombia (Castillo and Moreno, 1986). While the improved varieties were initially seen as a breakthrough towards ensuring a sustainable and economic production of Arabica coffee, their suitability for the top-class (speciality) coffee markets became a subject of scrutiny during the early 90's. A number of exporters/importers, specially those dealing in speciality coffees raised concerns to the effect that the quality of the new breeds were yet to be as good as those of the traditional varieties they were meant to replace. This paper examines the probable reasons for these importer concerns and the possible solutions which could be taken by the breeders to ensure that future varieties closely meet the expectations of the gourmet markets.

COFFEE BREEDING AND SELECTION

Until early 60's, high yields, good quality and adaptability were the main pre-occupation of most coffee selection programmes. A number of varieties including Caturra, Mundo Novo and Catuai in Brazil; Typica in Colombia and Central American countries, SL 28 and SL 34 in Kenya and N39 in Tanzania resulted from these efforts and have since formed the backbone of coffee production world wide. With the advent of diseases such as Coffee Leaf Rust (Berkeley, 1969) and Coffee Berry Disease (McDonald, 1906), disease resistance became a major breeding goal. Consequently, a number of breeding programmes shifted their attention towards incorporating disease resistance and compact growth into the commercial varieties. Tremendous progress has been made and has been the subject of many reviews (Graaff, 1981; Van der Vossen, 1985; Agwanda and Owuor, 1989; Carvalho and Monaco, 1989; Echevezzi and Fernandes, 1989; Balharmont, 1995). In Kenya, for example, a CBD and Leaf Rust resistant varieties, cultivar Ruiru 11, was released to the farmers for commercial exploitation in 1985. Around the same period, the Colombian programme resulted into the release of variety Colombia, a compact and leaf rust resistant cultivar.

During the early 90's however, some importers, particularly those dealing in speciality coffees raised concerns regarding the quality of newly bred varieties. Breeders, on the other hand, maintain that the quality of the disease resistant varieties are as good as those of the disease

susceptible traditional varieties. Indeed, published data based on quality evaluation by a variety of professional liquorers reveals no significant differences between the traditional varieties and the disease resistant cultivars hence supporting the breeders' claims (Owuor, 1988; Njoroge et al., 1995).

A number of reasons could be put forward to explain the two stand points. Firstly, it s possible that the liquor quality criteria used by the Breeders does not adequately cover the range of quality profiles which are of significance to the speciality coffee Roasters. Quality evaluations for Research purposes is based on sensorial analysis of the key liquor quality indicators, namely Acidity, Body and Flavour (Devonshire, 1956). Defects related to processing, insect damage, and faulty agronomic practices are also considered in the approach. However, no direct evaluation of other biochemical components such as levels of caffeine, chlorogenic acids, carbohydrates etc. which could have adverse effects on the fineness of the liquor quality is normally carried out. Since most of the resistance genes exploited in the main breeding programmes originate from germplasm of fairly different genetic backgrounds from those of the traditional varieties, it may be necessary to extend the evaluation criteria used in coffee breeding programmes to include the biochemical traits whose presence or absence may have negative effects on the cup quality of a given variety. This would be in line with the observation by Charrier, (1982) who recognised the important part played by these traits and the variability which exist amongst varieties for the bio-chemical characters. Cases where undesirable quality traits passed from parent to offspring have also been reported by Walyaro, (1983).

To effectively incorporate the bio-chemical trait into the selection work, it is necessary to establish the relationship between the major biochemical traits such as caffeine content, chlorogenic acids total carbohydrates and potassium with the final quality. Depending on the findings, breeding programmes could be reorganised to accommodate such traits and to ensure that the offending biochemical traits are eliminated through negative selection whereas the intensities of those which add value to the cup quality increased.

By its mature, organoleptic evaluation of liquor quality is highly subjective. Sampling techniques and storing methods used by workers in this area also vary (Charrier,1982). Consequently, quality evaluation results of a given green coffee sample may vary from panel to panel depending on a number of factors including the average level of training of the panellists, the types of coffees they deal in and the requirements of their target market. This means that what is considered as superior by dealers of the mainstream top quality coffee markets may not necessarily be rated similarly by buyers of speciality coffees. The lack of consistency in rating between liquorers is thus a major handicap to the breeders since it necessitates the use of results from a large number of liquorers before any meaningful conclusions can be reached. This is not only time consuming but is also expensive and frequently give results which have no direct relationship with one another and hence not useful for selection purposes (Table 1).

One way to overcoming the problems related to the subjective nature of the organoleptic procedures could be to develop common standards for sampling, preparation, tasting and scoring. Nevertheless, such standards may not significantly contribute to the development of varieties of superior qualities unless meaningful partnerships are developed between the breeders and the trade.

These partnership would ensure active participation by the buyers at all levels of breeding and selection activities and could take one or more of the following forms:

- Development of complementary criteria for determining liquor quality.
- Establishment of dependable liquoring panels.

- Infrastructure and capacity development.
- Comprehensive evaluation of existing germplasm for superior quality traits.

Table 1. Liquor quality results for Cultivar Ruiru 11 as assessed by four panels of liquorers

Code no.	Liquorer	Liquor quality results			
		Acid	Body	Flavour	Overall std
1	1	1.00	1.00	2.00	2.00
	2	2.00	2.00	5.33	5.00
	3	4.00	2.00	4.00	3.66
	4	2.00	2.00	3.00	3.00
22	1	1.00	1.00	3.00	2.00
	2	2.00	2.00	5.33	5.00
	3	2.00	2.00	3.66	3.66
	4	2.00	2.00	3.00	3.00
57	1	2.00	2.00	4.00	3.33
	2	2.00	1.00	5.33	5.00
	3	2.00	3.00	4.00	4.00
	4	2.00	2.00	3.00	3.00
143	1	4.00	4.00	6.00	6.00
	2	2.00	1.00	5.33	4.00
	3	3.00	3.00	4.00	4.00
	4	1.00	1.00	2.00	2.00

The services of Tylor Winch (K), KPCU, JENEM coffee & COSMOS companies in evaluating the liquor quality of the samples is acknowledged with thanks

DEVELOPMENT OF COMPLEMENTARY ASSESSMENT CRITERIA

Although organoleptic procedures will continue to be the backbone of liquor quality evaluation for the foreseeable feature (Gopel, 1997), their utility in breeding for improved quality decreases as homogeneity within the breeding population is approached. Further progress could however be expected if biochemical techniques and electronic approaches are integrated at such stages to detect finer differences between the breeding lines and the traditional cultivars. A lot of work has already been done in attempting to understand the biochemical composition of green and roasted coffee beans and to associate such chemicals with the cup quality (Hamphrey and Macrae, 1986; Cohem, 1993). The link between such studies and the genetic improvement of quality is however lacking due to reasons ranging from lack of facilities and trained man power to lack of interest in the part of the breeders in such studies.

Although still not completely developed, the use of electronic sniffing (Bartlet et al., 1993) could also contribute considerably in improving the aroma constitution of varieties through breeding. Their advantage over the human nose include consistency in rating and presentation of data on standard formats. Nevertheless, as pointed out by (Gopel, 1997), no amount of improvements in the use of electronic nose will rival the use of human nose in determining the final quality of coffee brew for human consumption. Consequently the use of electronic noses should only be developed as supplementary methods to the existing sensorial procedures.

The main draw backs of the organoleptic procedures include low heritability, how variability and heavy dependence on other factors such as the environment and processing. One method which could be used to overcome these constraints is the use of marker assisted selection. Progress has been made in the area of genetic diversity (Lashermes et al., 1996c; Orozco Castillo, 1993) and

disease resistance (Agwanda et al., 1997). Work targeting Arabica coffee quality is however scarce. To ensure rapid development in the area of quality, it is therefore necessary to focus equal attention on quality.

Assisted selection could be used for example, in selecting against the genetic background of the donor parents (Melchinger, 1990; Michelmore, 1995) or in developing markers related to the major cup quality traits. This approach should be given greater attention by molecular genetics if faster progress is to be realised.

ESTABLISHMENT OF PANELS OF LIQUORERS

The ultimate judge of liquor quality is the consumer. Results obtained from panels of liquorers may nevertheless closely reflect the feelings of the market. Given the diversity which exist within the market place, it would be difficult for any given panel to adequately assess the superiority of a given quality sample. To overcome this set-back, it may be important to have a jury comprised of dealers representing the major market sectors. Such panellists could be identified through various trade associations and should be composed of those who are willing to collaborate with the breeders in the struggle to develop improved varieties freely and in a confidential manner.

INFRASTRUCTURE AND CAPACITY DEVELOPMENT

Inadequate financing is one of the major constraints to research in most of the producer nations. Lack of analytical equipment and specialised manpower is therefore common in the coffee breeding programmes around the world. In the past, the problem of manpower and infrastructure development has been solely the burden of the producers. Participation by the importers in this area has been minimal. It is however desirable that the buyers of quality take more active role in these areas by providing training opportunities and access to specialised equipment.

GERMPLASM EVALUATION

So far, the quality attributes present in the old varieties are considered as the standards for determining the value of improved varieties for commercial use. No deliberate attempt is being made to identify unique quality traits which could be useful either presently or in future. This is indeed a dangerous situation since it is not identified and protected by public institutions, unique quality traits run the risk of being identified and patented by private institutions thereby making them unavailable to most breeders.

ACKNOWLEDGEMENTS

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REFERENCES

Agwanda C.O., Lashermes P., Trouslot P., Combes M-C. and Charrier A., 1997. Identification of RAPD markers for resistance to coffee berry diseasee, Colletotrichum kahawae in Arabica coffee. Euphytica **97**: 241-248

Bertlett P.N., Blair N. and Gardner J.W., 1993. Electronic noses. Principles, applications and outlook. In: 15th Int. Scient. Coll. On coffee, Montpellier, pp. 616-625. ASIC, Paris.

Carvalho A. and Monaco L.C., 1969. The breeding of arabica coffee. In: Ferwerda F. P. and Wit F. (Eds.), Outlines of perennial crop breeding in the tropics. Misc. Paper 4. Landbouwh. Wageningen pp. 198-216.

Charrier A., 1982. Quelques réflexions sur les possibilités d'amélioration génétique de la qualité des cafés. In: 10th Int. Scient. Coll. On coffee, Salvador, pp. 369-374. ASIC, Paris.

Cohen G., 1993. The analysis of sulfur compounds in coffee aroma by sulfur chemiluminescence detection/gass chromotography. In: 15th Int. Scient. Coll. On coffee, Montpellier, pp. 528-536. ASIC, Paris.

Devonshire C.R., 1956. Explanation of the coffee report form. Coffee Board of Kenya Monthly Bull. **21**: 186-187.

Gopel W., 1997. Electronic noses: State of the art and the science. In: 17th Int. Scient. Coll. On coffee, Nairobi, in press. ASIC, Paris.

Graaf de J., 1986. The economics of coffee. In: Economics of crops in developing countries, No. **1**, Pudoc, Wageningen, Netherlands, 294 p.

Kushalappa A.C., 1989. Importance. In: A.C. Kshalappa & A.B. Eskes (Eds.), Coffee rust: epidemiology, resistance and management. CRC press, Inc., Boca Raton, Florida, pp. 2-11.

Lashermes P., Trouslot P., Anthony F., Combes M-C. and Charrier A., 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. Euphytica **87**: 59-64.

Melchinger A.E., 1990. Use of molecular markers in breeding for oligogenic disease resistance. Plant Breeding **104**: 1-19.

Michelmore R.W., 1995. Molecular approach to manipulation of disease resistance genes. Ann. Rev. Phytopathol. **15**: 393-427.

Orozco-Castillo C., Chalmers K.J., Waugh R. and Powell W., 1994. Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. Theor. Appl. Genet. **87**: 934-940.

Coffee Breeding Assisted by Somaclonal Variation: Case of 'Bourbon LC' Variety

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SUMMARY

Somaclonal variation is the expression of the naturally occurring variability of plant cells, or the result of *in vitro* induced variation of cells following plant regeneration. Somaclonal variation is an excellent method for shortening breeding programs, since it can provide access to genetic variability within existing cultivars.

With the availability of alternative sources of natural variability and a strong consumer demand for quality coffee, attention has being directed to breed for new coffee varieties with enhanced aroma and taste. Green coffee quality is determined by *Genetics, Environment* and *Processing* and so, there is an opportunity to develop new varieties with superior cup quality traits.

Laurina is a natural mutation from Red Bourbon plants found in Reunion Island by mid-1800's and cultivated with success due to its drought tolerance and superior beverage properties. Laurina plants have small leaves, thin lateral branches, short-stature, elongated fruits and beans and it has being referred in the literature as "Laurina", "Leroy", "Bourbon Pointu" and "Smyrna" coffee.

A total of 800 Laurina somaclones were raised to maturity under normal coffee growing conditions in Brazil and superior plants were selected based on vigor, yield, and reduced caffeine content. Second and third generations of selected Laurina somaclone lines were evaluated in experimental plots in different coffee farms in Brazil leading to the scale-up of a new coffee variety called 'Bourbon LC'. Bourbon LC has a mild/sweet beverage type and it has a 50% natural reduced caffeine level (0.6% vs. 1.2%). Bourbon LC is a new coffee variety that resulted from natural somatic variation of *C. arabica* cv. Laurina, isolated from a Somaclonal Variation program, followed by standard breeding methods.

INTRODUCTION

Variability and interspecific hybrids

Coffea arabica is the only tetraploid and self-pollinated species in the genus *Coffea*. All other species of *Coffea* are diploid and out crossed (Carvalho et al., 1969; Medina Filho et al., 1984). This genetic isolation has hindered the utilization in Arabica breeding programs of natural sources of variability existing in the genus *Coffea* like morphological traits, resistance to diseases and insects, ripening time, caffeine content and many other characteristics. A few Arabica improvement programs have relied on spontaneous or artificial doubling of *C. canephora* for synthesizing interspecific hybrids with *C. arabica* leading to the release of new varieties: 'Icatu' 'Arabusta' and 'Catimor' (Carvalho, 1988; Fazuoli, 1991). Other interspecific crosses were made by A. Carvalho in Brazil including *C.canephora x*

C.eugenioides, C. racemosa x C. arabica, *C.arabica* x *C.dewevrei* dp (Carvalho and Monaco 1967; Medina 1963; Guerreiro Filho et al., 1991).

Somaclonal Variation

Variation among plants regenerated from *in vitro* cultures was first described from tobacco callus cultures by Butenko et al. (1967). However, this *in vitro* variability was not clearly recognized and defined until the review made by Larkin and Scowcroft (1981). Somaclonal variants can appear when an explant (any plant part) is subjected to a tissue culture cycle. This cycle includes establishment of a dedifferentiated cell or tissue culture under defined conditions and the subsequent regeneration of plants (Harmmerschlag, 1992). This phenomenon was further defined to include *in vitro* variability from cultivated haploid cells and named "Gametoclonal Variation" (Evans et al., 1984). Somaclonal variation is the expression of the naturally occurring variability of plant cells, or the result of *in vitro* induced variability of cells following plant regeneration (Larkin and Scowcroft, 1981; Evans and Sharp, 1986). Most of this spontaneous variability from *in vitro* plants is associated with chromosome alterations like breakage, translocation, deletions, aneuploidy, polyploidy and somatic crossing-over. In addition, somaclonal variation can also have a single gene origin, e.g. point mutation, alteration in gene copy number, activation of transposon elements and variation in DNA methylation (Karp et al., 1982; McCoy et al., 1982; Orton, 1983; Phillips et al., 1990).

Somaclonal variation is an excellent method for shortening breeding programs, since it can provide access to genetic variability within existing cultivars (Evans and Sharp 1986). Somaclones carry only a few genetic alterations and so, the overall genetic integrity of the original commercial cultivar is preserved. Somaclonal variation has contributed to the release of improved varieties of sexually (tobacco, tomato, rapeseed, corn, blackberry, celery, coffee) and non-sexually propagated species (potato, sweet potato, sugarcane) (Evans, 1988; Hammerschlag, 1992; Sondahl and Lauritis, 1992).

With the availability of other sources of variability for breeding and a strong consumer demand for cup quality coffee, attention has being directed to breed for new coffee varieties with enhanced aroma and taste. Green coffee quality is determined by *Genetics, Environment* and *Processing*. The aim is to develop new varieties with superior cup quality and agronomic traits.

Laurina is a natural mutation from Red Bourbon plants found in Reunion Island by mid-1800's. These mutated plants had small leaves, thin lateral branches, short-stature, elongated fruits and beans and it has being referred in the literature as "Laurina", "Leroy", "Bourbon Pointu" and "Smyrna" coffee (Raoul, 1897; Boutilly, 1900; Coste, 1955). This Laurina sport was immediately introduced into commercial plantations in Africa (and transferred to South America) because its drought tolerance and superior beverage properties (Raoul 1897; Krug et al., 1954). It was not until much later (mid-1950's) that studies of coffee collections reported that Laurina plants had a natural 50% reduced caffeine (Lopes, 1971). More recently, Bauman et al. (1998) explained that the reduction in caffeine content in Laurina is due to a reduced synthetic activity, most likely at the level of the 2nd and 3rd methylation steps.

This paper reports the development of an Arabica variety – Bourbon LC, which has a mild/sweet beverage characteristics and 50% natural reduced caffeine. Bourbon LC is a coffee variety of *C. arabica* cv. Laurina, isolated from a Somaclonal Variation program, followed by standard breeding methods.

MATERIAL AND METHODS

Plant material and growing conditions

Tissue culture was initiated from mature leaf explants of *C. arabica* cv Laurina following the Sondahl and Sharp (1977) protocol, using donor plants maintained in a greenhouse collection in Cinnaminson, New Jersey, USA. Donor plants were produced from seeds collected at different germplasm collections: Institute of Agronomy (Campinas, Brazil), CATIE (Turrialba, Costa Rica) and former Mexican Institute of Coffee (Xalapa, Mexico). Plantlets were recovered from both the "low-frequency pathway (HFSE)" and "high-frequency pathway (LFSE)". Plantlets were hardened in greenhouse conditions in the USA and then, transferred to a coffee nursery in Brazil, with the assistance of the Quarantine Service in Brasilia (Embrapa, Cenargen). *In vitro* derived plants were transferred to experimental fields as they reach transplanting size. Due to their somatic origin, the resulting plants were called "somaclones".

A total of 800 Laurina somaclones were raised to maturity under normal coffee growing conditions. Plants were established under field conditions in 1989, using a spacing design to facilitate single plant selection: 3.5 x 2.0 m spacing; 1,429 plants/ha; one plant per hill. The Experimental field was located at 21° latitude South, 1040 m altitude with 3-6% declivity. Seed-derived plants of *C. arabica* cv. Laurina were also introduced in the experimental fields, next to Laurina Somaclones, as "Control Plants". Normal coffee fertilization and disease-control practices were used in the experimental somaclone field.

Single plant selections of S_o somaclones begun at the time of first flowering. From individual somaclones, ripe cherries were harvest and parchment coffee prepared for sensory and chemical evaluation. Seeds of the selected plants were used to establish S_1 and S_2 generations. The progeny of each selected S_o somaclone were carried forward in the breeding program by the pure line selection method.

A second Experimental Area was established in another coffee farm using progenies of selected individual Laurina somaclones in 1992. Seeds of selected plants were used to evaluate the performance of each somaclone line in replicated blocks. A total of 360 plants per line were established, using nine random replicated blocks for the somaclone lines plus one block for the Control Plants (seed-derived plants from donor Laurina). This field was planted at 3.5 x 1.5m spacing, 1905 hills/ha, with two plants per hill (3810 plants/ha), at 21° latitude South, at 1,000m altitude. Planting was completed in March/92. Each replicated block (3,150 m^2) was represented by 40 hills (80 S_1 plants of each line) and a total of 15 lines were established per replicated block. The Control block was represented by a total of 440 hills (880 plants; 2,310 m^2). The replicated blocks were isolated by at least two-rows of border plants in a total of 3,117 hills. Growth pattern, yield, sensory analysis and caffeine content were monitored for each of these 15 Laurina somaclone lines during first 5 years under field conditions (three successive crops). Thereafter, just yield performance of best somaclone lines continue to be monitored. The selection process of somaclone lines and their progenies leading to seed production and scaling-up is illustrated in Figure 1.

Sensorial and Chemical analyses

After removal of the parchment, coffee beans from each somaclone line (R_o, R_1, and R_2 generations) were submitted to controlled roasting for sensory evaluation, according with the standard procedures described by Petracco (1997). Green coffee samples were submitted to

chemical analysis according with the standard procedures for each compound (Illy and Viani 1995).

Agronomic evaluations

The somaclones and their progenies were evaluated under normal coffee field conditions at the R_o, R_1 and R_2 generations. Any deviation from the phenotype of the original donor material was classified as a "variant". Seeds of the most interesting variant types were used to study the subsequent generations.

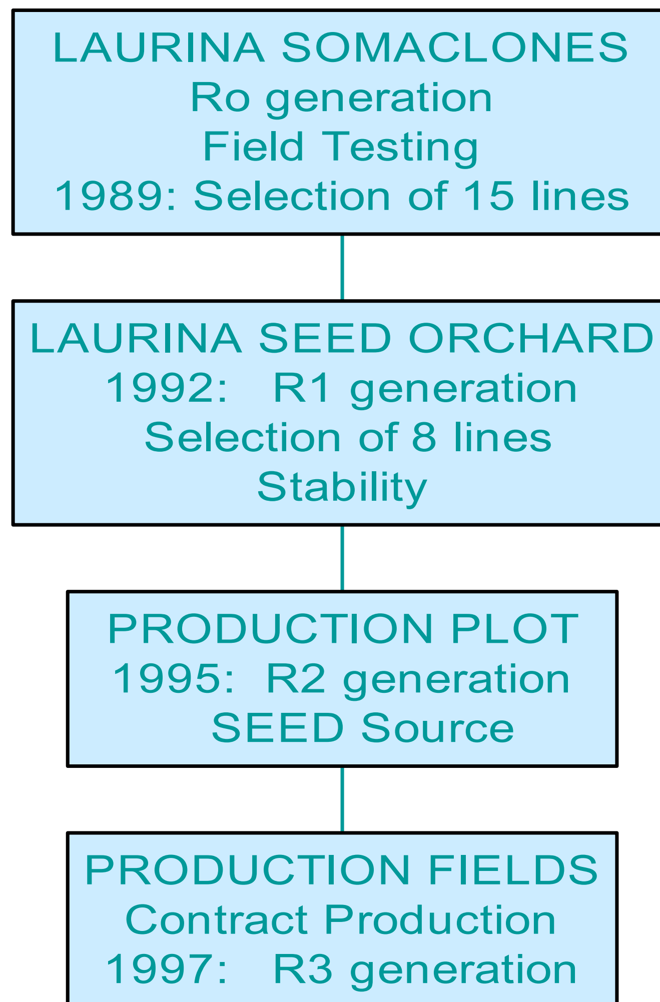

Figure 1. Selection Process of Laurina Somaclone Lines and Seed Production. A total time of 5 years since the initial field selection (1992) to beginning of commercial fields (1995). Total Coffee Improvement Program time equal to 10 years, since the establishment of leaf cultures (1987) for somaclone production to the establishment of production fields (1997)

RESULTS

Among more than 800 *in vitro*-derived plants of Laurina, 15 elite plants were selected from Laurina Somaclone experimental fields at Cajuru Farm, at the R_o generation, in June 1991. These selected plants were clearly more vigorous than sister plants and donor controls as demonstrated by greater leaf area, lateral branches, plant height, plant diameter and superior yield.

At the 2nd experimental field with R_1 plants, it was observed that caffeine content was stable and equal to the donor plants. Within each progeny line, vegetative growth pattern was uniform and stable (no segregation). Total yield from selected somaclone lines was twice as high than the Control plants. Yield evaluation of selected lines was carried in the 2nd experimental field with R1 plants up to the 6th harvest (1994-99 crops; Figure 2).

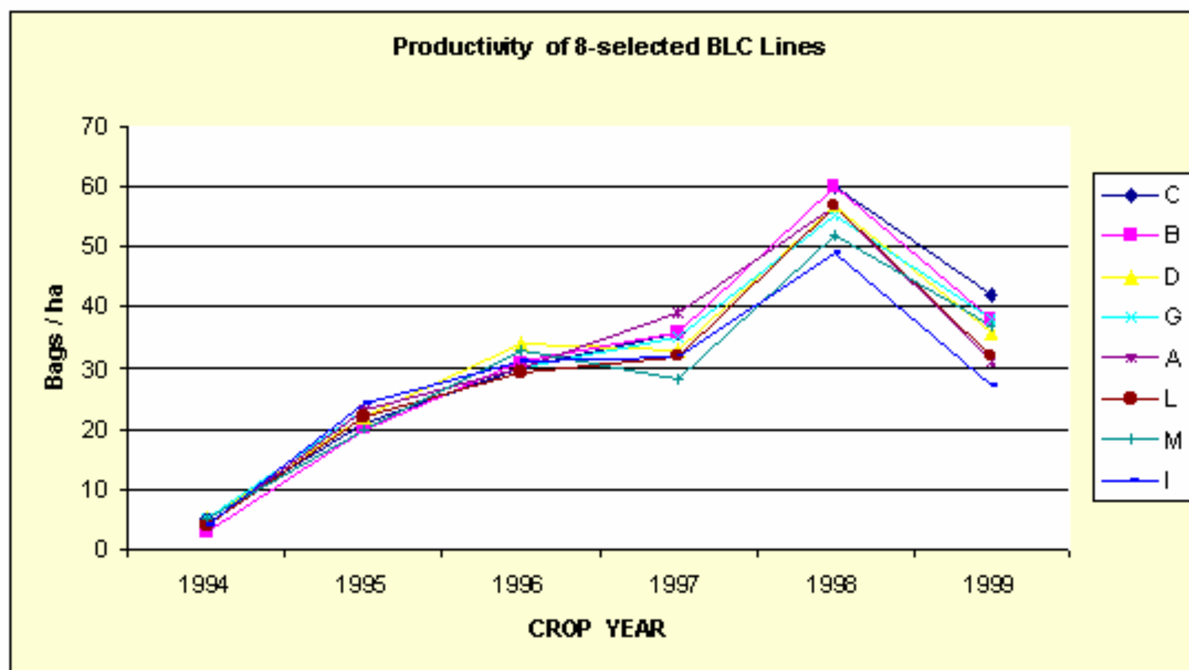

Figure 2. Comparative Yield of top 8-Selected Lines of Laurina Somaclones at R1 generation during six consecutive harvests. Data in 60 kg bags

The average yield data of selected lines for six consecutive crops was equal to 30 bags/ha (1.8 ton/ha), which confirmed the superiority of the single plant selection made at Ro level. This yield value is still about 30% inferior to high-yielding varieties like Catuai and Mundo Novo, growing at the same conditions. The yield pattern of selected somaclone lines was different than high-yielding Arabica varieties: biannual cycle begun at the 5th harvest in contrast with the normal pattern at the 3rd harvest (Figure 3). This fact can be explained by the initial reduced vegetative growth and lower yielding for these Laurina selected somaclone lines.

Third generation of selected Laurina somaclone lines was established in another coffee farm in Brazil, at 19° Latitude South and 1200 m altitude. Now, a semi-commercial plot design of 25 ha in size was established, using a spacing of 3.5 x 0.5 m with one plant/hill (total of 5,714 plants/ha). Seeds from top 8 high-yielding Laurina somaclone lines are being bulked for establishing commercial plantations under the name of '**Bourbon LC<**'.

At the time the first round of selection of elite Laurina somaclones was completed, filings for patent protection were made. An USA utility patent was awarded in July 25, 1995 (Sondahl et al., 1995).

The Bourbon LC is the first example that an utility patent has been awarded for a coffee variety and also it is the first case of a release of a coffee variety derived from natural variability isolated from somatic embryo cultures. 'Bourbon LC' is the only natural-reduced caffeine (–50%) variety being produced in commercial quantities these days.

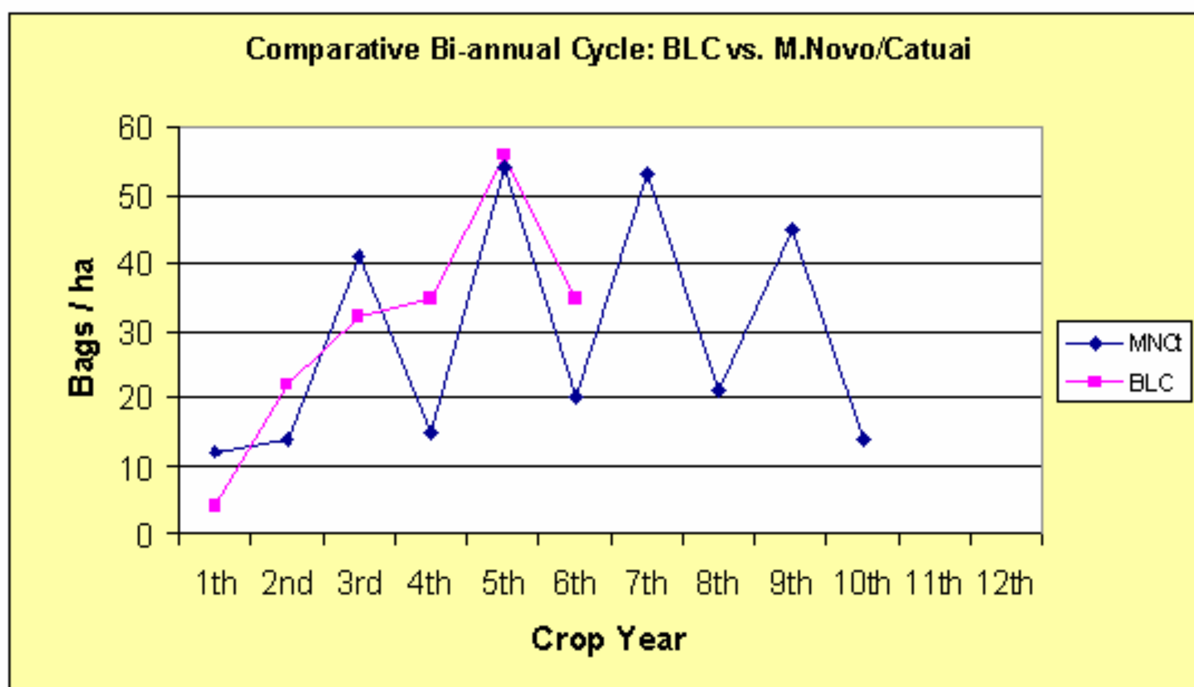

Figure 3. Biannual Cycle of Bourbon LC vs. Commercial Arabica Varieties (Mundo Novo and Catuai). Bourbon LC has reduced vegetative growth and lower yields at initial stages of plant development and only begun to show biannual yield pattern after the 5th crop

REFERENCES

Bauman T.W., Sondahl M.R., Simone O., Mösli Waldhauser S. and Kretschmar J.A., 1998. Non-destructive analysis of natural variability in bean caffeine content of Laurina coffee. Phytochemistry **49** (6): 1569-1573.

Boutilly V., 1900. Cafeier de Liberia. Sa Culture et sa manipulation. Bibliotheque de la Revue des Cultures Coloniales. A. Challamel, Paris, pp. 1-3.

Butenko R. G., Shemina Z. B. and Frolova L. Y., 1967. Induced organogenesis and characteristics of plants produced in tobacco tissue culture. Genetika **3**: 29-39.

Carvalho A., 1988. Principles and practice of coffee plant breeding for productivity and quality factors: *Coffea arabica*, p. 129-165. In Coffee Agronomy, Clarke R.J. and Macrae R. (eds.), Vol. **4**. Elsevier Appl.Sci., London.

Carvalho A. C., Ferwerda F. P. and et al. (1969). Coffee. In Outlines of perennial crop breeding in the tropics. F. P. Ferwerda and F. Wit (eds.). Veenmann H. and Zonen N.V. Publ., Wageningen, The Netherlands, pp. 189-241.

Carvalho A. and Monaco L. C., 1967. Relações genéticas de espécies selecionadas de *Coffea*. Ciencia e Cultura (S.Paulo), **19** (1): 151-165.

Coste R., 1955. Les cafeiers et les café dans le monde, Chapitre II, Tome 1$^{er.}$ Editions Larose, Paris, pp. 15-31.

Evans D. A., 1988. Applications of somaclonal variation. In: Biotechnology in Agriculture. Alan R. Liss, Inc., pp. 203-223.

Evans D. A., Sharp W.R. and Medina-Filho H.P., 1984. Somaclonal and gametoclonal variation. Amer. Journal of Botany **71**: 759-774.

Evans D. A. and Sharp W. R., 1986. Somaclonal variation in agriculture. Biotechnology **4**: 428-532.

Fazuoli L. C., 1991. Metodologias, Criterios e Resultados da Selecao em Progenies do Café Icatu com Resistencia a *Hemileia vastatrix*. Doctor Dissertation, Unicamp, 322 p.

Guerreiro Filho O., Medina Filho H. P. and Carvalho A., 1991. II. Genetica e Melhoramento de Plantas. Fontes de Resistencia ao Bicho-Mineiro, *Perileucoptera coffeella*, em *Coffea* spp. Bragantia (Campinas) 50 (1): 45-55.

Hemmerschlag F. A., 1992. Somaclonal Variation. In: Biotechnology of Perennial Fruit Crops, Hammerschlag F.A. and Litz R.E. (eds.), CAB Intl., UK, pp. 35-55.

Illy, A. and Viani, R., 1995. Expresso Coffee: the Chemistry of Quality. Academic Press, London. 253 p.

Karp A., Nelson R. S., Thomas E. and Bright S. W. J., 1982. Chromosome variation in protoplast-derived potato plants. Theor.and Appl. Genetics **63**: 265-272.

Krug C. A., Carvalho A. and Antunes Filho H., 1954. Genetica de Coffea. XXI – Hereditariedade dos caracteristicos de Coffea arabica var. Laurina (Smeathman) DC. Bol Tech. Div. Exper. Pesq., Inst. Agronomico, Campinas, Brazil, Vol. **13** (21): 247-255.

Larkin P. J. and Scowcroft W. R., 1981. Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor.and Appl. Genetics **60**: 197-214.

Lopes M. H., 1971. Teor em cafeína de cafés espontâneos de Moçambique. 5th Colloque Intern. Sur la Chimie de Cafes, Lisbon. ASIC, Paris, pp. 63-69.

McCoy T. J., Phillips R. L. and Rhines H. W., 1982. Cytogenic analysis of plants regenerated from oat (Avena sativa) tissue culture: high frequency of partial chromosome loss. Canadian Journal of Genetics and Cytology **24**: 37-50.

Medina D. M., 1963. Microsporogenese em um híbrido triploide de *Coffea racemosa* Lour x *C.arabica* L. Bragantia (Campinas), **22** (24): 299-318.

Medina Filho H. P., Carvalho A., Sondahl M. R., Fazuoli L. C. and Costa W. M., 1984. Coffee Breeding and Related Evolutionary Aspects, p. 157-193. In Plant Breeding Reviews, J. Janick (ed.), AVI Publ. Co, Conn., USA.

Orton T .J., 1983. Experimental approaches to the study of somaclonal variation. Plant Molecular Reporter **1**: 67-76.

Petracco M., 1997. Influence of Coffee Botanical Variety on Espresso Cuptesting Quality. In 17th International Conference on Coffee Science, Nairobi. ASIC, Paris.

Phillips R. L., Kaeppler S. M. and Peschke V. M., 1990. Do we understand somaclonal variation? In: Progress in Plant Cellular and Molecular Biology, Nijkamp H.J.J. et al. (eds), Kluwer Acad. Publ., Dordrecht, pp. 131-141.

Raoul E., 1897. Culture du cafeier. In: Manuel des cultures tropicales, Tome II, 1er Partie, E.Raoul and P.Sagot (eds.). A. Challamel Publ., Paris, pp. 86-95; 167-239.

Sondahl M. R. and Lauritis J. A., 1992. Coffee. In: F.A. Biotechnology of Perennial Fruit Crops, Hammerschlag F.A. and Litz R.E. (eds), CAB International, UK, pp. 401-420.

Sondahl M. R., Romig W. R. and Bragin A. 1995. Induction and selection of somaclonal variation in coffee. US Patent Office, no. **5**, 436, 395. 33 p.

Sondahl M. R. and Sharp W. R., 1977. High frequency induction of somatic embryos in cultured leaf explants of Coffea arabica L. Z. Pflanzenphysiol. **81**: 395-408.

Direct Regeneration of Double Haploid Plants from Coffee Isolated Microspores

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INTRODUCTION

Achieving an acceptable level of homozygocity in perennial species like coffee, may take about 15 to 20 years by conventional breeding methods. In this context, haploid methods offers great promise in reducing the time and enhancing the efficiency of field selection. Moreover, other potential applications in analysis of gene expression, development of mapping populations and genetic transformation could be envisaged. Although anther and pollen culture techniques have been developed in a wide range of species, they remain unattainable for majority of perennial crops. Unlike anther culture, isolated pollen culture permits plant regeneration directly from microspores, assuring a pure gametophytic origin and avoiding contamination from proliferating somatic cells. Induction of pollen embryogenic (androgenic) response could be attained by different ways (temperature pretreatment, starvation by modified media, osmotic shock or microtubule disruption agents). Positive effect on androgenic response by colchicine treatment of microspores has been demonstrated in *Brassica napus* (Iqbal et al., 1994; Zaki and Dickinson, 1995; Zhao et al., 1996). Although microspore culture has been tested in coffee, obtained microcolonies have failed to produce embryogenic tissue or plants (Neuenschwander et al., 1993; Neuenschwander and Baumann, 1995). Here we present for the first time, a method for embryo induction and plant regeneration from *C. arabica* L. isolated microspores, using a colchicine treatment.

MATERIALS AND METHODS

Donor plants

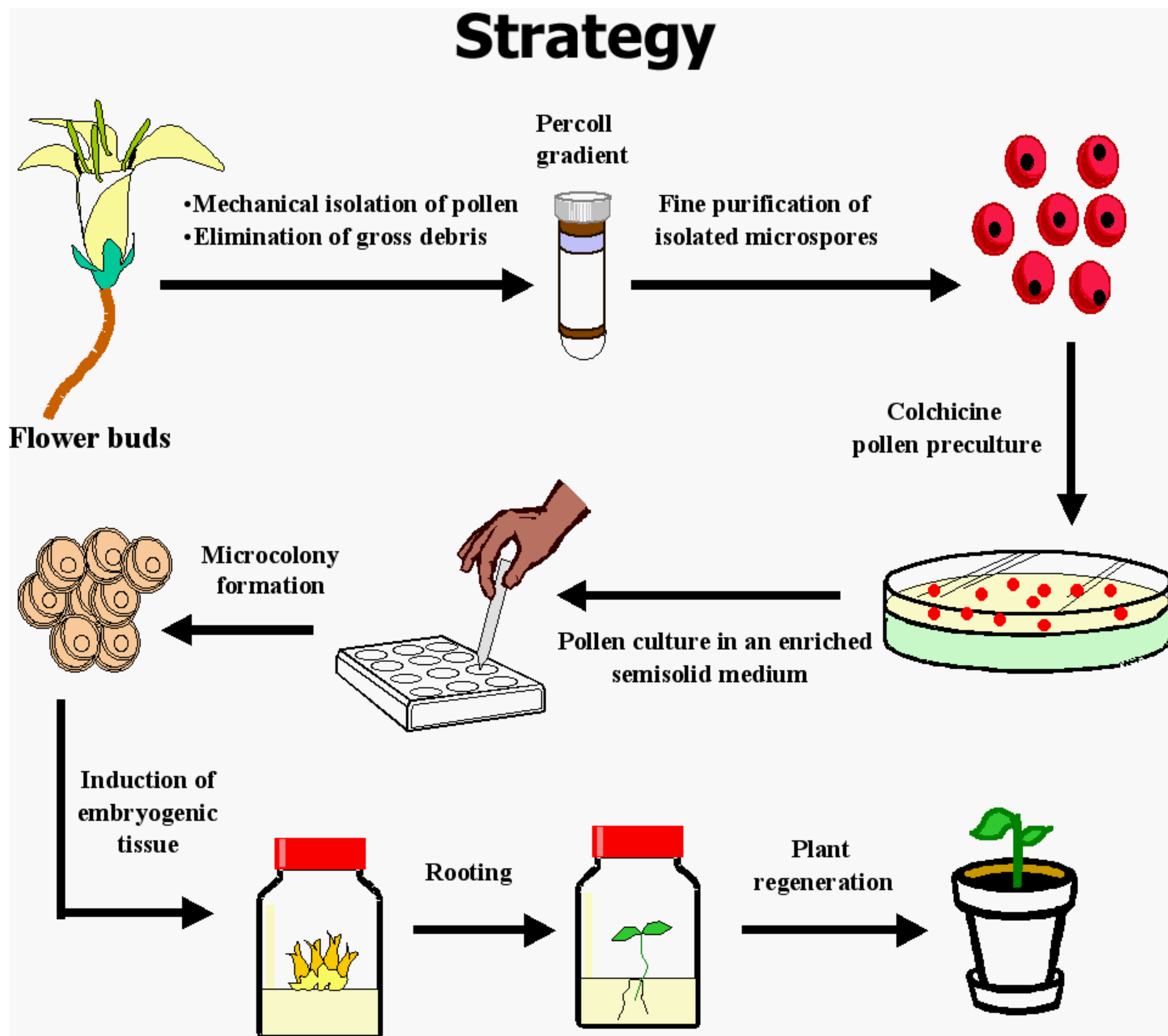
Plants of *C. arabica* cv caturra growing under field conditions were used. Flower buds were collected three days before flowering (15-18 mm corolla length) with anthers containing late-uninucleated and early-binucleated microspores (Herrera, 1995).

Microspore isolation and purification

Microspores were isolated by mechanical maceration of anthers. In order to obtain a debris free population of microspores, suspension was filtered through a fine mesh (45 µm) and immediately purified using a percoll gradient (50% v/v).

Colchicine treatment

Microspores were pre-cultured for 18, 24 and 48 h in an isolation medium supplemented with 25, 50 and 100 mg/l of colchicine. Culture dishes were incubated at 27°C in dark and subjected to moderate shaking.



Microspore culture and plant regeneration

After the colchicine treatment, microspores were washed by centrifugation. For further culture and regeneration, isolated microspores were resuspended in a semi-solid medium (agarose, 0,4%) supplemented with: Gamborg's B-5 salts medium (Gamborg et al.,1968) 0.5 X; coconut water, 16% v/v; maltose, 20 %; NAA (naphthaleneacetic acid), 300 mg/l; 2,4-D (2,4 dichlorophenoxyacetic acid), 100 mg/l; kinetin, 100 mg/l. Microspores were plated at a density of $1x10^5$ cells/ml. Embryo development was performed in a hormone free medium. Optimal humidity and photoperiod conditions were adapted progressively during *ex-vitro* development in order to assure adequate plant regeneration.

Ploidy level

Ploidy level of regenerated plants was scored by flow citometry analysis and morphological measures (length/wide leaf ratio and stomata density).

RESULTS AND DISCUSSION

Androgenic response was observed only when late-uninucleated or early-binucleated pollen were used, suggesting that in coffee, positive effect of colchicine is associated with a particular developmental stage of the microspores. Formation of microcolonies was observed after 21 days of culture in microspores pretreated with high colchicine concentrations (50-100

mg/l). Only treatments including longest colchicine exposure (24-48 h) resulted in continuous microspore division. The best results were obtained with the treatment of 100 mg/l for 48 h.

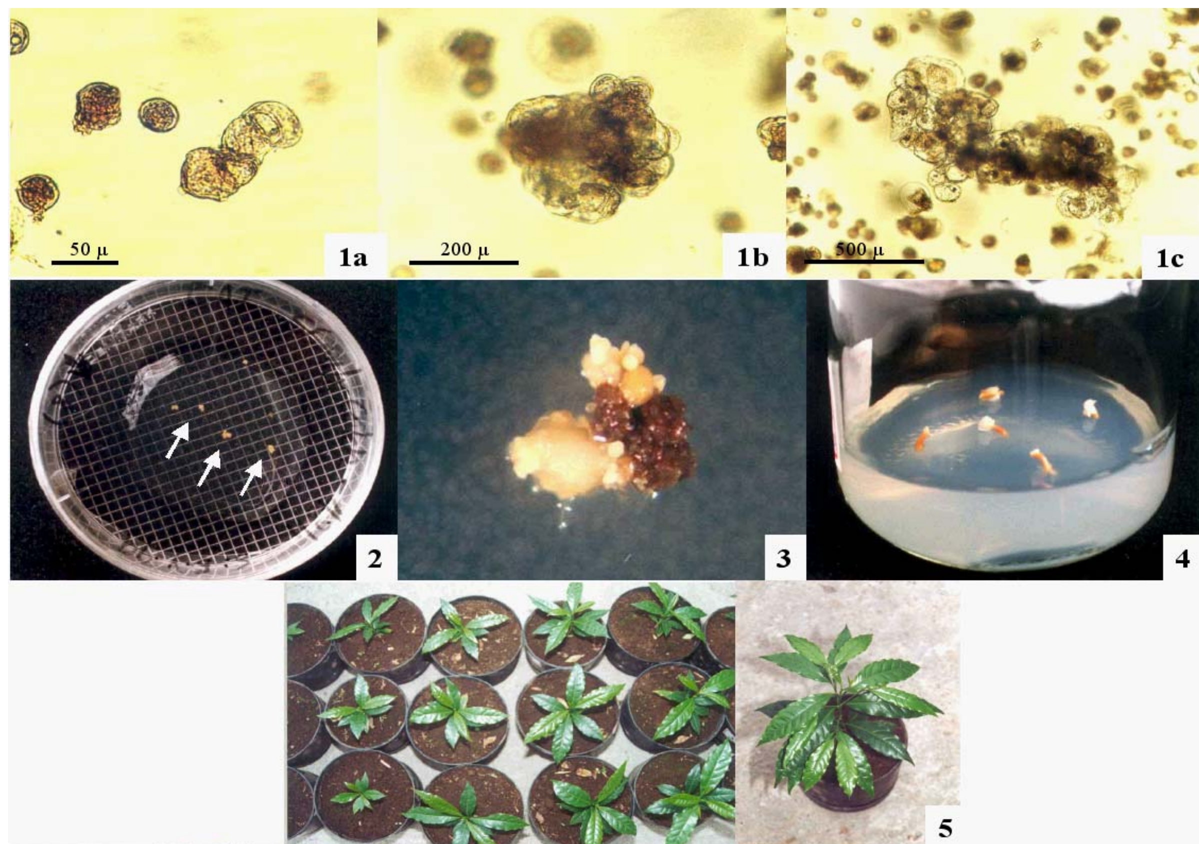


Figure 1. Embryogenesis from microspores of *Coffea arabica* L. cv. Caturra: 1(a, b and c) different stages in pollen microcolonies formation; 2. Visible pollen microcolonies after 45d in culture (arrows); 3. Early embryo formation from pollen embryogenic tissue; 4. Germinating pollen embryos; 5. Regenerated plants

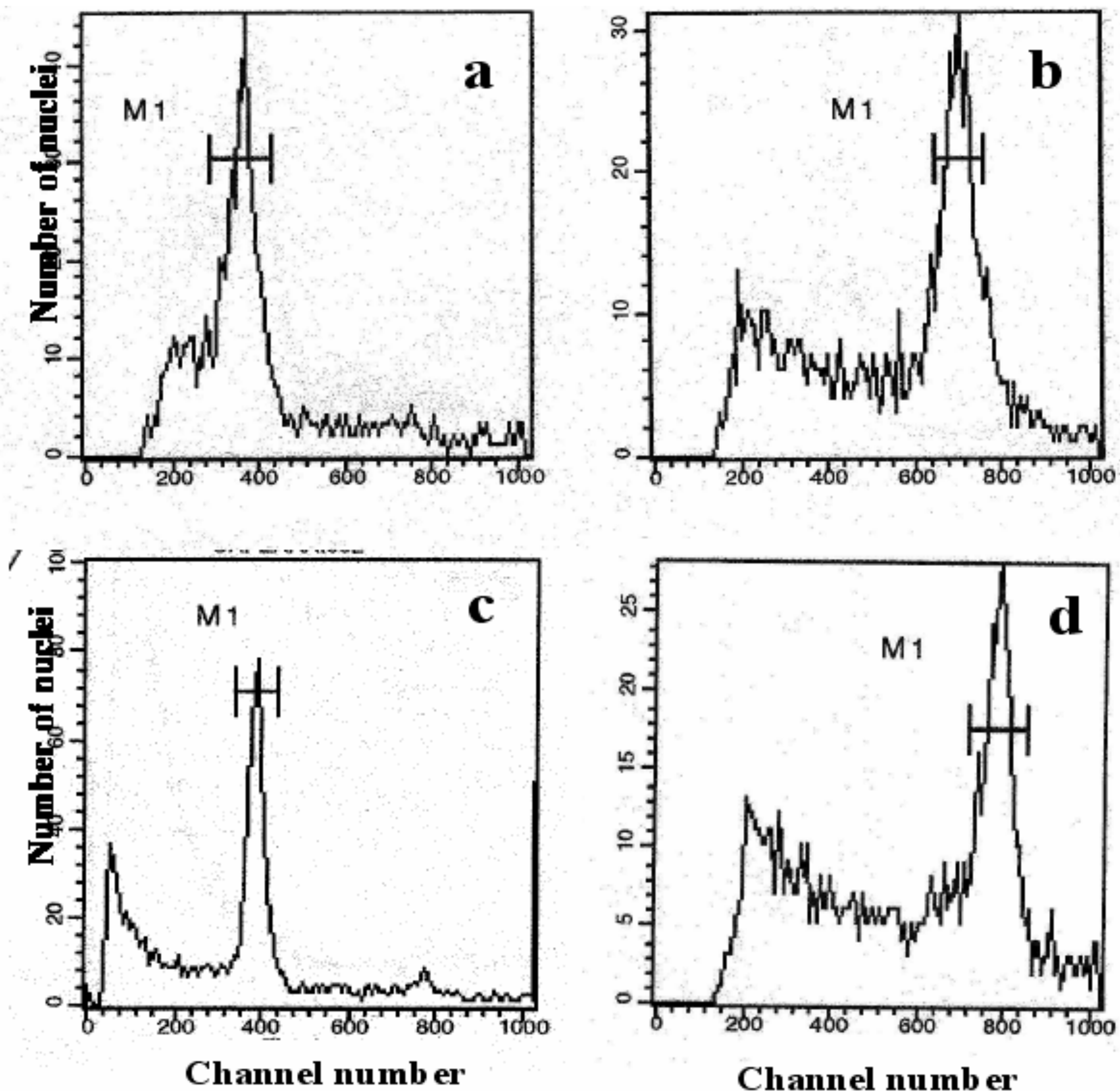
In our protocol, transfer of microcolonies into a semi-solid medium allowed enhanced growth of both microcolonies and embryogenic. Moreover, semisolid medium avoided clumping of microspores, thus facilitating the easy monitoring. No signs of toxicity were observed during early development of microspores as product of colchicine treatments, however, a low percentage of abnormal embryo-like structures were found.

At present, a first group of nineteen normal plants have been regenerated from these preliminary experiments. Flow citometry analyses as well as morphological measures, have showed that 95% (18 plants) are dihaploids (2n=2x=22). However obtention of one normal tetraploid (doubled-dihaploid) plant suggest that not only androgenic induction but also chromosome duplication could be expected from colchicine exposure of microspores.

CONCLUSION

A new protocol for induction of positive androgenic response in coffee (*C. arabica* L. var. caturra) is presented. We show that colchicine exposition (100 mg/l) of isolated microspores during a period of 48 h allowed feasible embryogenic induction and regeneration of normal dihaploids plants. Obtention of some tetraploid regenered plants suggest that direct *in vitro* duplication by this system is also possible. The success of the haploid induction in *C. arabica*

opens the opportunity to real utilization of this technology in coffee genetic studies and breeding.



Example of flow cytometer histograms of a dihaploid (a) and a doubled dihaploid (tetraploid) plant (b) regenerated from isolated microspores of *C. arabica* cv. Caturra. For comparison, DNA contents of a *C. arabica* haploid (c) (as diploid control) and a *C. arabica* cv. Caturra plant (d) (as tetraploid control are also indicated

REFERENCES

Gamborg O.L., Miller R.A., Ojima K. (1968) Exp. Cell Res. **50**: 151-158.

Herrera J.C. (1995) Cenicafe **46** (1): 21-31.

Iqbal M.C.M., Möllers C., Röbbelen G. (1994) J. Plant Physiol., **143**: 222-226.

Neuenschwander B., Baumann T.W. (1995) Plant Cell Tiss Org. Cult. **40**: 49-54.

Neuenschwander B., Dufour M., Baumann T.W. (1993) 15th Conference of ASIC, France. pp760-761.

Zaki M.A.M., Dickinson H.G. (1995) Plant Cell Tiss. Org. Cult. **40**: 255-270.

Zhao J-P., Simmonds D.H., Newcomb W. (1996) Planta **198**: 433-439.

Biosynthesis and Catabolism of Caffeine in Low Caffeine-containing Species of *Coffea*

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SUMMARY

Leaves of *Coffea salvatrix*, *C.eugenioides* and *C.bengalensis* contain ca. 3-7-fold lower levels of caffeine than those of *C.arabica*. There was more extensive biosynthesis of caffeine from [8-^{14}C]adenine in young leaves of C. arabica than in *C.salvatrix*, *C.eugenioides* and *C.bengalensis*. Degradation of [8-^{14}C]caffeine, which is negligible in leaves of *C.arabica,* was also very slow in *C.salvatrix* and *C.bengalensis*. In contrast, [8-^{14}C]caffeine was catabolised rapidly by young leaves of *C.eugenioides* primarily by a caffeine → theophylline → 3-methylxanthine → xanthine → uric acid → allantoin → allantoic acid → urea → CO_2 + NH_3 pathway. These results indicate that the low caffeine accumulation in *C.salvatrix*, *C.eugenioides* and *C.bengalensis* is a consequence a slow rate of caffeine biosynthesis while *rapid degradation of caffeine also contributes to the low endogenous caffeine pool in C.eugenioides*. The genes that regulate caffeine accumulation appear to be those encoding N-methyltransferase and caffeine (7-N) demethylase activities.

INTRODUCTION

Coffee is one of the worlds most valuable agricultural products because of its widespread use as a beverage. Fruits of *Coffea arabica* and *C.canephora* are the raw materials for the production of Arabica and Robusta coffee. These coffee beans contain relatively high concentrations of caffeine which is perceived by some members of the general public as having adverse effects on health. As a consequence, there is an increasing demand for decaffeinated coffee which is produced by solvent extraction or, more recently, supercritical fluid extraction with CO_2. An alternative source of decaffeinated coffee would be the use of *Coffea* species that contain much lower levels of caffeine than *C. arabica* or *C.canephora*. A number of such species exist but they either produce few fruits or the beans yield a poor quality beverage and are, therefore, intrinsically unsuitable for commercial development. Nor is a breeding programme to transfer the low caffeine trait to *C.arabica* a straight forward proposition because C. arabica and C. canephora are polyploid while other *Coffea* species are diploid. Under these circumstances, genetic engineering to produce transgenic caffeine-deficient *C.arabica* plants may be a more practical long term proposition than a breeding programme. The key genes in this regard are the N-methyltransferases associated with caffeine biosynthesis and the N-demethylases that catabolize caffeine. Caffeine-deficient transgenic coffee could be produced by expression of the gene encoding the appropriate demethylase or alternatively through the antisense expression of an N-methyltransferase gene (Kato et al., 2000). For this approach to be taken, detailed information is first required on the enzymes and genes controlling key steps in the biosynthesis and/or catabolism of caffeine.

There is much evidence that caffeine and other purine alkaloids are synthesized in *C. arabica* from purine nucleotides, primarily via the pathway illustrated in Figure 1 (Ashihara et al., 1996).

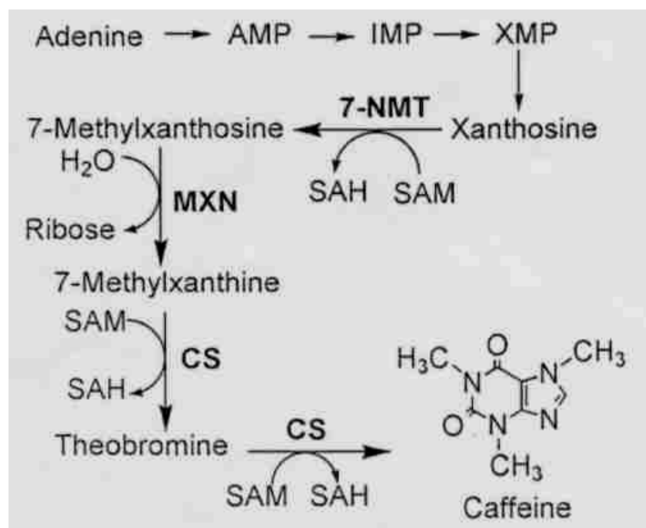

Figure 1. Biosynthetic pathway of caffeine

Xanthosine is metabolised to 7-methylxanthosine, which is the initial step in the caffeine biosynthesis pathway in which theobromine is the immediate precursor of caffeine (Ashihara, and Crozier, 1999). Caffeine biosynthesis occurs in both fruits and leaves of coffee. However, as they are obtained more easily, especially in non-coffee producing countries, most research has been carried out with leaves. Caffeine biosynthesis is especially active in young leaves of *C.arabica* and declines as the leaves age. Caffeine accumulates in *C. arabica* due to extremely slow catabolism to theophylline (Ashihara et al., 1996). In order to investigate the mechanisms that regulate the accumulation of purine alkaloids, the biosynthesis and catabolism of caffeine has been compared in leaves of *C. arabica* and three low caffeine-containing species of coffee, *C. salvatrix*, *C. eugenioides* and *C. bengalensis*.

RESULTS AND DISCUSSION

Endogenous Purine Alkaloids in Coffea Leaves

The levels of caffeine in *C. salvatrix*, *C. eugenioides* and *C. bengalensis* in young and mature leaves were 13-36% of those found in *C.arabica*. In all four species, young leaves contained higher concentrations of caffeine than mature leaves (Table 1). Theobromine, which was not detected in extracts from *C. salvatrix*, *C. eugenioides* and *C. bengalensis*, was present in young and mature leaves of *C. arabica* at concentrations of 1.8 mg and 0.1 mg g^{-1} (fr.wt.), respectively. No other endogenous purine alkaloids were detected in any of the leaf extracts.

Metabolism of [8-^{14}C]Adenine.

As plants readily convert adenine to AMP, isotopically-Iabelled adenine can be used to investigate the AMP-derived caffeine biosynthetic pathway (Figure 1). Table 2 shows results of the overall metabolism of [8-^{14}C]adenine. There was little difference in the incorporation of label into nucleotides in the four *Coffea* species although the level of radioactivity associated with theobromine and caffeine was much higher in young leaves of *C. arabica* than it was in *C. salvatrix*, *C. eugenioides* and *C. bengalensis*. In *C. arabica* 46.7% of the radioactivity

taken up by the leaf segments was incorporated into the two purine alkaloids while the figures for *C. salvatrix*, *C. eugenioides* and *C. bengalensis* were 15.0%, 0.4% and 0.4% respectively. Extensive labelling of the purine catabolites, allantoin, allantoic acid and CO_2, was observed only in the low caffeine-containing *Coffea* plants. In *C. eugenioides*, more than 30% of total radioactivity was released as $^{14}CO_2$, while the ureides, allantoin and allantoic acid, were the most heavily labelled compounds in *C. bengalensis*. Thus, in *C. arabica*, [8-^{14}C]adenine is metabolised preferentially to 7-methylxanthine and converted to caffeine via theobromine while in the low caffeine-containing Coffea species, it appears to be converted primarily to xanthine and enters the purine catabolism pathway.

Table 1. Caffeine content in young and mature coffee leaves[a]

species	young leaves	mature leaves
C. arabica cv. Kent	7.1 ± 2.6 (100)	2.1 ± 0.34 (100)
C. salvatrix	0.92 ± 0.08 (13)	0.30 ± 0.01 (14)
C. eugenioides	1.3 ± 0.11 (19)	0.37 ± 0.03 (18)
C. bengalensis	1.2 ± 0.01 (17)	0.76 ± 0.01 (36)

[a]Data are expressed as mg^{-1} of fresh weight. Values are the means ± SD (n = 3). The figures in parentheses represent caffeine levels expressed as a percentage of the amount detecyed in C. arabica

Table 2. Metabolism of [8-^{14}C]adenine by young coffee leaves[a]

metabolite	*C. salvatrix*	*C. eugenioides*	*C. bengalensis*	*C. arabica*
methanol-soluble	55.4 ± 1.3	37.4 ± 0.0	64.3 ± 1.3	63.2 ± 1.0
nucleotides	8.2 ± 1.6	20.4 ± 1.5	6.9 ± 0.4	4.4 ± 0.5[b]
adenine	2.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	
theobromine	12.3 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	26.2 ± 3.2
caffeine	2.7 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	20.5 ± 3.5
xanthine	7.1 ± 1.2	3.3 ± 0.1	3.0 ± 0.3	8.4 ± 1.7
allantoin	10.2 ± 1.2	18.5 ± 0.5	33.8 ± 1.4	
allantoic acid	10.2 ± 0.2	5.1 ± 0.4	6.6 ± 0.5	
unknown	2.1 ± 0.1	1.7 ± 0.4	4.6 ± 0.3	
CO_2	17.6 ± 1.1	31.9 ± 2.3	5.4 ± 1.0	3.2 ± 0.2
methanol-insoluble	27.0 ± 0.2	30.8 ± 2.4	30.3 ± 2.3	35.0 ± 0.7
total uptake (kBq)	83.4 ± 6.0	189.9 ± 16.6	155.0 ± 13.4	123.0 ± 3.0

[a]Segments of young leaves were incubated with 9.4 μM [8-^{14}C]adenine for 18 h. Values for C. arabica were taken from Ashihara et al. (1996a). Incorporation of radioactivity into metabolites is expressed as percentage of total uptake ± SD (n = 3). Total uptake of radioactivity is expressed as kBq g^{-1} of leaf (fresh weight). [b]This value includes incorporation into nucleotides, allantoin, allantoic acid, adenine, and unidentified methanol-soluble compounds

Catabolism of [8-^{14}C]Caffeine

Data on the catabolism of [8-^{14}C]caffeine by young and mature leaves from *C. salvatrix*, C.eugenioides and *C. bengalensis* are presented in Table 3. Little or no catabolism occurred in leaves of C. salvatrix and *C. bengalensis*. Similar results were obtained in an earlier study with *C. arabica* leaves (Ashihara et al., 1996), the data from which are included in Table 3 for comparative purposes. In contrast, very rapid catabolism of [8-^{14}C]caffeine was observed in leaf segments from *C.eugenioides*. More than 75% of [8-^{14}C]caffeine taken up by the segments was catabolized in both young and mature *C. eugenioides* leaves, with radioactivity recovered as theophylline, 3-methylxanthine, I-methylxanthine, xanthine, ureides, urea and CO_2 This indicates that [8-^{14}C]caffeine undergoes demethylation, probably via the routes indicated in Figure 1, resulting in the production of xanthine which is degraded to CO_2 and NH_3 via the conventional purine catabolic pathway. The rate of uptake of [8-^{14}C]caffeine by

leaf segments of C. eugenioides was much higher than those by the segments of *C. salvatrix* and *C. bengalensis* (Table 3). A similar trend was also observed when [8-^{14}C]theophylline was used as a precursor (Table 4). The reasons for this are unclear although it is possible that passive transport along a concentration gradient, from the incubation medium into the cells of the leaf, is enhanced in keeping with the extent to which the absorbed purine alkaloid is catabolized In order to obtain further information on the pathway utilised for the catabolism of caffeine in *C. eugenioides*, pulse-chase experiments with [8-^{14}C]caffeine were carried out using mature leaves (Table 4).

Table 4. Metabolism of [8-^{14}C]caffeine in a pulse-chase experiment with mature leaves of *C. eugenioides*[a]

metabolite	4 h (pulse)	8 h (chase)	24 h (chase)
residual caffeine	52.0 ± 0.1	4.9 ± 0.1	3.4 ± 0.1
theophylline	21.4 ± 0.9	24.2 ± 0.6	8.1 ± 0.5
3-methylxanthine	16.0 ± 0.1	44.0 ± 0.2	46.9 ± 1.9
1-methylxanthine	6.1 ± 0.6	6.6 ± 0.4	7.4 ± 0.0
xanthine	nd[b]	3.9 ± 0.4	5.8 ± 0.2
allantoin	0.9 ± 0.1	18 ± 0.0	1.4 ± 1.1
allantoic acid	1.1 ± 0.0	2.5 ± 0.0	nd
urea	1.2 ± 0.1	3.5 ± 0.1	2.9 ± 0.1
CO_2	1.3 ± 0.2	8.3 ± 0.9	24.2 ± 2.0

[a]Leaf segments (100 mg of fresh weight) were incubated with 18 µM [8-^{14}C]caffeine for 4 h (pulse), and then the incubation medium was replaced by fresh medium without tracer. The radioactivity was "chased" for a further 4 and 20 h. Incorporation of radioactivity into each compound is expressed as a percentage of the total radioactivity recovered. Mean values ± SD (n = 3) shown. Total radioactivity taken up by the tissues was 31.2 ± 4.0 kBq g^{-1} of leaf. [b]nd, not detected

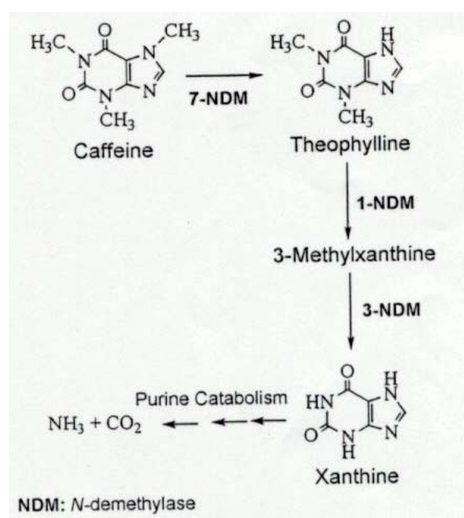

Figure 2. Major catabatic pathway of caffeine

Caffeine, theophylline and 3-methylxanthine were the most heavily labelled compounds after the 4-h pulse. The radioactivity associated with caffeine declined after the leaves were transferred to the non-radioactive medium. In contrast, ^{14}C-labelled theophylline, 3-

methylxanthine, l-methylxanthine, xanthine, ureides, urea and CO_2 increased after the 4-h chase, with more than 40% of the radioactivity taken up during the pulse being incorporated into 3-methylxanthine. After a further 20-h chase radioactivity associated with theophylline, ureides and urea declined, while the level of ^{14}C associated with 3-methylxanthine, 1-methylxanthine and xanthine changed little and $^{14}CO_2$ evolution increased from 8.3% to 24.2% of the recovered radioactivity.

Catabolism of [8-^{14}C]Theophylline.

C. arabica leaves catabolise theophylline much more rapidly than caffeine (Ashihara et al., 1996). It was, therefore, of interest to investigate the fate of theophylline when incubated with young and mature leaves of *C. salvatrix*, C.eugenioides and *C. bengalensis*. The data obtained are presented in Table 5. [8-^{14}C]Theophylline was converted to a range of catabolites, most extensively by *C. eugenioides* with the evolution of $^{14}CO_2$ from mature leaves being three times greater than that from young leaves where more than half of radioactivity was recovered as 3-methylxanthine. More label was associated with 3-methylxanthine than 1-methylxanthine indicating that the main route for catabolism of theophylline to xanthine is via 3-methylxanthine in *C. eugenioides*. Metabolism of [8-^{14}C]theophylline was slower in *C. bengalensis* with proportionally more radioactivity being recovered as unmetabolised theophylline and less as $^{14}CO_2$ and intermediates of purine catabolism. There was relatively little ~atabolism of [8-^{14}C]theophylline by leaves of *C. salvatrix*, .with more than 90% of the total radioactivity taken up leaves bemg the unmetabolised substrate and only minimal incorporation of label into purine catabolites.

Table 5. Metabolism of [8-^{14}C]theophylline by young and mature coffee leaves[a]

species	leaves	distribution of recovered radioactivity (% total ± SD)								total uptake of radioactivity
		Tp	3-mX	1-mX	X	Alln	Alla	urea	CO_2	(kBq ± SD)
C. salvatrix	young	99.5 ± 0.0	nd[b]	nd	nd	nd	nd	nd	0.5 ± 0.0	54.8 ± 12.2
	mature	95.2 ± 0.7	2.2 ± 0.2	nd	0.5 ± 0.1	nd	nd	0.5 ± 0.0	1.0 ± 0.4	50.2 ± 5.2
C. eugenioides	young	26.6 ± 1.0	53.8 ± 1.0	0.8 ± 0.1	1.3 ± 0.2	4.8 ± 0.7	1.0 ± 0.0	3.3 ± 0.1	7.9 ± 2.9	86.3 ± 14.2
	mature	36.4 ± 0.3	26.2 ± 0.7	0.8 ± 0.0	4.8 ± 0.3	0.8 ± 0.1	1.3 ± 0.0	3.5 ± 0.3	23.5 ± 0.7	85.7 ± 1.5
C. bengalensis	young	82.4 ± 1.0	6.9 ± 0.0	2.0 ± 0.4	nd	3.4 ± 0.2	nd	nd	1.3 ± 0.4	41.7 ± 1.8
	mature	78.2 ± 0.3	14.2 ± 0.2	3.1 ± 0.2	nd	3.7 ± 0.4	nd	nd	0.9 ± 0.1	34.5 ± 3.6

[a]Segments of young leaves were incubated with 9.1 μM [8-^{14}C]theophylline for 18 h. Incorporation of radioactivity into metabolites is expressed as percentage of total uptake ±SD (n = 3). Total uptake of radioactivity is expressed as kBq^{-1} of leaf (fw). Theophylline (Tp), 3-methylxanthine (3-mX), 1-methylxanthine (1-mX), xanthine (X) allantoin (Alln), allantoic (Alla). [b]nd, not detected

CONCLUSION

The results of the present study are useful for the long-term aims of using biotechnology to produce transgenic, caffeine-deficient *C. arabica* plants. *C. eugenioides* degrades caffeine via theophylline and so, unlike the other species, contains a specific 7 N-demethylase activity. The substrate specificity of this demethylase seems to be different from that of the N-demethylase isolated from bacteria, such as *Pseudomonas putida* and *Pseudomonas cepacia* (Ashihara and Crozier 1999) which catabolizes caffeine to theobromine rather than theophylline. Expression of the bacterial demethylase in *C. arabica* is unlikely to result in caffeine-deficiency as caffeine will be degraded to theobromine which is the immediate precursor of caffeine in coffee. In contrast, insertion of the 7N-demethylase encoding gene from *C. eugenioides* into the genome of *C. arabica* is much more likely to produce caffeine-

deficiency because the Eugenioides gene product will catalyse the conversion to caffeine to theophylline and the native Arabica enzymes have the capacity to rapidly degrade theophylline. Work on the isolation and characterization of this novel N-demethylase from *C. eugenioides* is in progress.

EXPRIMENTAL

Seeds of various *Coffea* plants were obtained from the Instituto Agronomico, Campinas, Sao Paulo, Brasil. The leaves used in this study were from two-year old plants two-year old plants growing under a natural photoperiod in a greenhouse at the University of Glasgow. Young leaves were the most recently emerged primary leaf while mature leaves comprised the fully expanded, second and third leaf below the apex. [8-^{14}C]Caffeine (specific activity 2.07 MBq μmol^{-1}), [8-^{14}C]adenine (1.96 MBq μmol^{-1}) and [8-^{14}C]Theophylline (2.04 MBq μmol^{-1}) were used. Segments ofCojfea leaves were incubated in 2 ml medium, comprising 30 mM potassium phosphate buffer, pH 5.6, 10 mM sucrose and a radiolabelled substrate (37 kBq), in a 30 ml Erlenmeyer flask, in a shaking water bath for 18 h at 27°C. Analysis of ^{14}C-metabolites was carried out as described elsewhere (Ashihara and Crozier, 1999).

REFERENCES

Ashihara H. and Crozier C. (1999) Adv. Bot. Res. **30**: 117-205.

Ashihara H. and Crozier C. (1999) J. Agric. Food Chem. **47**: 3425-3431.

Ashihara H., Monteiro A.M., Moritz T., Gillies F.M. and Crozier A. (1996) Planta **198**: 334-339.

Ashihara H., Monteiro A.M., Gillies F.M. and Crozier A. (1996) Plant Physiol. **111**:747-753.

Kato M., Mizuno K., Crozier A., Fujimura T. and Ashihara H. (2000) Nature **406**: 956-957.

Air Layering: An Ancient Vegetative Propagation Technique with a Modern Application for the Conservation of a Genetically Diverse Germplasm Collection of *Coffea* Secies and Complex Hybrids*

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SUMMARY

One of the largest and most diverse coffee germplasm bank in the world is located in the Centro de Café of Instituto Agronômico de Campinas, Brazil, where its conservation and active utilization is the foundation of a solid and sucessfull breeding program supporting the leading coffee production country in the world. Maintenance and rejuvenation of the germplasm collection is a defying task, provide the large number, the genetic diversity of acessions and also, the differences in age and growing conditions of each entry.

Air layering of orthotropic branches of plants growing in an irrigated and shadded lathhouse or in full sun fields without irrigation was attempted during one year by a single unexperienced worker. This has yielded 201 clones of 140 endangered acessions, comprising genetic, chromosomal and somatic mutants, F_1 and complex intra and interspecific hybrids and other representatives of the germplasm bank involving 10 genera of *Coffea*, one of the close related *Psilanthus* and one of the far related *Tricalisia*. The results are clear evidences of the easiness, efficiency and broad applicability of this ancient and inexpensive technique of vegetative propagation of plants to the noble use for conservating and preserving the integrity of the genetic variability of *Coffea* and related genera. Details, problems, advantages over alternative vegetative methods of propagation and further needed research are discussed.

INTRODUCTION

Since the foundation of the Instituto Agronômico de Campinas in 1887, coffee has been a crop intensively studied. Indeed, 30 years after the rediscovery of Mendel's laws of inheritance it was started a comprehensive program on taxonomy, cytology, evolution, genetics and breeding (Krug, 1936). Testimony of the success of such investigations are the published results during the 20 century (Carvalho, 1988; Fazuoli et al., 1999) and the impressive fact that presently over 95% of cultivars grown in Brazil – the world's largest producer – is a direct result of this program.

The cornerstone of all this work was the establishment and concomitant active use of the diverse resources of a large Germplasm Bank. Today it is represented in Campinas a living collection comprising 18 species of the genus *Coffea*, 3 of *Psilanthus* and thousands of simple and complex hybrids, derivatives of them, genetic, chromosomal and somatic mutants, ploidy level series and a myriad of unique combinations of genotypes, including alleles conferring distinct plant architectures, flowering times, branching characteristics, bean and bean types, stress, diseases, insects and nematodes tolerances or resistances. Many of these unique

individuals synthesised or selected many decades ago are still under study, used in hybridizations, or must be maintained as strategic genetic resource for future work. In some cases they are maintained in the field or, alternatively, in a special lathhouse where they grow in 200 liters metal barrels. Despite all the care, they are subjected to occasional diseases, die back, natural exaustion or they need to be locally rearranged, reasons why they must to be cloned, rejuvenated and reestablished in other conditions. Otherwise, unvaluable genetic resources might be irremedialy lost. This social and scientific responsability of saving endangered germplasm is a chalenging undertaking and methods must be devised for clonning, cheap and efficiently, hundreds or thousands of unique genotypes securing however just a few copies of each. With the exception of commercial varieties or pure breeding lines of *Coffea arabica* that are self-compatible, autogamous and thus breed true by seeds, their hybrids and the other species of *Coffea* as well as the large majority (Medina-Filho et al., 1984) of related genera are self-incompatible and therefore need to be vegetative propagated in order to maintain its genetic integrity.

PROBLEMS WITH THE MAINTENANCE OF GERMPLASM COLLECTION BY VEGETATIVE PROPAGATION

Methods available for vegetative propagation of plants are several (Toogood, 1999), but not all of them are amenable or appropriate for coffee, specifically in the situation explained above where it is needed very few copies of a large number of different genotypes.

The experience of almost 70 years of working with an increasinly diverse germplasm of coffee to be maintened at the Instituto Agronômico has indicated that there is not yet an ideal method for cheap and dependable propagation of coffee germplasm.

As to cuttings, a number of variations in ages, conditons, environments, procedures and hormone applications have been tested through the years. *Coffea canephora* usually roots easily, although several specific clones are surprisingly recalcitrant like many *C. arabica*. Some of them in very good conditions, under favorable temperature and moisture keep the original leaves of the cutting green for up to six months, eventually sprout, start to grow exuberantly but die during weaning because actually they had not callused nor rooted (Gonçalves, personal communication). Moist, temperature, season, physiological condition of the donnor trees besides the interaction with the species and specific genotypes surely influence the success of the operation (Cramer, 1957). Although theoretically any genotype can have the conditions for rooting optimized, for larger number of genotypes, it however becomes certainly not practical. Vigorous growth and healthy condition of the trees are usually benneficial and in most cases it is a required condition for rooting. Unfortunately, this is, most of the time, the opposite situation encountered in the individuals that need to be cloned and rejuvenated.

Grafting is usually the second choice of propagating plants that are difficult to do by cuttings. Since the begining of the establishment of the germplasm collection in Campinas, mature high apical wedge-grafting has been achieved by a few very skillfull and dedicate nurseryworkers, yet with variable and unreliable results. Spliced side-grafting, whip-and – tongue grafting, chip, T, rind budding and others tried gave rise to erratic, usually disappointing results. Again, as with cuttings, the process is genotype dependent with an additional complicating factor that, in this case, one deals with two different entities, the roostock and the scion that must be graft compatible on the long run. It should be mentioned that it is commercially used in Brazil and Central America the hypocotiledonar wedge grafting of *C. arabica* varieties onto nematode resistant *C. canephora* (Reina, 1966; Moraes and Franco, 1979). Millions of grafting with almost hundred percent of taking are done every year. However this is a very

special case inasmuch as both scions and rootstochs are actually very young seedlings from seeds of true-breeding lines, which leads to uniform fields but, in fact, biologically speaking it is not a vegetative propagation.

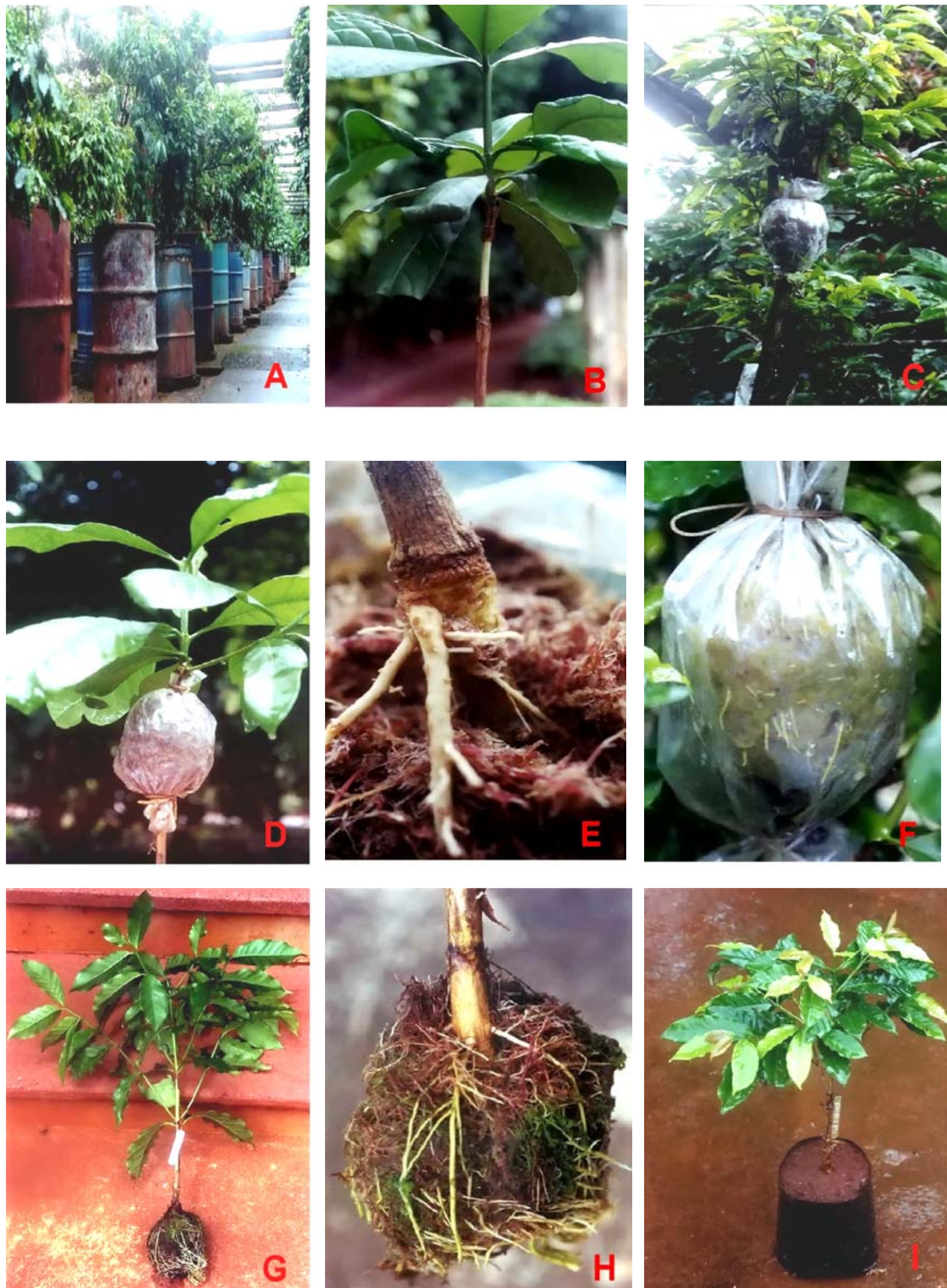

Figure 1. Air layering of coffee germplasm plants growing in barrels in lath-house {A}. A ring of bark is removed {B} from 30-100 cm orthotropic stems {C}, sphagnum moss packed around the stem and wrapped with plastic sleeve {D}. Callus are formed at the site of the wound and adventious roots developped {E}, some showing through the sleeve {F}. The ball is then cut through {G}, roots partially teased out {H} and the layer is potted in plastic bags {I}

Tissue culture technique have been intensively studied in the last three decades and sucessfully applied to coffee propagation (Ducos et al., 1999). It is a very appropriate procedure for massive reproduction of a few genotypes after the development of specific protocols which are, as in the previous cases, unfortunately genotype dependent. Interesting enough, the genotypes that are recalcitrant in cuttings are also the ones that do not calluse or propagate *in vitro* (Gonçalves, personal comunication; Ramos, 1993.). An additional difficulty of the *in vitro* propagation is the high level of contamination observed in explants from plants grown in the field or in lath house. Thus, this is also a technique unsuitable for the coffee germplasm propagation, in the sense and extent above mentioned.

On the course of the investigations, air layering has thus originally attempted in the Campinas germplasm with quite reasonable results. It is noteworthy that air layering is an ancient – it was routinely used in China 4000 years ago – natural and simple way of propagating a plant but to the knowledge of the authors, never used to extensively propagate coffee.

AIR LAYERING TECHNIQUE APPLIED TO COFFEE GERMPLASM PROPAGATION

Layering is the general process by which plants form adventious roots from the stems, either naturally or artificially induced (Bellair, 1934; Janick, 1968). Actually, it is a type of cutting where callusing and the subsequent formation of adventitious roots take place while the stem or layer is still attached to and nourished by the parent plant. It is therefore a less drastic process and thus, subjected to less influence of the environment than the required for cuttings. At first, a ring of bark is removed (Figure 1B), moist sphagnum moss or soil mix is then patched around and a plastic sleeve is taped in the stem (Figure 1C, 1D). The sap with nutrients and hormones that otherwise would go to the roots are thus trapped in the phloem at the site of the wound and induce callusing, eventually promoting the growth of adventitious roots (Figure 1E, 1H). Usually this takes several months but, during this time, little care is necessary. After roots are formed the layer is severed from the branch (Figure 1G) and potted (Figure 1I).

Coffee plants of the living collection in Campinas subjected to air layering were growing in two extreeme conditions, either in a wooden lathhouse in 200 liters metal barrels (Figure 1A) and watered daily, or were in the field in full sun condition without any irrigation. A band 5 to 10 cm wide of mature bark (Figure 1B) of 30-100cm stems (Figure 1C) was peelled off with a grafting knife, sphagnum moss firmely packed around the stem, 1cm above and below the wounds, moistened, wrapped with a transparent plastic sleeve and tied with a cotton twine at the extremeties (Figure 1D, 1F). Once a month the ball was checked externaly for rooting and 10-20 ml of water was injected into the balls with a syringe. Occasionally, slow rooting plants naturally reconstituted the bark which needed to be again removed for rooting. Layering was done year round but no difference among seasons was noticed.

Adventitious roots developed at the site of the wound (Figure 1E) and some showed through the sleeve after 3-6 months (Figure 1F). The stem was then cut through just below the ball (Figure 1G), the sleeve removed, roots teased out, the moss partially taken out (Figure 1H) and the layer labelled and potted in 5 liters plastic bags (Figure 1I) filled with regular nursery soil. It was watered well at planting time and regularly afterwards. Attempts to prune back the layers in order to help roots to sustain the plants during weaning invariably killed the potted layers. A number of plants were lost when this was done. To avoid excessive dehydration of the newly potted plants an ideal condition during early weaning would be to keep them under mist or fog and gradually hard them off.

The application of the above procedures has rendered 201 plants of 140 entries, a diverse germplasm of acessions, genetic, chromosomal and somatic mutants and variations, single, complex and derivatives of intra and interspecific hybrids as listed in Table 1. It comprises 10 species of the genus *Coffea*, one of the close related *Psilanthus* and one of the far related genus *Tricalisia*. The conservation of such a fantastic genetic variability that was previously endangered was achieved in one year by one full time worker that had no previous experience with the coffee plant.

This testifies the easiness and the universality of the technique as to a broad range of coffee genotypes. Besides its simplicity, additional relevant attributes of the technique are that it is relatively inexpensive, reliable, can be done year round and it is not dependent on the genotype. Evidently, this is not an appropriate procedure of clonning for the establishment of commercial fields as coffee is planted in very high densities, usually around 5000 plants/ha. This is very likely the reason why air layering, except for a brief comment on *Coffea canephora* (Chevalier, 1929), is not referred in the literature (Coste, 1955; Haarer, 1956; Cramer, 1957; Snoeck, 1988) as a method of propagating coffee. However, for the purpose of germplasm conservation one genotype may represent a unique genetic reservoir. Consequenly, every single plant worth to be preserved. The wealth of genetic resources preserved amongst the 140 genotypes layered can be seen in Table 1.

PRESENT PROBLEMS AND FUTURE IMPROVEMENTS OF THE TECHNIQUE

Although the results so far obtained can be considered a real achievement for the process of genetic conservation of coffee germplasm, it must be noticed that this was just an exploratory attempt to air layer some individuals of the living collection in Campinas. A number of tests and procedures should be experienced in order to improve the technique and broaden the knowledge of its application on coffee.

The shorter the layer, the better is the recovered plant. Several of the plants produced so far are too tall and will need to be forced to develop suckers, carefully pruned back or further layered. The effect of hormones commonly used for inducing rooting as well as different substrats and sleeves could also be tested. A major improvement in the efficiency of the technique could be achieved simply by carefull monitoring the shade, temperature and moisture during weaning. This phase is quite critical since new roots must form fast in order to nourish the shoots and avoid dehydration, formely provided by the parent plant while the layer was still attached to it.

A delicate process that has to be cared for, is the potting operation as the roots of the layer ball should be teased out, but not completely, otherwise damage of the roots surely occurs. Also, the soil should be very gently added around and on the top of the ball and never pressed on the top or laterally, as done with regular transplants. Rigid plastic pots should be prefered over regular flexible bags as they avoid the disturbance of the roots after potting.

Coffee as some other important tropical plants has two distinct and specialized types of branches (Cook, 1911) to which attention must be directed when cloning is effected. The upright or orthortropic branches form the main stem bearing the internodes from which bases develop the lateral, plagiotropic branches. The lateral branches form secondary lateral branches, leaves, inflorescences, but usually are not capable of producing uprights. So, the dimorfism of branches is of utmost importance in the vegetative propagation of coffee since only the orthotropic ones should be layered. They will reproduce a normal upright growing plant with the lateral reproductive plagiotropic branches. Instead, if the latter are used, a plant without orthotropic branch and an inadequate prostrate habit will result.

Table 1. General list of ex-endangered entries of the Coffee Germplasm Bank of the Centro de Café of the Instituto Agronômico de Campinas, Brazil preserved by the air-layering technique. It is indicated the species, type of germplasm and the biological diversity of the genetic backgrounds involved in the hybrids. Number in parenthesis indicates different genotypes of the same type, n is the ploidy level other than the normal 2n=44 chromosomes for *C. arabica* and 2n=22 for the remaining species

Coffea arabica			
Cultivars, acessions and others		**Genetic and chromosomal mutants, variations**	
Bourbon Vermelho	Icatu	Purpurascens (3)	Polysperma
Introdução CIFC	Icatu JCG	Bullata 4n	Bronze (3)
Arábia Saudita	Introdução 4192	Variação	Semperflorens
Introdução Etiópia	Maragogipe	Bourbon n	Nana → Nana
Obatã	H66	Catuaí n (2)	Angustifolia
Nanicão		Mokka	Cera
		Murta	Laurina
# of distinct genotypes..........................11		**# of distinct genotypes.................................19**	
F_1 and other intraspecific hybrids		**F_1, other interspecific hybrids and derivatives**	
Cera x Icatu	Bourbon x Laurina	Híbrido Timor	Catuaí x Catuaí x Híbrido de Timor (2)
Mundo Novo x Icatu	Catuaí x Tupi	Catuaí x BA x *C. salvatrix* 4n	Catuaí x Híbrido de Timor (2)
Catuaí x Icatu (2)	Catuaí x Crespa	Catuaí x *C. salvatrix* 4n (4)	Bourbon x *C. congensis* Bangelan
BA 16 x Catuaí	Crespa x Laurina	Icatu x Catuaí x *C. salvatrix* 4n	Purpurancens x Nacional x *C. congensis*
Catuaí x BA 10 x Mundo Novo	Angustifolia x Bourbon	Nacional x *C. eugenioides*	Catuaí x *C. congensis* 4n
Diminuta x Erecta	Angustifolia x Laurina	Nacional x *C. dewevrei* (2)	Mundo Novo x Amphilo x *C. canephora* guarini 4n x Catuaí
Angustifolia x Angustifolia	Icatu x Tupi	Amphilo x *C. canephora* 4n	Catuaí x *C. canephora* guarini 4n
KP x Goiaba	Tupi x Cera	Nacional x *C. salvatrix* 4n	Purpurascens x Híbrido de Timor x Mundo Novo x San Bernardo x *C. salvatrix*
San Ramon x Bourbon Vermelho x San Ramon	Bourbon x Catuaí (2)	Mundo Novo x *C. eugenioides*	Catuaí x *C. salvatrix* 4n x Catuaí
Angustifolia x Nacional	Catuaí x Mundo Novo x Mundo Novo (2)	Mundo Novo x Híbrido de Timor	Acaiá x Nacional x *C. racemosa* x Bourbon
Pacas x Caturra x Típica	Catuaí x Icatu	Mundo Novo x Catimor	Icatu x *C. salvatrix* 4n
Pacas x Nacional	Catimor x Laurina (2)	Catuaí x Mundo Novo x Mundo Novo x Catuaí x *C. eugenioides* 4n	Icatu x Catuaí x Nacional x *C. racemosa* x Bourbon x Icatu (3)
Pacas x Villa Sarchi	Purpurascens x Macrodiscus	Híbrido de Timor x Catimor x *C. dewevrei* x *C. kapakata*	Catuaí x *C. salvatrix* x *C. kapakata*
Villa Sarchi x Pacas (2)	Purpurascens x Crespa	Híbrido de Timor x Catimor	Catuaí x Angustifolia x *C. kapakata*

Coffea arabica (suite)

F$_1$ and other intraspecific hybrids		F$_1$, other interspecific hybrids and derivatives	
San Bernardo x Pacas	Catuaí x Catuaí x Icatu	Híbrido de Timor x Híbrido de Timor (2)	Catuaí x *C. eugenioides* x Mundo Novo
Laurina x Purpurascens	Icatu x Icatu		
Purpurascens x Nacional x Anomala x Purpurascens	Purpurascens x Nacional		
Mundo Novo x Laurina			
# of distinct genotypes................................40		**# of distinct genotypes.............................39**	

Coffea canephora

Cultivars, acessions and clones		Genetic and chromosomal mutants	
Robusta (3) Apoatã (24) Kouillou (37)	Laurentii (4) Bukobensis	Angustifolia	Robusta 4n
# of distinct genotypes...............................68		**# of distinct genotypes..............................2**	

F$_1$, other interspecific hybrids and derivatives	
Robusta x *C. liberica*	Listed under *C. arabica* (13)
Robusta x *C. eugenioides*	
# of distinct genotypes.................……........15	

Coffea congensis

Introductions and clones		Variation	
Congensis (12) Bangelan (7)	Challotii Uganda (7)	Folha pequena	
# of distinct genotypes...............................27		**# of distinct genotypes..............................1**	

F$_1$ and other interspecific hybrids	
Congensis x *C. eugenioides* Listed under *C. eugenioides* (1)	Listed under *C. arabica* (3)
# of distinct genotypes.................................5	

Coffea eugenioides

Acession		F$_1$, other interspecific hybrids and derivatives	
Eugenioides (4)		Eugenioides x *C. kapakata*	Listed under *C. canephora* (1)
		Eugenioides x *C. salvatrix*	Listed under *C. liberica* (1)
		Listed under *C. congensis* (1)	Listed under *C. arabica* (4)
# of distinct genotypes.................................4		**# of distinct genotypes.............................9**	

Coffea liberica (suite)		
Acession	**F_1 and other interspecific hybrids**	
Liberica	Liberica 4n x Maragogipe 4n x Mundo Novo x Cera	Liberica x *C. eugenioides* x *C. eugenioides*
	Liberica x *C. eugenioides*	Listed under *C. robusta* (1)
# of distinct genotypes.........................1	**# of distinct genotypes.....................................4**	

Coffea dewevrei		
Acession	**F_1 and other interspecific hybrids**	
Excelsa 63	Excelsa x *C. kapakata* (2)	Listed under *C. arabica* (3)
# of distinct genotypes.........................1	**# of distinct genotypes.....................................5**	

Coffea kapakata		
Acession	**F_1 and other interspecific hybrids**	
Kapakata	Listed under *C. dewevrei* (2)	Listed under *C. eugenioides* (1)
	Listed under *C. arabica* (3)	
# of distinct genotypes.........................1	**# of distinct genotypes.....................................6**	

Coffea stenophylla	**Tricalisia spp**
Acession	**Acession**
Stenophylla (2)	Tricalisia
# of distinct genotypes.........................2	**# of distinct genotypes.....................................1**

Coffea salvatrix	
Chromosomal mutant	**F_1 and other interspecific hybrids**
Salvatrix 4n	Listed under *C. arabica* (7)
# of distinct genotypes.........................1	**# of distinct genotypes.....................................7**

Coffea racemosa	
Acession	**F_1, other interspecific hybrids and derivatives**
Racemosa (3)	Listed under *C. arabica* (2)
# of different genotypes.......................3	**# of different genotypes 2**

Psilanthus bengalensis
Acession
P. bengalensis
# of distinct genotypes 1

Concluding, this first attempt to air layer coffee indicates that this technique is an asset for conservation activities of endangered genetic germplasm resources that has to be maintained in living collections. It can be easily improved. The universality and advantageous perspectives of the technique are evident.

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REFERENCES

Bellair G., 1934. Traité d'horticulture pratique. (G. Doin e Cie, Editeurs.) Paris. 1120p.

Carvalho A., 1988. Principles and practice of coffee plant breeding for productivity and quality factors: *Coffea arabica*. In: Coffee, vol. **4**. (Clarke, R.J. and Macrae, R., eds). Elsevier Appl. Sci. p. 129-165.

Chevalier A., 1929. Les caféiers du globe. Encyclopédie Biologique. (Lechevalier P., ed.). Ch. Monnoyer, Les Mans. 196 p.

Cook O., F. 1911. Dimorphic branches in tropical crop plants. U.S. Dept. of Agriculture. Bureau of Plant Industry. Bulletin n.º 198. 63 p.

Coste R., 1955. Les caféier et les cafés dans le monde. Tome Premier. Editions Larose. Paris. 381p.

Cramer P.J.S., 1957. A review of literature of coffee research in Indonesia, (Wellman, F.L., ed.) Miscellaneous publication, n.º 15 SCI Editorial Inter-American Institute of Agricultural Sciences. Turrialba, Costa Rica. 262 p.

Ducos J.P., Gianforcaro M., Florin B., Petiard V., Deshayes A., 1999. 18Th Intl. Sci. Colloq. on Coffee. ASIC. Helsinki p. 295-307.

Fazuoli L.C., Medina-Filho H.P., Gonçalves W., Silvarolla M.B., Lima M.M.A., 1999. Coffee cultivars in Brazil. 18th Intl. Sci. Colloq. on Coffee. ASIC. Helsinki p. 396-404.

Haarer A.E., 1956. Modern coffee production. Leonard Hill Ltd. London. 467 p.

Janick J., 1968. A ciência da horticultura, Livraria Freitas Bastos S.A. Rio de Janeiro, 485p.

Krug C.A., 1936. Genética de *Coffea*. Plano de estudos em execução do Departamento de Genética do Instituto Agronômico de Campinas. Boletim n.º 26. Instituto Agronômico. 39 p.

Medina-Filho H.P, Carvalho A., Sondahl M.R., Fazuoli, L.C., Costa W.M., 1984. Coffee breeding and related evolutionary aspects. In: Plant Breeding Reviews, vol. **2** (Janick, J., ed). Avi Publ. Co. p. 157-193

Moraes M.V., Franco C.M., 1979. Método expedito para enxertia em café. Ministério da Indústria e do Comércio. Instituto Brasileiro do Café. 16 p.

Ramos L.C.S., Yokoo E.Y., Gonçalves W., 1993. Direct somatic embryogenesis is genotype specific in coffee. 15th Intl. Sci. Colloq. on Coffee. ASIC. Montpellier p. 763-766.

Reina E.H., 1966. La técnica del injerte hipocotiledonar del cafeto para el control de nematodos. Turrialba **7**: 5-11.

Snoeck J., 1988. Cultivation and harvesting of the Robusta coffee tree. In: Coffee vol. **4** (Clarke, R.J. & Macrae, R., eds). Elsevier Appl. Sci. p. 91-128.

Toogood A., 1999. Plant Propagation. Amer. Hort. Soc. DK Publ, Inc. New York, 320 p.

Outturn of Tetraploid Arabusta Hybrids (*C. canephora* 4n x *Coffea arabica*)*

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SUMMARY

The germplasm bank maintained as a living collection at the Instituto Agronômico de Campinas pocess several fertile Arabusta hybrids that are being studied as to several aspects concerning its biology of reproduction. Such hybrids are more rustic and productive than *C. arabica* and have a cup quality superior to *C. canephora*. Arabustas might represent a crop option for regions with climatic aptitude for *C. canephora* but marginal to *C. arabica*. Among several characteristics, the outturn percentage coffee was studied in 39 different Arabustas, 89 lines of *C. canephora* and 18 of *C. arabica*, cultivated and processed under comparable conditions. Higher ratios were observed among *C. canephora* (54 ± 6,9%) as compared to *C. arabica* (45 ± 4,5%). The Arabustas displayed a much inferior ratio (28 ± 7,2%). A more detailed observation of the Arabusta fruits indicated that 67% were devoid of at least oneseed as they floated in water and the remaining yielded 47% of peaberry seeds. A test of 9 different *C. arabica* indicate that in fruits pocessing a peaberry the outturn is reduced 22% in average. Thus, both floaters and fruits with peaberry account for the low outturn of the Arabustas. However, the basic underlying reasoning should be traced back to its interspecific hybrid origin and to the additional fact that the parental *C. canephora* is a self incompatible species.

INTRODUCTION

Several attempts of research institutes in coffee producing countries have been done toward the creation and growth of tetraploid interspecific hybrids of *Coffea canephora* x *C. arabica* in order to exploit the possibilities of combining in the hybrids the distinct attributes present in the parental species (Capot et al., 1968; Capot, 1972; Capot and Ake Assi, 1975; Vossen and Owuor, 1981; Cambrony, 1988; Sreenivasan et al., 1993; Yapo, 1995). These have been specially stimulated by the heterotic effects on vigor and yield (Mônaco et al., 1974; Mônaco, 1977; Carvalho, 1982), dominant gene action of leaf rust, CBD and nematode resistance alleles and the intermediate to good technological characteristics of the beverage (Mônaco and Carvalho, 1957; Bettencourt and Carvalho, 1968; Carvalho and Mônaco, 1975; Gutman et al., 1977; Mônaco and Carvalho, 1975). Arabustas, the coinned name of such hybrids, would be theoretically a good crop option for cultivation in hot, low lands, inappropriate or marginal to Arabicas but with edaphoclimatic aptitude for Robustas. Brazil is the leading producing country of *C. arabica* and the third largest producer of *C. canephora* in the world. Only in São Paulo, which is a traditional Arabica producing state, there is over 200.000 ha of land marginal to Arabicas with climatic aptitude for Robustas (Camargo et al, 1999; Medina-Filho et al., 1999) that would be potentially suitable for experiencing the cultivation of Arabustas.

After the pioneer tetraploid Arabusta hybrid obtained in 1950 that gave rise to the Icatu lines upon several backcrosses to Arabica and pedigree selections (Monaco et al., 1974; Carvalho, 1982), several others have been developed and planted at the Instituto Agronômico de Campinas. Such hybrids show exuberant vigor and have been yielding very well for many years, reason why further attention is starting to be paid in its characteristics considering the possibilities of its commercial exploitation.

The outturn of coffee is an important characteristic for, among others, two main reasons. In breeding programs the yield of individual trees or progenies are usually evaluated by the weight of cherries as this is much more simple and economical than obtaining the correspondent weight of dry, clean beans. However, evaluations of yield of different lines or trees based on weight of cherries are evidently only unbiased estimates if the outturns are equivalent. Otherwise, they must be compensated for the eventual differences in their rates. For a practical point of view, the outturn becomes an even more important trait since the harvesting operation of cherries accounts for over 40% of the production cost. If one takes into consideration the subsequent handling, drying, dehusking and the other post-harvest operations, the ratio of cherries to clean beans surely has a proportionally greater impact in the final revenue of the coffee farmer.

In this paper it is presented data on the outturn of several Arabustas in comparison to Robustas and Arabicas grown in Campinas (670m altitude, 22º 54'S latitude, 47º 05' W longitude, Cwa Köeppen Intl. Climate Classif., Red Clay Soil).

MATERIAL AND METHODS

Outturns of coffees investigated here refer to the percent ratios of clean beans weight relative to the weight of the correspondent fruits sun dried in the patio. Available samples ranging from 100g to 4 Kg of 31 cross distinct tetraploid Arabustas (*C. canephora* 4n x *C. arabica*), 89 lines/cultivars of *C. canephora* and 18 acessions/cultivars of *C. arabica* were assayed. Prior to drying, the cherries were tested in a recipient filled with tap water in order to determine the percentage of floaters. As peaberries turned out to be almost half of the observed seed type in the Arabustas, the influence in the outturn of fruits containing this type of seed compared to ones with two regular flat seeds were determined in samples of 6 regular Arabica cultivars and 3 accessions from Ethiopia. Patio dried fruits were individually cut open, classifyed as containing two normal flat or one peaberry seed and the pulp weighted to calculate the outturn of each fruit. Thirteen to thirty eight fruits of each type were used in the evaluations of each sample. Tests of significance, means, medians, quartiles, standard deviations and box-plot graphics to characterize the samples were performed with MINITAB 13 statistical software.

RESULTS AND DISCUSSION

As depicted in Figure 1, the different *C. canephora* assayed displayed a very high percent of outturn (54 ± 6,9%) as compared to the *C. arabica* cultivars/acessions (45 ± 4,5%). This is in close agreement with known determinations for both species confirming that Robustas have in general a better outturn than Arabicas. This is due to their thinner pericarp in most of the lines with less mucilage and sugars than Arabicas, although there exist considerable variation for this characteristic among the different lines of *C. canephora* in the germplasm collection of Campinas. However, the Arabustas displayed a very low percentage of outturn (28 ± 7,2%). Those values are statistically high significant ($F = 210,6$ $p \leq 0,001$) Around 50% of the hybrids were in the range of 22-32%. This represents aproximately half the value obtained for the Robustas where 50% of lines showed values between 51-58%. This indicates that this

characteristc constitutes an extreemely important selection criteria in a breeding program of Arabustas provided its ultimate consequence on net yield and the implications in the final revenue of the farmers.

OUT TURN %

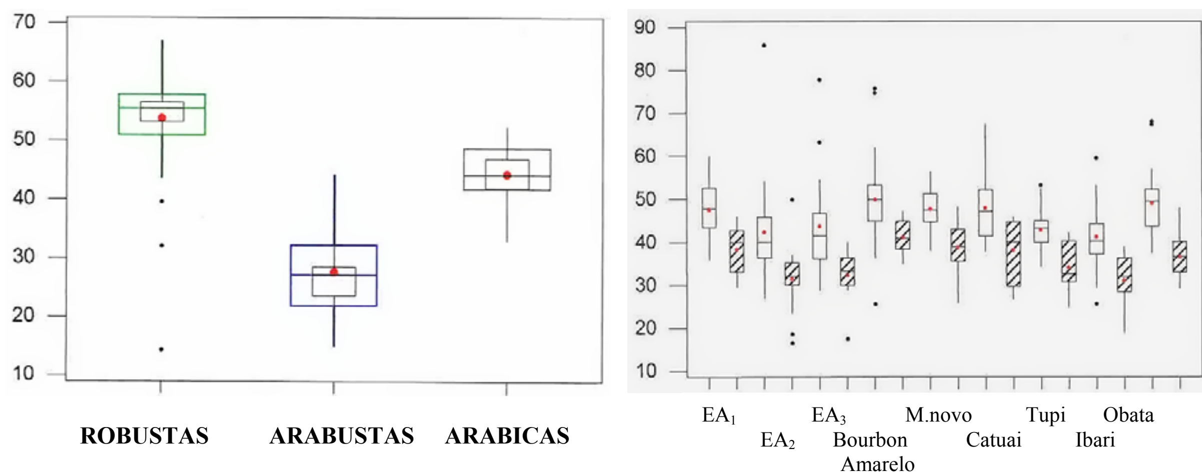

Figure 1. Left: Outturn percentage (clean bean/fruit patio dry weight x 100) of 89 lines of *C. canephora* (ROBUSTAS), 18 cultivars of *C. arabica* (ARABICAS) and 31 different tretraploid hybrids of *C. canephora* 4n x *C. arabica* (ARABUSTAS). Right: Outturn % of regular fruits pocessing 2 flat beans and of fruits with a peabean (slanted boxes) in 3 acessions from Ethiopia($EA_{1\text{-}3}$) and 6 cultivars of *C. arabica*. Box plots depict first and fourth quartiles (whiskers), median, mean (red dots), outliers (black dots) and 95% confidence intervals (inner boxes)

A more detailled observation of the Arabustas indicated a very high percentage (67%) of floaters as compared with Arabicas and Robustas (3-15%). This is probably the major reason for the low outturn of Arabustas. In a investigation with the Mundo Novo cultivar of *C. arabica* it was determined by Monaco (1960) that in an experimental sample constituted with 100% of floaters, the outturn was reduced 56% as compared to the sample without any floaters. Thus, it seems that floaters alone does not explain *in toto* the observed low outturn values obtained for the Arabustas. Indeed, the comparative study of the fruits containing two regular flat seeds relative to the ones with a single peaberry indicate (Figure 1) that in all nine Arabica coffees investigated, the outturn of fruit samples containing a peaberry seed was invariably lower (31-41%) than the ones with two normal flat seeds (41-50%), an average reduction of 22% ($p \leq 0{,}05$). Since peaberry containing fruits constituted 47% of the sinkers, these certainly reduced further the outturn of the Arabustas. Therefore, the low outturn of the Arabustas was due to the high percentages of both, floaters and fruits with peaberry seeds. Undesirable fruits containing one or both empty locules (with no or only one flat seed) normaly referred as floaters, as well as fruits containing one peaberry or mocha seed are due to interrelated phenomena. Krug (1937), Mendes (1946, 1949), Antunes Filho (1949), Mendes et al. (1954), report that empty locules result from the arrestment of the endosperm divisions after the ovary locule had already developed. Frahm-Leliveld (1940), Mendes and Bacchi (1940) pointed out that an early abortion of one of the two ovules or lack of proper pollination lead to the formation, due to mechanical reasons, of a peaberry seed in the other locule of the fruit. Probably, in the case of Arabustas, cytogenetically related collapse of endosperm or ovule abortion and lack of suitable pollinations might be factors causing high rates of peaberry seeds and floaters. Another, yet less likely cause, would be the ocurrence of alleles for endosperm abortion (Mendes and Medina, 1955). The basic reason of these problems lies in their own origin as interspecific hybrids made up of three genomes, being two from

C. canephora and the remaining one totally unbalanced from *C. arabica* (n=22 chromosomes) notwithstanding the fact that both are somewhat related (Medina Filho et al., 1984). Meiosis and fertility of tetraploid Arabustas have been studied (Grassias, 1977; Owuor and Van der Vossen, 1981; Boaventura and Cruz, 1987) indicating, as expected, chromosome pairing irregularities and low germination rates of pollen. Considering that *C. canephora* is a self incompatible species of gametophytic type (Mendes and Mendes, 1961), the presence of incompatibility alleles in the gametophytes of hybrids probably has additional effects lowering the fertility of the plants. In order to fully exploit the agronomic potentialities of the Arabustas, in depth studies of biology of reproduction must thus be undertaken.

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REFERENCES

Antunes Filho H., 1949. Melhoramento do cafeeiro. Bol. Suptda. Serv. Café. São Paulo. **24**: 241-246

Bettencourt A. J. and Carvalho A., 1968. Melhoramento visando a resistência do cafeeiro à ferrugem. Bragantia **27** (4): 35-68.

Boaventura Y.M.S. and Cruz N.D., 1987. Citogenética do híbrido interespecífico (*Coffea arabica* L. var Bourbon x *C. canephora* Pierre ex. Froehner (Linden) Chev.) que originou o café Icatú. Turrialba **37**: 171-178.

Camargo A. P, Camargo M. B. P., Fazuoli L. C. and Thomaziello R. A., 1999. Instituto Agronômico de Campinas (unpublished data).

Cambrony H.R., 1988. Arabusta and other interspecific fertile hybrids. In: Coffee. Vol. **4**. Agronomy. (Clarke, R.J. and Macrae, R., eds).Elsevier. Appl. Sci. p. 263-291.

Capot J., 1972. L'amélioration du caféier en Côte d'Ivoire. Les hybrides Arabusta. Café, Cacao, Thé **16**: 3-18.

Capot J. and Ake Assi L., 1975. Un nouveau caféier hybride de Côte d'Ivoire. Café, Cacao, Thé **19**: 3-4.

Capot J., Dupautex B. and Durandeau A., 1968. L'amélioration du caféier en Côte d'Ivoire. Duplication chromosomique et hybridation. Café, Cacao, Thé **12**: 114-126.

Carvalho A., 1982. Melhoramento do cafeeiro. Cruzamentos entre *C. arabica* e *C. canephora*. ASIC, 10º Colloque. Salvador. Brazil. p. 363-368.

Carvalho A., Monaco L.C. and Van der Vossen H.A.M., 1975. Café Icatú como fonte de resistência a *Colletotrichum coffeanum*. Ciência e Cultura. **27** (suplemento): 241-242.

Frahm-Leliveld J.A., 1940. Ontstaan en voorkomen van radboon en voosboon bij koffie. Bergcultures **14**: 1358-1362.

Grassias M., 1977. La fertilité chez les hybrides Arabusta. Méthodes d'estimation, influence du parent Arabica et relation avec le comportement méiotique. ASIC, 8º Colloque, Abidjan. p. 553-559.

Gutmann W., Werkhoff P., Barthels M. and Vitzthum O., 1977. Comparison by the headspace technique of the aroma profiles of the Arabusta coffee to those of the Arabica and Robusta varieties. ASIC, 8º Colloque, Abidjan. p. 153-161.

Krug C.A., 1937. Cytological observation in *Coffea*. III. J. Genet. **34**: 399-414.

Medina Filho H. P., Carvalho A., Sondahl M. R., Fazuoli L. C. and Costa W. M., 1984. Coffee breeding and related evolutionary aspects. In: Plant Breeding Reviews. Vol. **2** (Janick, J., ed) AVI Publ. Co. p. 157-193.

Medina Filho H.P., Fazuoli L. C., Guerreiro Filho O., Gonçalves W., Silvarolla M. B., Lima M. M. A. and Thomaziello R. A., 1999. Increasing the production of Robusta coffee in Brazil: The potential of 200.000 ha in the State of São Paulo. ASIC Colloque, Helsinki. P.390-395

Mendes A J. T. and Medina D. M., 1955. Controle genético dos frutos chochos no café Mundo Novo. Bragantia **14**: 87-99

Mendes A J. T., Medina D. M. and Conagin C.H.T.M., 1954. Citologia da ocorrência de frutos sem sementes no café Mundo Novo. Bragantia **13**: 257-279.

Mendes A.J.T., 1946. Partenogênese, partenocarpia e casos anormais de fertilização em *Coffea*. Bragantia **6**: 265-273.

Mendes A.J.T., 1949. Observações citológicas em *Coffea*. XIII. Uma nova forma tetraplóide. Bragantia **9**: 25-34.

Mendes A.J.T. and Bacchi O., 1940. Os grãos moca de café. Rev. Inst. São Paulo. **15**: 996-999.

Mendes C. H. T. and Mendes A. J. T., 1961. Pesquisas citológicas e genéticas em três espécies de *Coffea*: autoimcompatibilidade em *Coffea canephora* Pierre ex Froehner. Bragantia **20**: 787-804.

Monaco L.C., 1960. Efeito das lojas vazias sobre o rendimento do café Mundo Novo. Bragantia **19**: 1-12.

Monaco L.C., 1977. Consequences of the introduction of coffee rust into Brazil. Annals New York Acad. Sci. **287**: 57-71.

Monaco L.C. and Carvalho A., 1975. Coffee genetics and interspecific hybridization. ASIC, 7º Colloque. Hamburg. p. 497-504.

Monaco L.C. and Carvalho A., 1975. Coffee breeding for leaf rust resistance. In: ASIC, 7º Colloque. Hamburg. p. 437-445.

Monaco L.C., Carvalho A. and Fazuoli L.C., 1974. Melhoramento do cafeeiro: Germoplasma do café Icatu e seu potencial no melhoramento. 2º Congresso Brasileiro de Pesquisas Cafeeiras. Resumos. Poços de Caldas. p. 103.

Owuor J.B.O., and Van der Vossen H.A.M., 1981. Interspecific hybridization between *Coffea arabica* L. and tetraploid *C. canephora* P. ex Fr. I. Fertility in F_1 hybrids and backcrosses to *C. arabica*. Euphytica **30**: 861-866.

Sreenivasan M.S., Ram A.S. & Prakash N.S., 1993. Tetraploid interspecific hybrids in coffee breeding in India. ASIC 15º Colloque. Montpellier. p. 226-233.

van der Vossen H.A.M., Owuor J.B.O., 1981. A programme of interspecific hybridization between arabica and robusta coffee in Kenya. Kenya Coffee **46**: 131-137.

Yapo A., 1995. Amélioration qualitative de *Coffea canephora* Pierre par hybridation interspécifique: exploitation d'un nouveau schéme de sélection chez les Arabusta. ASIC, 16º Colloque, Kyoto. p. 655-662.

cDNA Cloning of Caffeine (Theobromine) Synthase from Coffee (*Coffea arabica* L.)

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SUMMARY

Caffeine synthase (CS), the *S*-adenosylmethionine(SAM)-dependent *N*-methyltransferase is a key enzyme for caffeine biosynthesis, since this enzyme is involved in the last two-methylation steps. The CS protein was purified from young tea leaves and subsequently the CS cDNA, named TCS, was isolated (Kato et al., 2000) To isolate cDNA clones encoding CS from coffee, we established a cDNA library from young coffee leaves and carried out screening of this. Oligonucleotides corresponding to the consensus sequences, which form the putative SAM binding region of TCS, were synthesized and used for RT-PCR as primers. The resulting PCR products were used to screen approximately 5.0×10^5 plaques from a coffee cDNA library. Finally, independent five cDNA clones, termed CCS clones, were isolated and analyzed those sequences. The predicted amino acid sequences of the CCS clones are over 80% identical among those of the clones and share almost 40% identity with those of TCS and salicylic acid carboxyl methyltransferase from *Clarkia breweri*, respectively. The molecular masses of these proteins were almost the same (approximately 43 kDa). These values agree well with that of TCS protein (41kDa). We have established expression systems of CCS cDNAs in *E. coli.* Some transformants have produced recombinant proteins. Theobromine producing activity was confirmed in some kinds of recombinant proteins.

INTRODUCTION

Extensive metabolic studies of purine alkaloids in leaves of coffee and tea (*Camellia sinensis*) have elucidated the caffeine biosynthesis pathway in some detail. The available data support the operation of a xanthosine → 7-methylxanthosine → 7-methylxantine → theobromine → caffeine pathway as the major route to caffeine. In addition, there is one report of an alternative entry in the caffeine biosynthesis pathway in coffee that involves conversion of xanthosine 5'-monophosphate (XMP) → 7-methyl XMP → 7-methylxanthosine. Little is known about the properties of enzymes that participate in the caffeine biosynthesis pathway; the pathway contains three *S*-adenosylmethionine (SAM)-dependent methylation steps, indicating that *N*-methyltransferases play an important role. Recently, we show that the gene encoding caffeine synthase, termed CS, which catalyzes the most crucial final two methylation steps of caffeine biosynthesis was cloned from young tea leaves (Kato et al., 2000; Kato et al., 1999). To isolate cDNA clones encoding CS from coffee, we established a cDNA library from young coffee leaves and carried out screening of this.

RESULTS AND DISCUSSION

To isolate coffee CS cDNA fragment, RT-PCR was carried out. We searched the databases for sequences similar to that of tea CS (TCS1), using BLAST 2.0 program. The search identified several proteins encoded by *Arabidopsis* genes of unknown function that were approximately 40% identical to TCS1. Three upstream and downstream degenerated oligonucleotide primers corresponding to conserved regions were synthesized and performed PCR for amplification of CS cDNA in coffee. The reactions were found to amplify DNA fragments of expected sizes. The deduced amino acid sequences of the two RT-PCR products showed approximately 40 % sequence similarity to the corresponding amino acid sequence from TCS1. The RT-PCR product was used as a CCS probe for screening of cDNA library. A cDNA library constructed using poly(A)$^+$RNA from young coffee leaves was screened to isolate full-length CCS cDNA clones. Approximately 5.0×10^5 pfu were screened with the CCS cDNA probe. Finally, five independent clones were isolated and named CCS1, CTS1, CTS2, CCS3, and CCS4, respectively. The CTS1 cDNA has 1303 nucleotides, and it has an open reading frame of 378 codons. The calculated molecular mass of the protein encoded by this open reading frame is 42,700. The value agrees well with that of TCS protein (41kDa). The predicted amino acid sequences of the other CCS (CTS) clones are over 80% identical among those of the clones and share almost 40% identity with those of TCS and salicylic acid carboxyl methyltransferase from *Clarkia breweri* (Ross et al., 1999), respectively (Table 1).

Table 1. Sequence similarity and the degree of relatedness among CS family and the related methyltransferases

	Motif A	Motif B	Motif C
CCS1 (Coffee)	VADLGCASG	---------------	PGSFYSRLFP
CTS1 (Coffee)	VADLGCASG	---------------	PGSFYGRLFP
TCS1 (Tea)	AADLGCAAG	---------------	PGSFHGRLFP
SA-MT (*Clarkia breweri*)	IADLGCSSG	---------------	PGSFYGRLFP
NTR1 (Chinese cabbage)	IADLGCSSG	---------------	PGSFYGRLFP
BA-MT (Snapdragon)	MMDMGCSSG	---------------	PGSFYGRLLP
Putrescine NMT (Tobacco)	VLIIGGGIG	---------------	ANFNDPRVTL
Caffeoyl-CoA OMT (Tobacco)	LVKIGGLIG	--VAPPDAPLRKY--	ALAADSRIEI
Catechol OMT (Tobacco)	IVDVGGGTG	--VPKADAIFMKW--	ALPANGKVII
myo-inositol OMT (Ice plant)	LVDVGGNIG	--IPQADAIFMKW--	SLAKGGKIIL
Consensus OMT	LVDVGGXXG	--VAXADAXXXKW--	ALAXXAKVEL
	ILKI C	IP P Y	GIG GRIII
	V	FE G F	PVP P V
			S S S

Three amino acid sequence motifs that could be involved in the binding of the methyl donor SAM are conserved in most of the plant SAM-dependent *O*-methyltransferases (Joshi and Chiang, 1998) (Figure 1).

However there is no motif B in the amino acid sequences of CCS (CTS) series and the related methyltransferases, these enzymes define a new class of plant methyltransferases. Northern blot analysis of total RNA extracted at various stages of leaf expansion showed that the CCS (CTS) transcripts were present at higher levels during the early stages of leaf expansion (Figure 2).

1	2	3	4	5	6	7	8	9	
100 %	93.2	84.4	80.8	80.0	39.5	39.7	38.6	37.1	1:CTS1
	100	85.2	82.9	82.3	38.4	39.1	39.2	37.2	2:CTS2
		100	82.9	81.6	39.5	40.6	37.7	37.4	3:CCS1
			100	95.6	40.7	39.5	39.0	36.3	4:CCS3
				100	40.3	40.0	39.0	37.1	5:CCS4
					100	41.8	38.4	38.5	6:TCS1
						100	42.8	43.9	7:SA-MT
							100	41.3	8:NTR1
								100	9:BA-MT

CTS1 and CTS2 : *Coffea arabica* theobromine synthase

CCS1, CCS3 and CCS4 : *Coffea arabica* caffeine synthase

TCS1 : *Camellia sinensis* caffeine synthase (AB031280)

SA-MT : *Clarkia breweri* *S*-adenosyl-L-methionine (SAM):salicylic acid carboxyl methyltransferase (AF133053)

NTR1 : *Brassica rapa subsp. pekinensis* floral nectary-specific protein (AAF22289)

BA-MT : *Antirrhinum majus* SAM:benzoic acid carboxyl methyltransferase (AF198492)

Figure 1. Comparison of amino acid sequences of the CCS (CTS) series with related methyltransferases

The Sequences of CCS1 at residues 57-65, and 138-147 are compared with those of the proposed SAM-binding motifs of typical methyltransferases. There is no motif B in the amino acid sequences of CS family and the related methyltransferases.

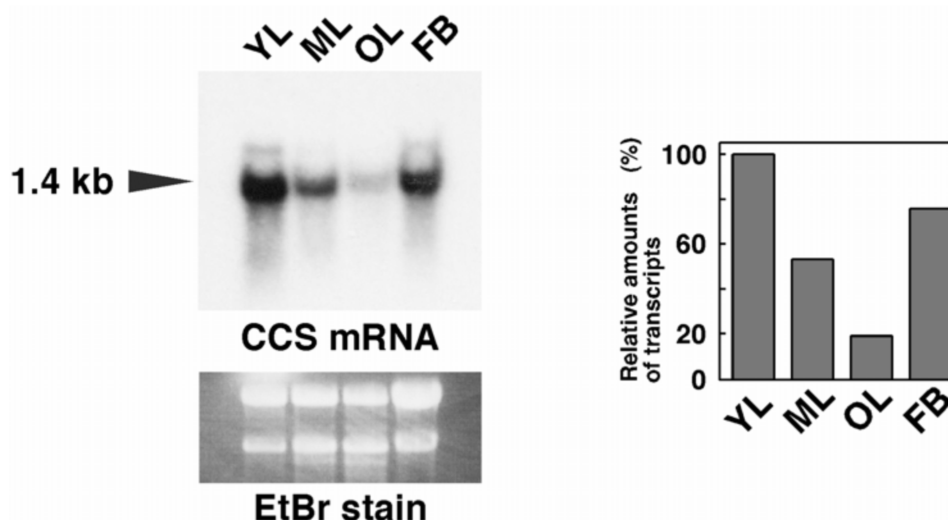

Figure 2. Northern blot analysis of total cellular RNAs from coffee tissues. Abbreviations: YL, young leaves; ML, mature leaves; OL, old leaves; FB, flower buds

Total RNAs (10 μg) of leaves at various stages and of flower buds were separated by agarose gel electrophoresis, transferred onto membrane, and then probed by ^{32}P-labeled cDNA fragment encoding CCS1. There are many transcripts of the genes for CCS (CTS) series in young leaves and flower buds.

And besides, there are many transcripts of the genes for CCS (CTS) in flower buds (Figure 2). Expression plasmids for CTS1 and CTS2 were constructed in a pET-23d (Novagen) vector. Recombinant CTS1 and CTS2 were produced in *E. coli* BL21 (DE3). The recombinant proteins were extracted by sonication of the transformed cells in 50 mM Tris-HCl (pH 7.5) containing 1 mM EDTA-Na_2 and 0.1 M NaCl. When the crude extracts were used for enzyme assay, CTS1 and CTS2 have only 3-*N*-methylation activity (Table 2).

Table 2. Substrate specificity of recombinant coffee enzymes

Substrate	Methylated product	*N*-methylation position	CTS1	CTS2	TCS1(tea)[1)]
Monomethylxanthines					
7-Methylxanthine	Theobromine	*3*	100	100	100
3-Methylxanthine	Theophylline	*1*	n.d.	n.d.	1.0
1-Methylxanthine	Theophylline	*3*	n.d.	n.d.	12.3
Dimethylxanthines					
Theobromine	Caffeine	*1*	n.d.	n.d.	18.5
Theophylline	Caffeine	*7*	n.d.	n.d.	<0.1
Paraxanthine	Caffeine	*3*	1.4	1.1	230
Others					
Xanthosine	7-Methylxanthosine	*7*	n.d.	n.d.	n.d.

N-Methyltransferase activity is expressed as percentage of the activity on 7-methylxanthine.

The cloning of the caffeine (theobromine) synthase gene is an important advance towards the development of transgenic caffeine-deficient *Coffea arabica* and *Camellia sinensis* plants through antisense mRNA technology or by gene silencing.

REFERENCES

Joshi C.P. and Chiang V. L. *Plant Mol. Biol.* **37**, 663-674 (1998)

Kato M. et al. *Plant Physiol.* **120**, 579-586 (1999)

Kato M. et al. *Nature* **406**, 956-957 (2000)

Ross J.R. et al. *Arch. Biochem. Biophys.* **367**, 9-16 (1999)

Progeny Analysis by Microsatellites in Crosses of *Coffea arabica* L.

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SUMMARY

Genetic polymorphisms are a fundamental tool for studying the rules governing inheritance as well as for tracing specific genes in breeding programmes. In the last decade it was discover that DNA itself is a major source of polymorphism. *Coffea arabica* is expected to show polymorphic DNA sequences as any other species even if, this species could show a low level of genetic variability because of the restricted genetic base of the cultivated varieties and because of self-fertility. Nevertheless we could identify some DNA sequences which show a high degree of polymorphism, namely microsatellites.

Microsatellites, also known as simple sequence repeats (SSRs), are produced by tandem repetition of sequences from 1 to 6 bp long and constitute highly informative markers. They are inherited in a codominant Mendelian manner and they are somatically stable. The polymorphisms are the result of the variation in the number of the repeated monomers. Primers can be designed on the single sequences flanking the microsatellite and then used to amplify the locus by PCR. Simple gel electrophoresis reveals polymorphic variations in the size of the amplification product. Microsatellites have already been found to be useful as genetic markers in a number of plants such as soy bean, *Arabidopsis*, barley, rice, corn, tomato and other plants.

Here we report the analysis of two *C.arabica* crosses with some microsatellite to show their Mendelian inheritance and their use in molecular assisted breeding programmes.

In the first cross we analysed 51 microsatellite and the parents showed different alleles for 4 systems. All the progeny had the same alleles as expected by Mendelian inheritance. For instance, the analysis of the E10-3CTG microsatellite gave the following results: the mother was homozygote for the 135bp allele and the father was homozygote for the 137bp allele, all the progeny was heterozygote 135/137. This cross was performed with the aim of selecting plants resistant to nematodes and we should be able to follow the inheritance of the resistance gene/s when more polymorphisms become available.

The second cross was analysed with 30 microsatellites, two of which proved to be homozygote in the parents while all the progeny was heterozygote as reported in the table below.

Microsat.	PARENTS		F_1 PROGENY					
	Icatuaì	IAPAR-59	I-30-1-1	I-30-1-2	I-30-2-1	I-30-2-2	I-30-3-1	I-30-3-2
34-6CTG	108/108	112/112	108/112	108/112	108/112	108/112	108/112	108/112
37/6CTG	121/121	119/119	119/121	119/121	119/121	119/121	119/121	119/121

More microsatellite should be developed and their association to interesting gene should be established for a practical use in breeding programmes.

INTRODUCTION

The cultivated varieties of *Coffea arabica* show a very low level of genetic diversity (Bertraud and Charrier, 1988) due to autogamy and the limited number of original plants from which the main cultivars were derived. Consequently, there are very few allelic variants, making it difficult to find polymorphisms. Numerous techniques have been applied to study polymorphisms in *C. arabica*, such as RFLP (Lashermes et al., 1996a), RAPD (Orozco-Castillo, 1994; Lashermes et al., 1996b), and AFLP (Lashermes et al., 2000), and positive results have in fact been obtained. Nonetheless, the same techniques applied to other species have provided a greater number of polymorphic loci.

Another approach to studying variability in *C. arabica* and identifying polymorphism is the analysis of microsatellites. These highly polymorphic repeated sequences are very informative molecular markers because they are codominant and therefore, in contrast to the abovementioned techniques, enable the heterozygous samples to be distinguished from the homozygous. Due to these characteristics microsatellites are powerful tools for following specific genes in assisted cross programmes.

In this paper we describe the analysis by microsatellites of different crosses of *Coffea arabica* and of its progeny, F_1 or F_2.

Furthermore, we begun a project of RFLP analysis of different varieties of *C. arabica* and of *C. canephora* for coding sequencies. The aim of this is twofold: firstly, to find markers for the construction of a low density genetic map and secondly, to create the possibility of finding differences within the genes of *Coffea arabica*, that is, polymorphisms capable of marking the expressed genes

MATERIALS AND METHOD

Amplification of the microsatellites

The microsatellites used for the analysis of the crosses were identified and amplified as described in Rovelli et al. (2000), beginning with two genomic libraries of *C. a*. var Caturra enriched in di- tri- nucleotides TG and ATC.

Identification of the polymorphic microsatellites

The primers which amplified the microsatellites were tested on DNA of the parent samples of three crosses; if the microsatellites presented different alleles from their parents, we proceeded with the analysis of the progeny.

The amplification products were analysed on sequencing gel with an ABI automatic sequencer and the length of the fragments containing microsatellites was calculated with the GENESCAN 672 (Perkin Elmer) programme.

Samples

The samples examined were: 1) *C. a.* var Caturra x *C. a.* var Ethiopica ET-30 cross, and 96 plants of the F2 population, originating from IRD (Montpellier, France); 2) *C. a.* introgressed genotype Catimor x *C.a.* var Icatuaì cross, and 6 plants of the F1 population, originating from IAPAR (Londrina, Brazil); 3) *C. a.* introgressed genotype Sarchimor x *C. a.* var Ethiopica ET-6 cross and 17 plants of the F1 population, originating from CATIE (Turrialba, Costa Rica).

The DNA was extracted with a modification of the method of Murray and Thompson (1980) and Orozco-Castillo et al. (1994).

RFLP analysis

The fragments of DNA examined are traits of genomic DNA amplified with primers designed on EST sequences; the EST were derived from a genomic library of radical meristems of *C. a.* var Bourbon red.

For the RFLP analysis we chose those fragments which provided an amplification from genomic DNA of greater length than the corresponding cDNA, and which therefore presumably contained introns.

RESULTS

Analysis of the crosses

The *C. a.* var Caturra x *C. a.* var Et-30 cross was analysed with 59 microsatellites. Only five of these proved to be polymorphic in the parental samples. The F_2 population of 96 plants was analysed with 5 microsatellites to examine the distribution of the alleles. Table 1 summarises the results.

Three out of 28 microsatellites analyses in the introgressed genotype Catimor x *C. a.* var Icatuaì cross demonstrated different alleles in the parents, and therefore we analysed the 6 plants of the F_1 population. Table 2 shows the alleles, expressed in bp, relative to the three polymorphic microsatellites.

The introgressed genotype Sarchimor x *C. a*. var Et-6 cross was analyses with 51 microsatellites and four were polymorphic in the parents, and of these we analysed the 17 F_1 plants. The alleles of these are shown in Table 3.
In total 8 polymorphic microsatellites were identified.

RFLP analysis

48 EST sequences were amplified of which 13 produced a genomic amplification with a length greater than the corresponding EST. We therefore analysed these 13 genomic loci, presuming that they also contained introns, where mutations are more likely to accumulate. Table 4 shows the names of the genes with which the EST sequences were the most homologous in the database.

Varieties of *C. arabica* (Ethiopica, Caturra, Mundo Novo, Laurina and a wild variety) and *C. canephora* were examined.

These traits of genomic DNA were analysed with 8-11 restriction enzymes (AciI, AluI, BamHI, EcoRI, FokI, HindIII, HinfI, HphI, MboI, MnlI, MspI, Tsp45I, Tsp509I). The analysis of the restriction patterns to date have not uncovered differences between the varieties of *C. arabica* in the size and number of bands.

Table 1.

Microsatellite	**Alleles (expressed in bp)**		
	C.a. Caturra	C.a. Et-30	F 2
I9-3CTG	200-214	198-200-214	36 samples: 200-214 48 samples: 198-200-214 12 samples: 198-214
17-2CTG	204-215	202-215	39 samples: 204-215 32 samples: 202-204-215 25 samples: 202-215
32-2CTG	121-128	119-126	40 samples: 119-121 56:121-126
E10-3CTG	135	137	23 samples: 135 43 samples: 135-137 30 samples: 137
14-2CTG	130	128-130	96 samples: 130

Table 2.

Microsatellite	**Alleles (expressed in bp)**		
	Catimor	C.a. Icatuaì	F1
20-6CTG	105-109	105-107-109	2 samples: 105-109 4 samples: 105-107-109
37-6CTG	119	121	6 samples: 119-121
24-4CTG	112	108	6 samples: 108-112

Table 3.

Microsatellite	**Alleles (expressed in bp)**		
	Sarchimor	C.a. Et-6	F1
I9-3CTG	200-214	198-200-214	10 samples: 200-214 7 samples: 198-200-214
14-2CTG	204-217	202-217	17 samples: 202-204-217
E10-3CTG	135	137	17 samples: 135-137
20-6CTG	104-106-108	104-106	17 samples: 104-106-108

Varieties of *C. arabica* (Ethiopica, Caturra, Mundo Novo, Laurina and a wild variety) and *C. canephora* were examined.

Nonetheless, we could identify 5 different patterns between the two species of *Coffea arabica* and *Coffea canephora*. Table 5 shows the size of the different bands of the two species.

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Table 4.

Clone	Homology	Clone	Homology
RM-0-L19	unknown	RM D04	thioredoxin h
RM B11	translationally controlled tumor protein (TCTP)	RM-0-I05	antimicrobial peptides precursor
RM A11	A.thaliana hypothetical protein	RM-0-E12	germin-like protein
RM C05	chlorophyll a/b-binding protein	RM B08	unknown
RMi-5-B10	60S ribosomal protein L22	RM B10	40S ribosomal protein S23
RMi-1-E03	cysteine proteinase	RM A01	60S ribosomal protein L34
RM C11	A.thaliana hypothetical protein		

DISCUSSION

In total we identified 8 polymorphic microsatellites in varieties of *C. arabic*a and 5 polymorphic restriction sites amongst *C. arabica* and *C. canephora*.

Table 5.

EST	ENZYME	*C. canephora* (in bp)	*C. arabica* (in bp)
RM A11	MnlI	600	400 + 200
Rmi-5-B10	MspI	not cut	900 + 800
RM C11	AluI	600	500 + 100
RM C11	MboI	not cut	700 + 300
RM D04	MspI	800 + 100	not cut

With regard to the microsatellites, we found between one and three alleles in the parental samples. Where there were two or three alleles, one of these, such as the microsatellites I9-3CTG or 17-2CTG, was always present both in the parents and the progeny. We therefore hypothesised that another locus was involved which became coamplified. Only the microsatellite 20-6CTG in the *C. a*. Sarchimor x *C. a*. Et-6 cross presents three alleles in one parent and the progeny, but two alleles in the other parent. In this case there could be three alleles for the same locus; however, double haploid plants would be necessary to verify this.

The analysis of the microsatellites such as E10-3CTG, 37-6CTG or 24-4CTG suggests that even in this allotetraploid species the microsatellites are inherited in accordance with Medel's

laws: when the parental samples displayed one allele in homozygosis but the parents had different alleles, then the F_2 displays the classic Mendelian distribution of 1:2:1.

The microsatellites can be used to follow the traces of certain genes within specific crosses. Indeed, the *C. a*. Sarchimor x *C. a*. Et-6 cross was produced with the intention of selecting plants resistant to nematodes. With the identification of new polymorphic microsatellites we should be able to verify the distribution of the progeny of genes linked to resistance.

In general it is worth noting that the parental samples are heterozygotes only in 7 out of 24 loci: this demonstrates the high level of homozygosity in which the species *C. arabica* is found and explains the high genetic uniformity of the species.

The RFLP polymorphisms to date have not provided positive results within the species *C. arabica*, but even polymorphisms between the two species are highly useful. Moreover, each polymorphism found in this manner is related to differences in coding regions which could account for the phenotypic differences between the two species, as well as identify the genes originating from *C. canephora* in inter-specific crosses, in which attempts are made to bring the positive qualities of resistance of *C. canephora* to the cultivated varieties of *C. arabica*.

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REFERENCES

Bertraud J. and Charrier A., Genetic Resources in Coffea. 1988 In “Coffee”, Vol. **4**, Agronomy, R.J. Clarke and R. Macrae Eds, Elsevier Applied Science, London, pp. 1-41.

Lashermes P., Cros J., Combes M. C., Trouslot P., Anthony F., Hamon S. and Charrier A., 1996a “Inheritance and restriction fragment length polymorphism of chloroplast DNA in the genus *Coffea* L.” Theor Appl Genet **98**: 626-632.

Lashermes P., Trouslot P., Anthony F., Combes M. C. and Charrier A., 1996b “Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*.” Euphytica **87**: 59-64.

Lashermes P., Andrzejewski S., Bertrand B., Combes M. C., Dussert S., Graziosi G., Trouslot P. and Anthony F., 2000 “Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.)” Theor Appl Genet **100**: 139-146.

Murray M. G. and Thompson W.F., 1980 “Rapid isolation of high molecular weight plant DNA” Nucleic Acis Res **8**: 4321-4325.

Orozco-Castillo C., Chalmers K. J., Waugh R. and Powell, 1994 “Detection of genetic diversity and selective gene introgression in coffee using RAPD markers” Theor Appl genet **87**: 934-940.

Rovelli P., Mettulio R., Anthony F., Anzueto F., Lashermes P., Graziosi G., 2000 “Microsatellites in *Coffea arabica* L.” In “Coffee Biotechnology and Quality” Sera T., Soccol C. R., Pandey A. and Roussos S. Eds, Kluwer Academic Publishers, pp 123-133.

Relations between and Inheritance of Chlorogenic Acid Contents in an Interspecific Cross between *Coffea pseudozanguebariae* and *C. liberica* var *dewevrei**

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INTRODUCTION

In coffee trees, chlorogenic acids (CGA) mainly includes three classes: the caffeoylquinic acids (CQA), the dicaffeoylquinic acids (diCQA) and the ferulylquinic acids (FQA), each class containing three isomers). An interspecific cross between *Coffea pseudozanguebariae* (PSE) with low CGA content and *C. liberica* var *dewevrei* (DEW) high CGA content was investigated for chlorogenic acid (CGA) contents in F1 and back-cross hybrids. The aim was to analyse CGA inheritance and relationships between CGA classes and isomers. Results constitute a model for the genetic improvement of *C. canephora* using interspecific crosses with PSE.

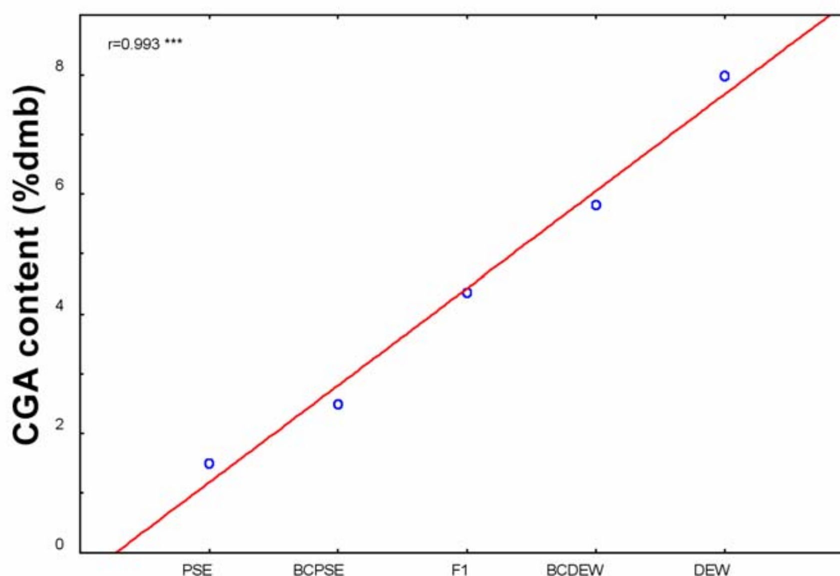

Figure 1. Regression analysis showing additivity of CGA content

CGA INHERITANCE

1. The importance of environmental effects (years + interactions) varies from 22% (5-CQA) to 65% (3,4-diCQA).
2. Additivity is found for 5-CQA, 3,4-diCQA and 4,5-diCQA isomers.
3. Additivity is verified, but after data transformation, for 3-CQA, 3,5-diCQA, and 5-FQA.

RELATIONSHIP BETWEEN CGA ISOMERS

1. Relationships between CGA isomers are curvilinear (Figure 2) or curvilinea (Figure 3).
2. The shape of the relation explains the use of transformation for additivity test.
3. The presence and the importance of the relation agree with our current knowledge on CGA pathways.

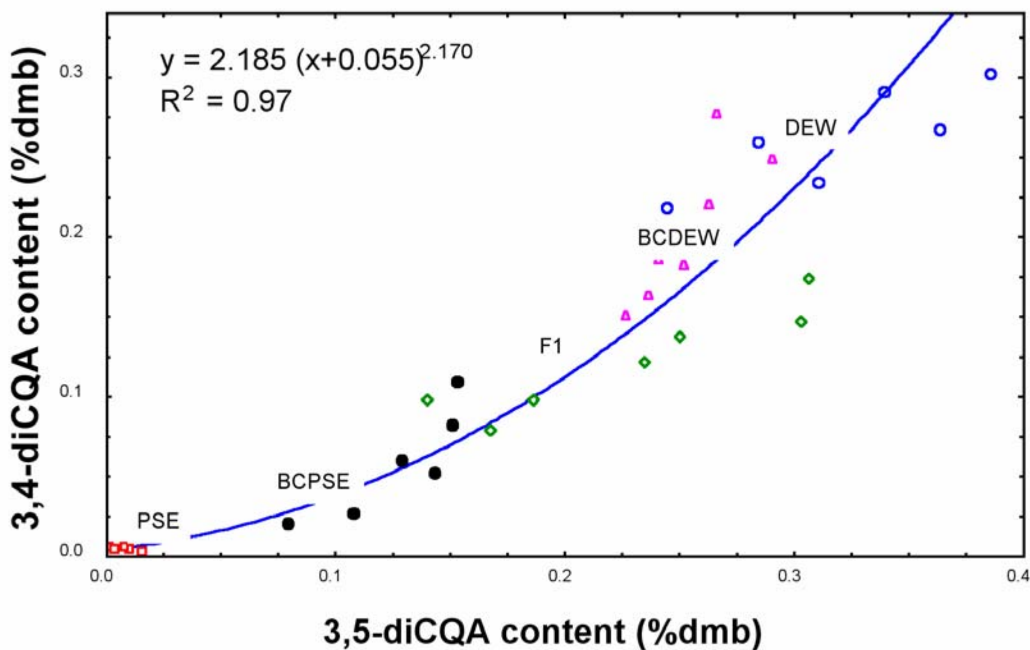

Figure 2. Curvilinear relationship between 3,4-diCQA and 3,5-diCQA contents

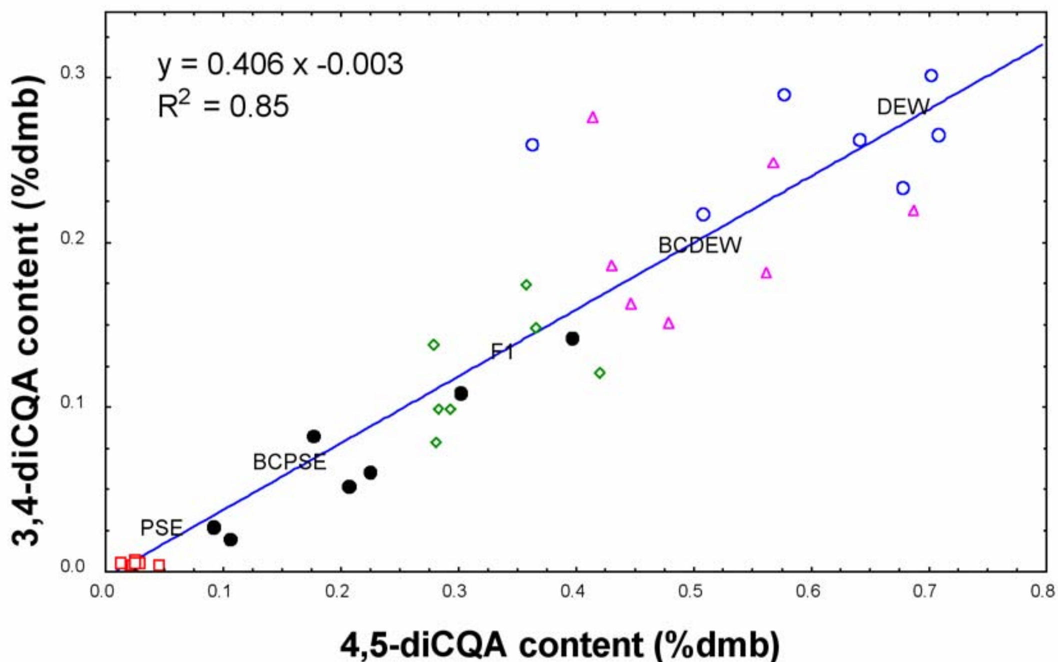

Figure 3. Linear relationship between 3,4-diCQA and 4,5-diCQA contents

Inheritance of Caffeine and Heteroside Contents in an Interspecific Cross between a Cultivated Coffee Species *Coffea liberica* var *dewevrei* and a Wild Species Caffeine-free *C. pseudozanguebariae**

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INTRODUCTION

Interspecific hybrids were obtained between *C. pseudozanguebariae* (PSE), a wild caffeine-free species and a formerly cultivated species *C. liberica* var. *dewevrei* (DEW) for which caffeine content (CAF) is about 1% dmb. The wild species is also the only species having a diterpenic heteroside (HET) in their beans, this compounds leading to a strong bitterness. The aim was to describe the inheritance of caffeine and heteroside contents in the first and second generations of this cross, as well as relationship between the two compounds. The preliminary results presented here constitute a model for the genetic improvement of *C. canephora* using interspecific crosses with PSE.

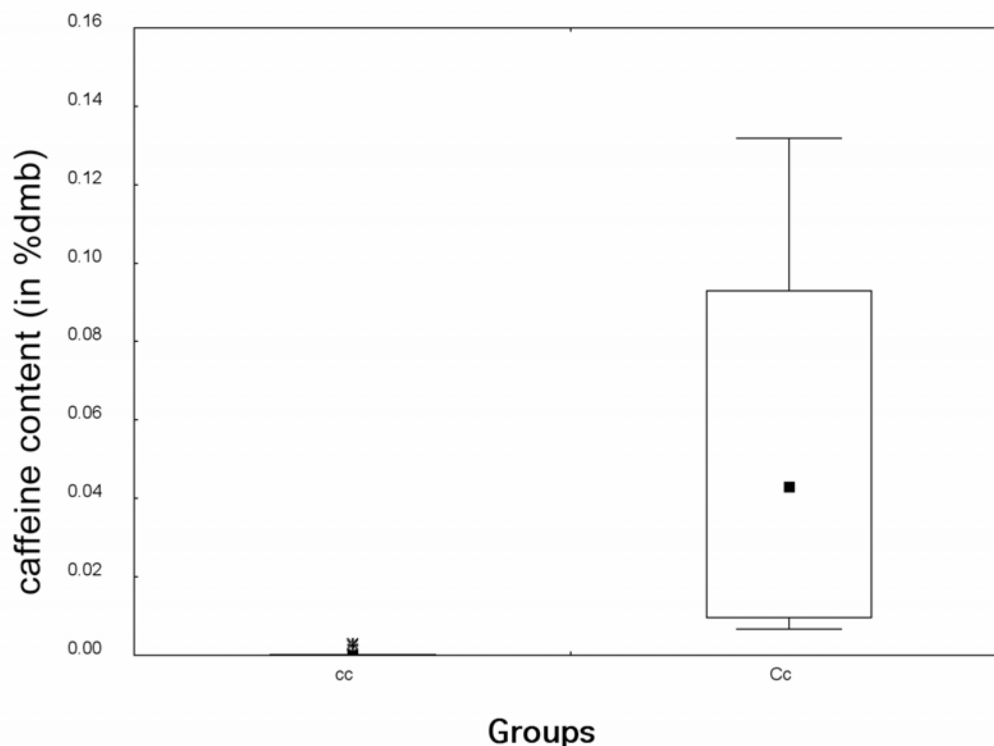

Figure 1. Distribution of caffeine content in the BCPSE backcross. Two types of hybrids: 10 trees close to zero (cc) and 7 trees with higher amount of caffeine (cC)

CAFFEINE INHERITANCE

1. CAF heritability *s.l.* is high (94.6%) showing the little effect of environment on its variation.
2. Caffeine absence is controlled by one recessive gene (Figure 1).
3. CAF is additive: in F1 hybrids, CAF is 0.17% dmb, i.e. 60% lower than the parental average. Nevertheless, additivity is respected for the square root of CAF as well as for
4. F1 hybrids than for backcross hybrids with caffeine.

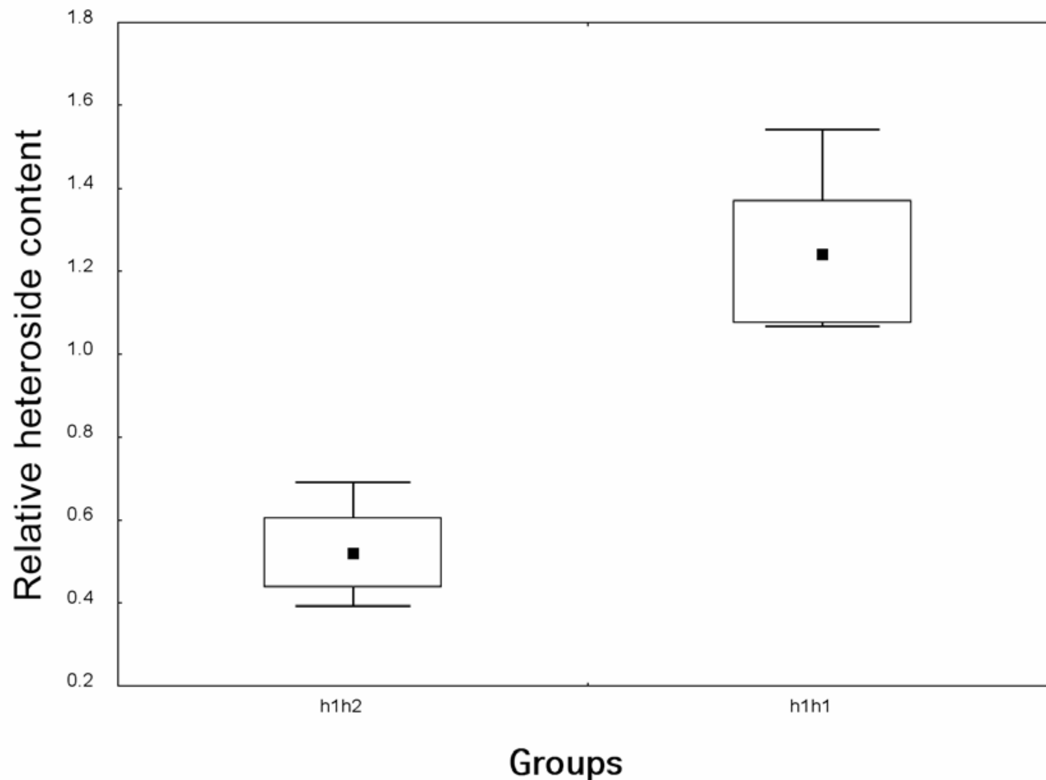

Figure 2. Distribution of heteroside in the BCPSE backcross. Two types of hybrids: h1h2 (nine genotypes) close to the F1 average and h1h1 (eight genotypes) close to the PSE average

HETEROSIDE INHERITANCE

1. HET heritability *s.l.* is high (92%) showing the little effect of environment on its variation.
2. Heteroside is controlled by one major gene with two co-dominant alleles (Figure 2).
3. HET is an additive trait in F1 hybrids as well as in backcross hybrids.

RELATIONSHIP BETWEEN CAFFEINE AND HETEROSIDE CONTENTS

In second-generation hybrids with caffeine and heteroside, an hyperbolic relationship (Y = 0.03/X+0.08) exists between HET and CAF.

Interspecific Genetic Linkage Map, Segregation Distortion and Genetic Conversion in Coffee (*Coffea* sp.)*

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INTRODUCTION

An interspecific partial genetic linkage map of Coffea sp. based on 62 backcross hybrids is presented. F1 hybrids were generated by a cross between the wild *C. pseudozanguebariae* (PSE) and the formerly cultivated C. liberica var. dewevrei (DEW). Progeny derived from a backcross between F1 hybrid and DEW. The map construction consisted on a two-step strategy using 5.5 and 3.1 LOD score revealed by simulation file.

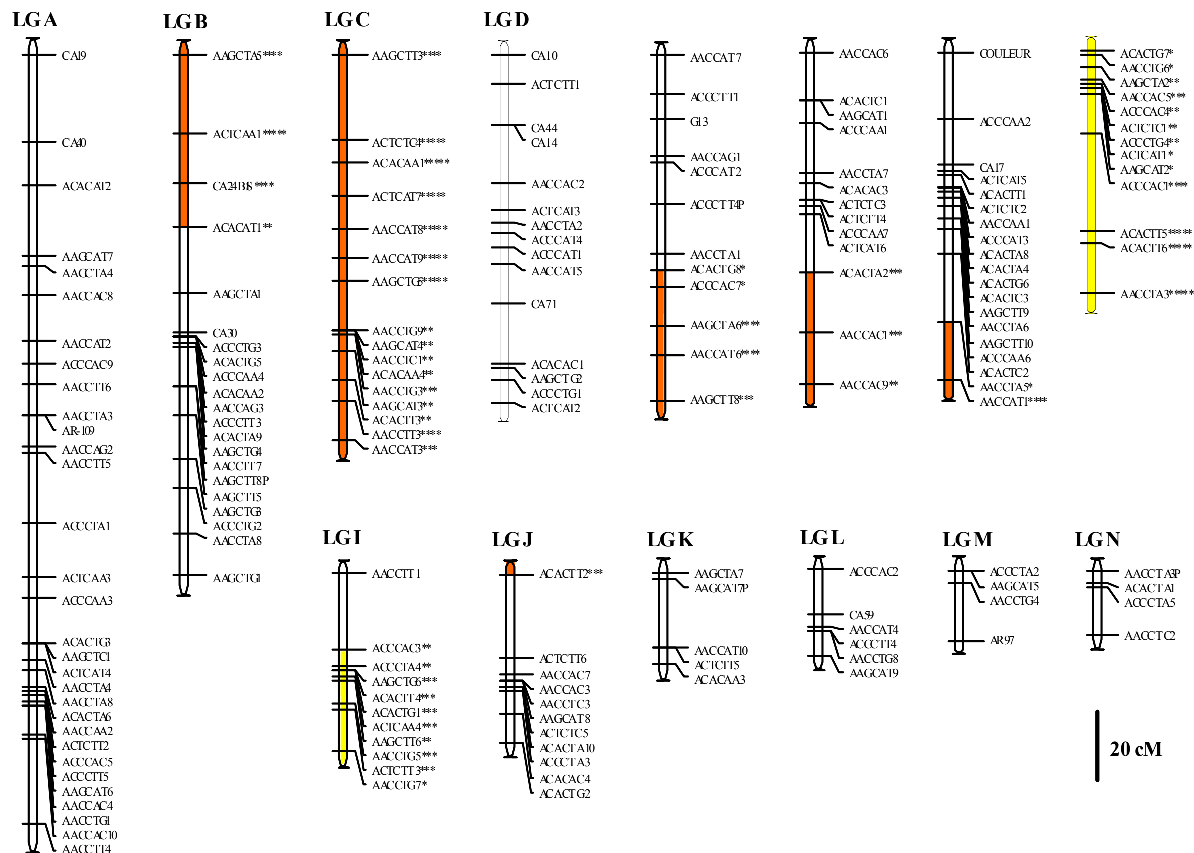

Figure 1. Genetic map obtained from an interspecific backcross BCDEW

THE GENETIC MAP

1. The map consisted of 180 loci: 167 AFLP and 13 RFLP.
2. The markers were assem-bled into 14 linkage groups, each with 4 to 31 markers covering 1144 cM.

3. The orange and yellow segments correspond to dis-tortion segregation in favour to PSE and DEW respecti-vely. Loci marked with stars (*) deviated from (1:1) ratio at $p < 0{,}01$ (Figure 1).

MAIN RESULTS CONCERNING SEGREGATION DISTORTION

1. The figure 2 clearly shows a predominant fraction of markers presenting a Mendelian segregation of (1:1), and two categories of segregation distortion following a (3:1)
2. ratio in favour to PSE and a (1:3) ratio in favour to DEW.
3. Segregation distortion was observed for 30% of all loci, with a particular (3:1) and (1:3) ratios, equally favouring each of the two parents.
4. The origin of such ratio suggests the implication of genetic conversion events, implying DNA heteroduplex formation. Indeed, conversion is known to give such symmetric and polymodal distribution.

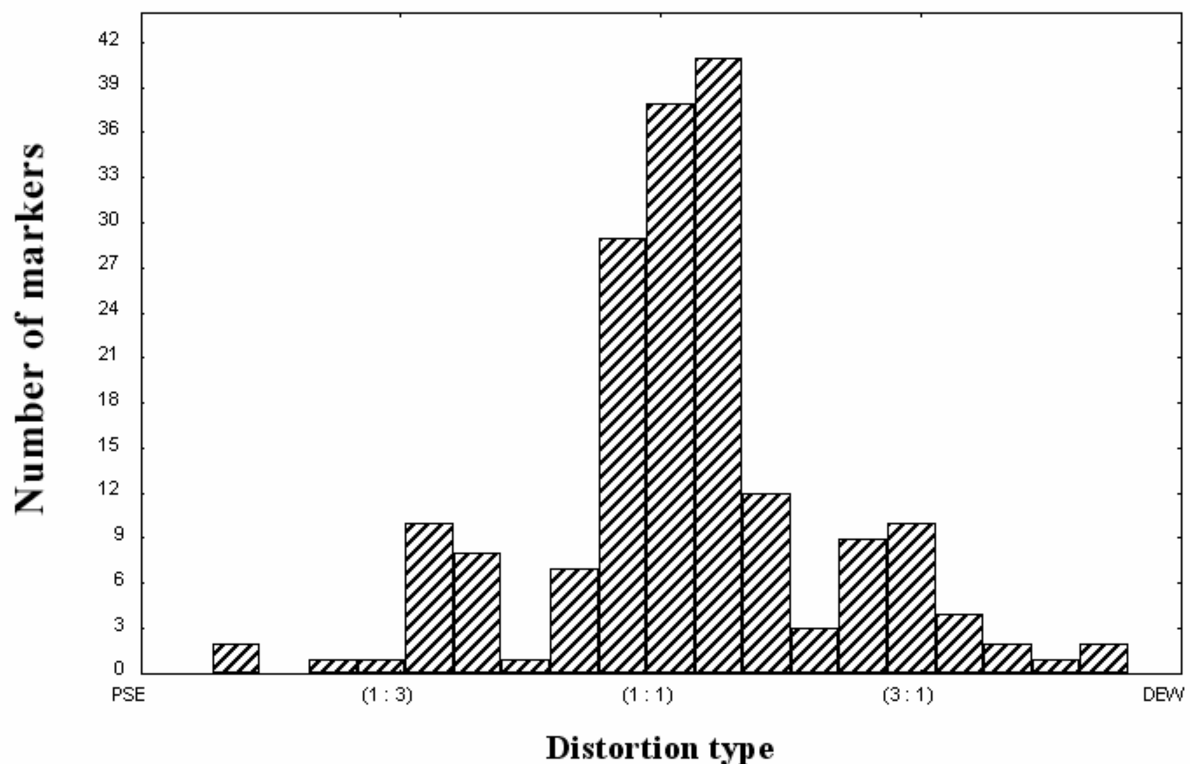

Figure 2. Distribution of the 180 markers in relation to distortion type

Inheritance of Coffee Bean Sucrose Content in the Interspecific Cross: *Coffea pseudozanguebariae* x *Coffea liberica dewevrei**

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INTRODUCTION

The sucrose content of coffee beans is an important component of the coffee flavour: the higher the sucrose content in green beans, the more intense coffee cup flavour. Beans of the formerly cultivated *Coffea liberica dewevrei* (DEW) have lower levels of sucrose (5.37% dmb) than beans of the wild species *Coffea pseudozanguebariae* (PSE) (7.57% dmb). In the present study, the genetic inheritance of sucrose accumulation in green beans is analysed in an interspecific cross including F1 and backcross hybrids between these two species.

Table 1. Sucrose content variation between hybrids and years (1995 and 1997)

Trees	95	97	Means
9/8	5.34	5.22	5.28
14/3	5.33	4.36	4.84
1/9	3.96	3.67	3.82
12/12	8.25	8.57	8.41
19/13	5.55	6.54	6.04
20/17	5.90	6.54	6.22
9/3	3.32	4.04	3.68
Means	5.38	5.56	

MAIN RESULTS CONCERNING GENETIC INHERITANCE

1. Between-tree variation exists within species.
2. There is no between-year variation, but an interaction “between-year x tree” (Table 2).
3. Additivity is emphasised for sucrose content in F1 and BCDEW hybrids (Figure 1).
4. Discrepancy to additivity in BCPSE hybrids (Figure 1) is due to a segregation distortion eliminating hybrids with sucrose content >5.2% dmb.

CONCLUSIONS

Our results clearly indicate that, using an interspecific cross with PSE, we can expect an improve-ment of the sucrose content in *C. canephora* beans. The intraspe-cific variability of *C. pseudozangue-bariae* and the genetic additivity allow to select genotypes as parent for breeding. As an example, the PSE genotype having 9.25% dmb of sucrose is of special interest. The large within-BCDEW variance, coupled with additivity, strongly suggests that the choice of parents for the next generation BC2 should be efficient.

Photo 1. ***C. pseudozanguebariae*** **flowers**

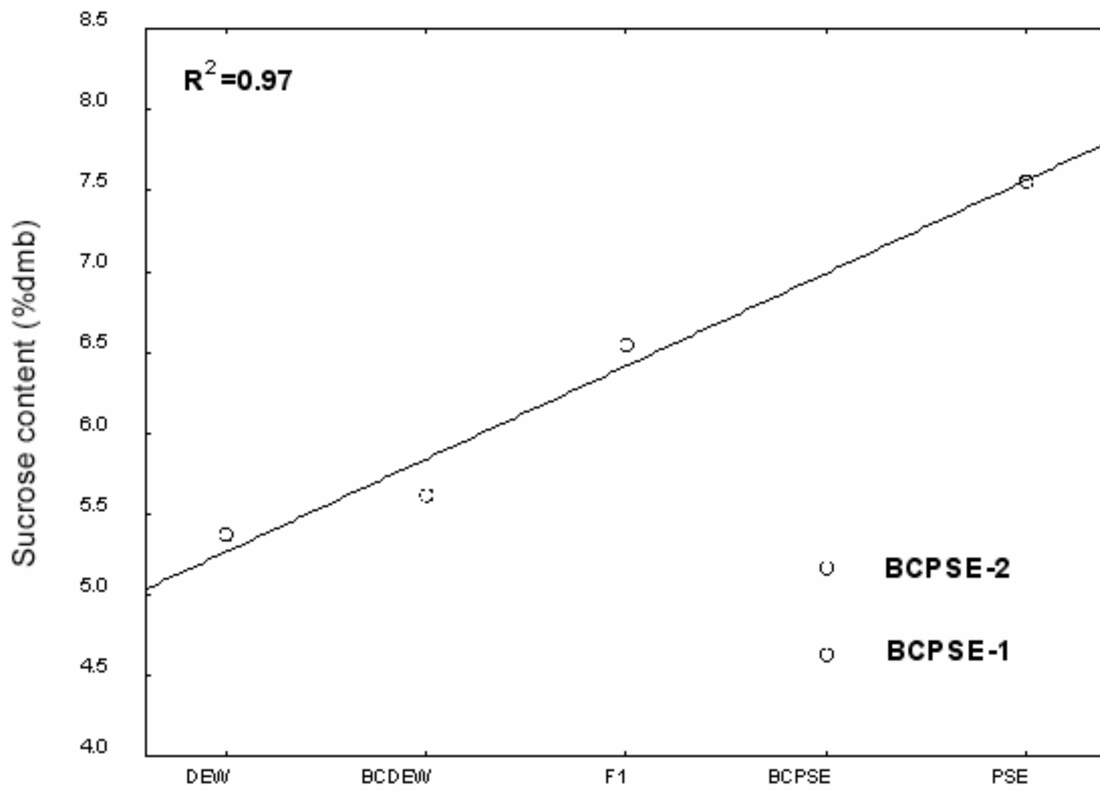

Figure 1. Inheritance of bean sucrose content (regression analysis). Discrepancy to additivity in BCPSE was verified on two harvests (BCPSE-1 and -2)

Trigonelline Inheritance in the Interspecific Cross between *Coffea pseudozanguebariae* x *C. liberica dewevrei**

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INTRODUCTION

Coffee trees are characterised by the presence of the trigonelline alkaloid in their beans. During roasting, trigonelline leads to major coffee aroma compounds (several alkyl-pyridines and pyrroles). In the present study, the genetic inheritance of trigonelline accumulation, in green beans, was investigated in an interspecific cross between a wild East African species *C. pseudozanguebariae* (PSE) and the formerly cultivated West African species *C. liberica dewevrei* (DEW) (the two species are allogamous). Trigonelline content was measured by HPLC in both parental species, F1 and reciprocal backcross hybrids (BCDEW and BCPSE).

Objectives were i) to evaluate trigonelline content in green beans of parental species and hybrids, ii) to estimate between year variation, iii) to analyse the genetic inheritance of its content and iv) to map QTL(s) for trigonelline.

MAIN RESULTS CONCERNING GENETIC INHERITANCE

1. Trigonelline was 0.57% in DEW and 1.02% in PSE and within-species range did not overlap. Between-tree variation existed also within species.
2. There was neither between-year variation nor interaction “between-year x tree”.
3. Trigonelline contents were similar in PSE, F1, BCDEW and BCPSE, i.e. in groups with PSE cytoplasm.
4. Between-tree variation was emphasised in F1 showing also the presence of nuclear genes.

MAIN RESULTS CONCERNING TRIGONELLINE QTL ANALYSIS

1. A QTL was located on the G genetic linkage group of the map developed in Ky et al. (2000), near the ACTCAT5 AFLP locus (LOD score=3.56) (Figure 1)
2. In BCDEW hybrids, there were two genotypes at the locus ACTCAT5: [tt] as for DEW and [Tt] as for F1 in a 1:1 ratio (54% *vs* 46%). These two genotypes differed by their trigonelline average contents: 0.86% and 1.11%, respectively.
3. Distribution study within [tt] and [Tt] classes showed recombination events between the ACTCAT5 locus and the QTL. Two alleles of the QTL were defined: *T* for PSE and *t* for DEW.
4. Average contents differed between [*tt*] and [*Tt*] classes: 0.83% and 1.20%, respectively.
5. The [*tt*] class average content was equal to (DEW + PSE)/2 showing additivity between nuclear and cytoplasmic effects.

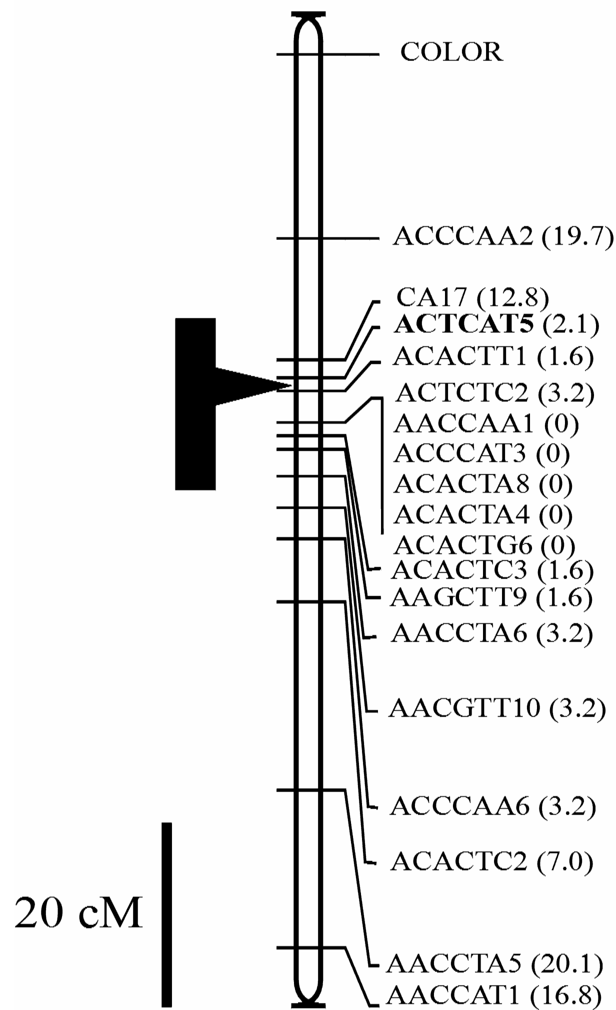

Figure 1. Trigonelline QTL location on the G linkage group

CONCLUSIONS

The importance of these results with respect to breeding is three-fold: i) trigonelline content can be increased in cultivated coffee using PSE as female parent and its cytoplasm in the next generations; ii) marker-assisted selection could be used in further backcross generations. MAS, at the plantlet stage, will avoid having to install large progeny populations in the field; iii) the existence of a small environmental effect and between-tree variations within [t t] and [Tt] groups, similar to the within-species variation, should facilitate clonal selection between hybrids.

Evolution of Disease-resistance Gene in Coffee Trees (*Coffea* L.)

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SUMMARY

The ability of plant species to survive over evolutionary time depends to some instance on their capability to maintain and generate useful diversity at resistance loci. Therefore, analysis of diversity and evolution of resistance genes (R) in plant species could be of particular interest in the elaboration of strategies for developing durable resistance mediated by major genes. Molecular analysis of R-gene evolution concern so far mainly plants with short life cycle while perennial plants have retained little attention.

The majority of R-genes isolated so far encode a predicted nucleotide-binding site (NBS) domain. NBS domains related to R-genes show a highly conserved backbone of amino acid motifs offering the possibility of isolating resistance gene analogous sequences (RGAs) by polymerase chain reaction (PCR). Multiple combinations of primers with low degeneracy designed from two conserved motifs in the NBS regions of R-genes of various plants were used in coffee trees. Nine distinct classes of NBS-like resistance gene analogs (RGAs) representing a large diversity were isolated from *Coffea arabica* and *C. canephora* species. The analysis of one coffee RGA family suggested point mutations as the primary source of diversity. With one exception, coffee RGA families appeared closely related by sequence to at least one cloned R-gene. In addition, deduced amino acid sequences of coffee RGAs were identified showing strong sequence similarity with almost all known non-TIR type R-genes. The high similarity between particular coffee RGAs and R-genes isolated from other angiosperm species such as *Arabidopsis*, tomato and rice indicated an ancestral relationship and the existence of common ancestors. As revealed in coffee trees, the evolution of NBS-encoding sequences seems to involve accumulation and slow divergence mechanisms within distinct R-gene families rather than a fast-evolving process. Functional inference of the suggested NBS domain type of evolution is also discussed.

INTRODUCTION

In the gene-for-gene resistance, the rapid changes that occur in the virulence characteristics of pathogen populations raise a continuous threat to the effectiveness of individual major genes of resistance (R). Consequently, the ability of plant species to survive over evolutionary time depends to some instance on their ability to maintain and generate useful diversity at resistance loci. Therefore, analysis of diversity and evolution of R-genes in plant species could be of particular interest in the elaboration of strategies for developing durable resistance mediated by major genes.

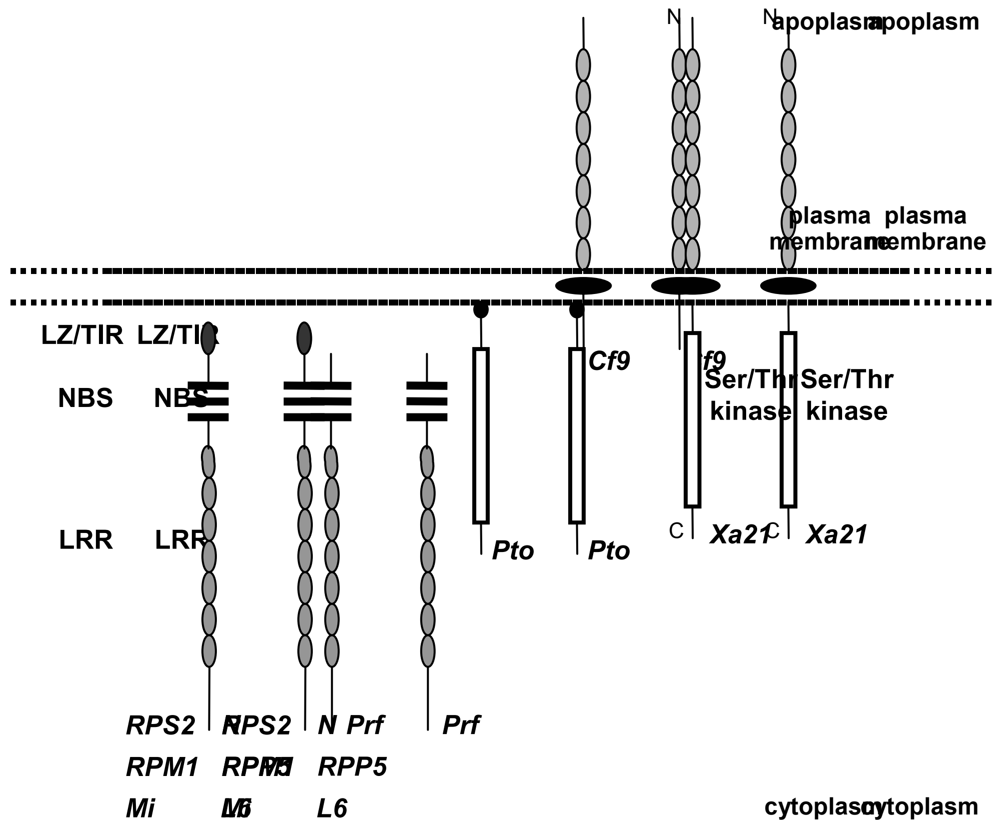

Figure 1. Schematic representation and putative location of conserved protein domains of cloned R-genes. Five mains peptidic conserved domains were distinguished: NBS (Nucleotide Binding Site), LRR (Leucine Rich Repeat), TIR (Toll-Interleukin Receptor), LZ (Leucine Zipper) and Serine/Threonine Kinase

A growing number of disease resistance genes conferring resistance to a wide range of pathogens have recently been isolated from several plant species. Analysis of the sequences of some R-genes revealed conservation of specific amino acid domains in the putative products (Figure 1). Otherwise, remarkable similarities in the general structure of R loci have also been observed. They are generally members of multigene families and show a complex physical organization of repeated sequences (i.e. cluster). These recent data have shed light on the molecular evolution of R-genes. The organization of these R-gene families suggests indeed that novel sequences and therefore novel specificities are generated by various evolutionary events such as substitutions, different mechanisms of recombination (i.e. unequal crossing-over, gene conversion) and more exceptionally, transposable elements (2). However, these analysis concern mainly plants with short life cycle while perennial plants have retained so far little attention.

Coffea arabica, an important tropical crop, is characterized by a low genetic diversity. This allotetraploid species shows a high susceptibility to many pests and diseases, and the diploid species *C. canephora* constitutes the main resistance source for breeding purposes. Our project displays two principal objectives:

- to precise the molecular organization and the evolution of R-genes in this perennial plant
- to improve the coffee tree for disease resistance, especially against *Meloidogyne* root-knot nematodes and *Hemileia vastatrix* rust fungi.

MATERIALS AND METHODS

Plant material

Plant material involved the accessions of both species *C. arabica* and *C. canephora*. Genomic DNA and mRNA extracted from leaves were used.

Amplification with degenerate primers

NBS domains related to R-genes show a highly conserved backbone of amino acid motifs offering the possibility of isolating resistance gene analogous sequences (RGAs) by polymerase chain reaction (PCR) with degenerate primers. Multiple combinations of primers with low and without degeneracy were designed from two conserved motifs (i.e. P-Loop and GLPL) in the NBS regions of R-genes of various plants. These primers were used in PCR amplification from coffee genomic DNA. The amplified products were cloned and sequenced.

Amplification with coffee RGA family-specific primers

Primers specific to the identified coffee RGA families were tested on mRNA samples by RT-PCR analysis.

RESULTS AND DISCUSSION

Isolation of coffee RGAs

Twenty five combinations of degenerate or non-degenerate primers were tested. From the amplified products showing the expected size considering the NBS domain length of known R-genes (~500 bp), 120 clones were isolated and sequenced. On the base of several features, 40 PCR-derived coffee NBS sequences were identified as RGAs. These sequences contained uninterrupted open reading frames. Moreover, all sequences contained the characteristic conserved motifs of NBS R-genes. Most coffee RGAs were closely related by sequence to at least one known R-gene.

A high coffee RGA diversity

The NBS-encoding RGAs isolated from coffee trees showed considerable sequence variation.

Nine distinct families of NBS-like RGAs were identified in both *C. arabica* and *C. canephora* species (Figure 2). More particularly, the analysis of one coffee RGA family suggested point mutations as the primary source of diversity. Moreover, by transcription analysis based on RT-PCR experiments, cDNAs corresponding to 8 different coffee RGA families were detected in coffee leaves of both studied species. Otherwise, RGAs belonging to the 9 distinguished families were observed in a same individual.

Coffee RGA phylogenetic analysis

Phylogenetic relationships between deduced amino acid coffee RGA sequences and a representative set of NBS domains of known R-gene products (isolated from Arabidopsis, tomato, potato, pepper, lettuce, maize and rice) were investigated. According to the reported distinction between the TIR class and the non-TIR class of R-genes (3), all isolated coffee RGAs seemed to belong to the non-TIR class type of R-genes (Figure 3). In addition, all non-TIR type NBS domains of R-genes considered in this study (excepted Dm3) were associated to one of the isolated coffee RGA families. Lastly, the alignment between coffee RGA peptidic sequences of a particular family and the NBS domain of R-genes related to this

family shows that similarities were shared beyond characteristic conserved NBS motifs of R-genes (Figure 4).

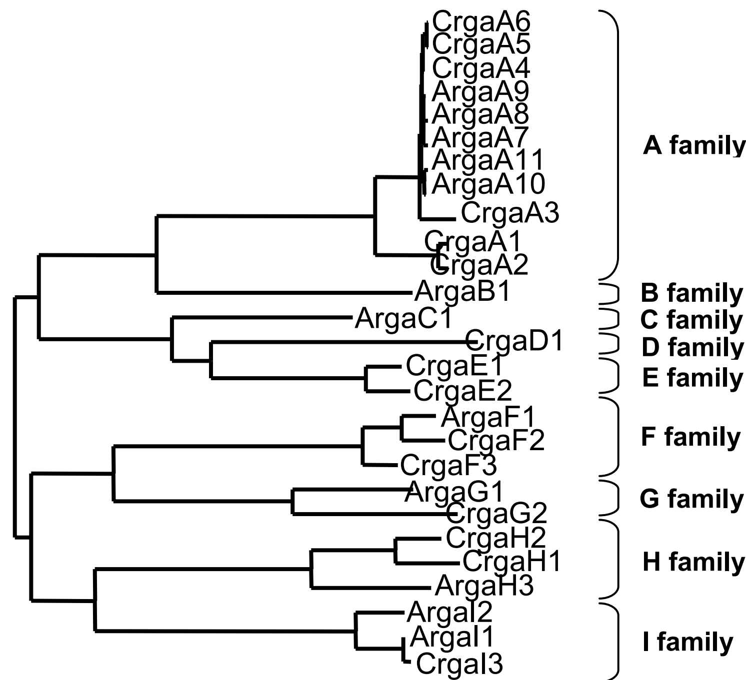

Figure 2. Phylogenetic tree for nucleotide RGA sequences isolated from *C. arabica* (A) and *C. canephora* (C) species. This tree was constructed by Neighbor-Joining method. Coffee RGA families (high sequence identity) are labeled A to I

CONCLUSIONS

In the coffee tree, the genetic diversity analysis between RGA families suggested an independent evolution of these different families. In this perennial plant, the evolution of NBS-encoding sequences seems to involve accumulation and slow divergence mechanisms within distinct R-gene families rather than a fast-evolving process. Otherwise, the high similarity between particular coffee RGAs and R-genes isolated from other angiosperm species such as *Arabidopsis*, tomato and rice indicated a common ancestral origin. This also supports a duplication and primary diversification of NBS-LRR R-genes more ancient than the divergence of monocots and dicots. The maintenance since dicot differentiation of different R-gene families showing sequence divergence and specific signature might result in fitness superiority and suggests a functional family-specificity.

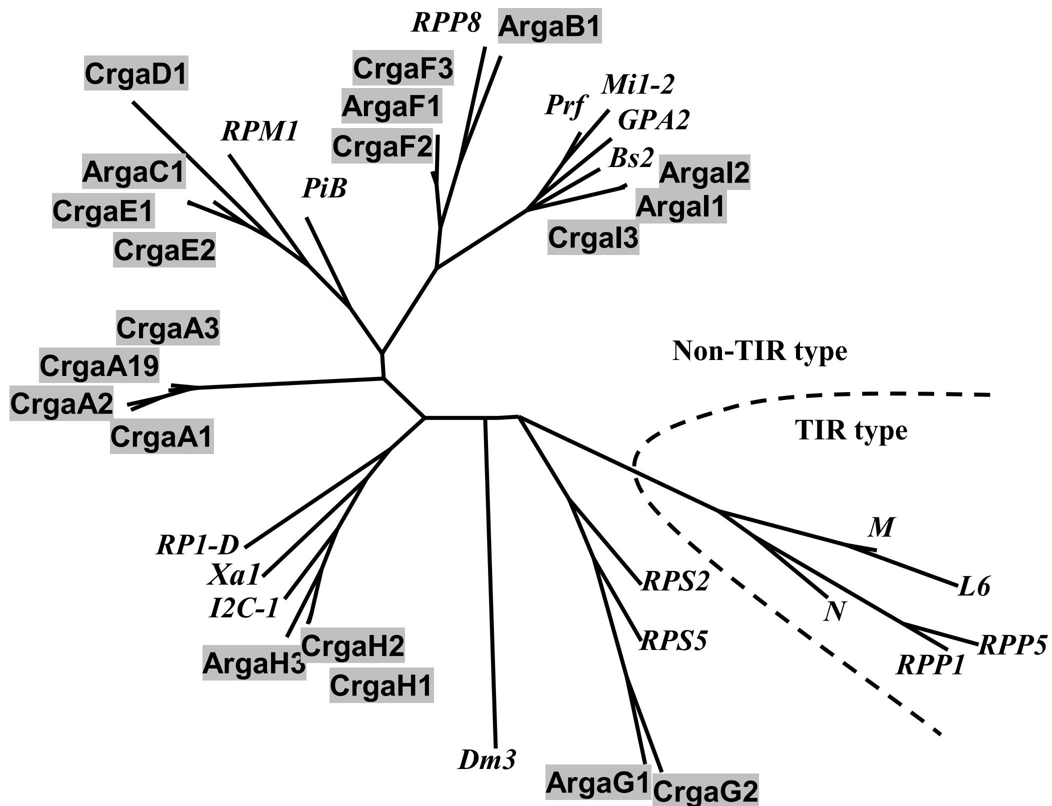

Figure 3. Neighbor-Joining tree based on alignment of amino acid sequences of representative coffee RGAs and NBS domains of cloned R-genes. Coffee RGAs are shaded gray. The dotted line distinguishes TIR and non-TIR type sequences

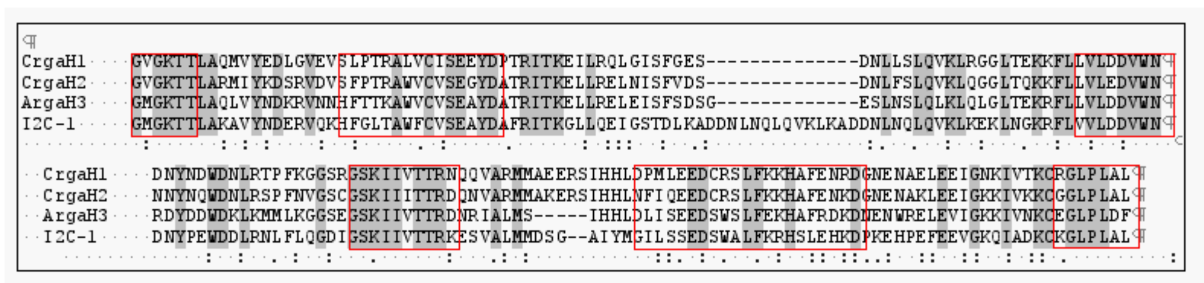

Figure 4. Multiple amino acid sequence alignment of coffee RGAs of the H family and the NBS domain of the closely related R-gene, *I2C-1*. Strict consensus residues are shown by shading. Residues sharing high (:) or low (.) physico-chemical proproperties are specified. Sequence blocks marked with boxes correspond to conserved motifs of NBS-encoding regregion R-genes

REFERENCES

Ellis J., Dodds P., Pryor T. (2000) Current Opinion in Plant Biology, **3**: 278-284.

Noir S., Combes M.-C., Anthony F., Lashermes P. (2001) Molecular Genetics and Genomics, DOI 10.1007/s004380100459.

Pan Q., Wendel J., Fluhr R. (2000) Journal of Molecular Evolution, **50**: 203-213.

Characterization of Extracellular Peroxidases Released by Coffee Suspension Cell Cultures

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SUMMARY

Cell suspension cultures release a broad and complex range of proteins into culture medium and, among these, peroxidases have been the most studied. The role of peroxidases has not yet been fully elucidated, but they have been shown to promote embryogenesis when added to non-embriogenic or tunicamycin-inhibited cell lines. The aim of this study was to characterize some properties of the extracellular peroxidases released by coffee (*Coffea arabica* cv Catuai) cell suspension cultures. During the growth of suspension cultures, extracellular proteins accumulated to a concentration of 63 $\mu g\ ml^{-1}$ medium within 24 days. Secretion started slowly, but increased rapidly after 12 days of culture. Extracellular peroxidases have an optimum pH of 5 and show a rapid loss of activity at higher pH values. Although high activity levels of peroxidases were detected at the beginning of the growth cycle, a loss of activity was detected after 6 days of culture. Following this decrease, activity reached a maximum at day 18. The peroxidase isoenzyme patterns obtained by isoelectric focusing revealed several bands distributed over the whole pH range (pH 3 to 10). One major band observed at pH 7.5 showed a similar pattern to the activity curve during the culture cycle. These results showed that there is a direct relationship between growth, peroxidase activity and isoenzymatic patterns in coffee suspension cell cultures.

INTRODUCTION

The majority of the *in-vitro* cultured plant species release a broad and complex range of proteins into the culture medium. More than 50 different proteins have been identified (Nielsen and Hansen, 1992). The proteins released from the cells into the culture medium have been identified as peroxidases (Fry, 1980; Hari, 1980; Bredemeijer et al., 1985), phosphatases (Ciarrocchi et al., 1981), proteases (Gavish et al., 1991), α-mannosidase (Kunze et al., 1998), chitinases (Kunze et al., 1998; Esaka et al., 1990; Kragh et al., 1996) and ’-1, 3 glucanase (Helleboid et al., 1998; Kunze et al., 1998). Proteins such as trypsin inhibitor (Carlberg et al., 1987), a protein homologous to the inhibitor of a cysteine proteinase (Satoh et al., 1995), a protein type extensin (Kawasaki, 1989), pathogenesis related proteins (Gavish et al., 1991; Stirn and Jacobsen, 1987), a lipid transfer protein (Sterk et al., 1991), and arabinogalactan-proteins (Fincher et al., 1983; Kreuger and Van Holst, 1993; Toonen et al., 1997) also have been reported. The majority of proteins released into the culture medium are related to defense mechanisms. Among these, the most studied have been the peroxidases (Fry, 1980; Chibbar and Van Huystee, 1984; Bredemeijer et al., 1985; Mellon, 1986; Moreno-Valenzuela et al., 1989; Moreno et al., 1990; Shetty et al., 1990). Plant peroxidases have different roles in lignin biosynthesis and oxidation of endogenous IAA. During somatic embryogenesis induction the presence of peroxidases has been shown to promote embryogenesis when added to a non-embryogenic or tunicamycin-inhibited cell lines (Cordewener et al., 1991). The aim of this study was to characterize some properties of the extracellular peroxidases released by cell suspension cultures of *Coffea arabica.*

MATERIALS AND METHODS

Plant material and concentration of extracellular proteins from liquid medium. Cell suspension cultures of coffee (Coffea arabica cv Catuai) were initiated and maintained as described previously by Huchín-May, (2000). Cell suspension cultures from 0 to 24 days old were vacuum filtered through three paper sheets (two Whatman # 1 and one Whatman # 42) and then through a 0.2 [m cellulose acetate filter (Sartorius®). The free cell medium was lyophilized, resuspended and then concentrated using Stirred Ultrafiltration Cells (Amicon, Danvers, Mass.) with a 10-kDa cut-off pore size (Diaflo® YM 10, Amicon, Beverly, Ma).

Protein and peroxidase assays. The amount of protein in the concentrates was determined using Peterson's method (1977) with BSA (Sigma, St. Louis, MO) as standard. Peroxidase activity was measured by Van den Berg's method (1984). The standard assay mixture contained 2.2 mM H_2O_2 and 4 mM guaiacol in 50 mM acetate buffer at pH 5, in a final volume of 3 ml. One unit of peroxidase activity will oxidize 1 [M guaiacol to tetraguaiacol per minute.

Isoelectric focusing of peroxidase isoenzymes. Isoelectric focusing was performed on thin-layer polyacrylamide gel (5%) containing carrier ampholytes in the pH range 3.5 to10 (Sigma, St. Louis, MO). Peroxidase isoenzymes bands were visualized by staining with 4-chloro-1-naphtol and hydrogen peroxide in phosphate buffer, at pH 7, as described previously (Robertson et al., 1987)

RESULTS AND DISCUSSION

During the growth of cell suspension cultures, extracellular proteins were detected after six h until 24 days (Figure 1). The concentration increased with the duration of culture, reaching up to 63 $\mu g\ ml^{-1}$ medium by day 24. At that same time, growth had increased 16-fold. FDA staining of cells showed that cells were highly viable. Furthermore the intracellular enzyme marker, glutamate dehydrogenase, was not detected among extracellular proteins, but was detected intracellularly (data not shown). Therefore, extracellular proteins come from cellular secretions and not from dead cells. Our results showed a direct relationship between growth and extracellular protein of coffee cell suspension cultures.

Protein patterns found in a time-course study from zero to 24 days, after subculture, presented notable changes during the growth cycle. They varied in size from 14 to 90 kDa (Figure 2). More than 30 bands were detected. These bands can be grouped according to their expression pattern 1) proteins expressed all the time, 2) proteins increasing in concentration during the growth cycle, 3) proteins decreasing in concentration and 4) proteins increasing-decreasing (or vice versa) during growth. Several proteins were abundant, for example 22, 28, 38, 40 and 70 kDa. The extracellular protein profiles of *C. arabica* were more complex than profiles observed in *D. carota*: 15 different proteins (De Vries et al., 1988a), *Spinacia oleracea*: 16 different proteins (Fry, 1980), and *Picea abies*: 20 different proteins (Egertsdotter et al., 1993). The difference between our data and those mentioned above may be due to sensitivity of the detection method used.

The effect of pH on the peroxidase activity was assayed with different buffers at pH values between 2.6 and 9. Peroxidases have an optimum pH of 5 at 30ºC and rapidly lost activity at pH between 2.6-4 and 6-9 (Figure 3). Our results are similar to those from cowpea (Moreno-Valenzuela et al., 1989) and *Gossypium hirsutum* (Mellon, 1986).

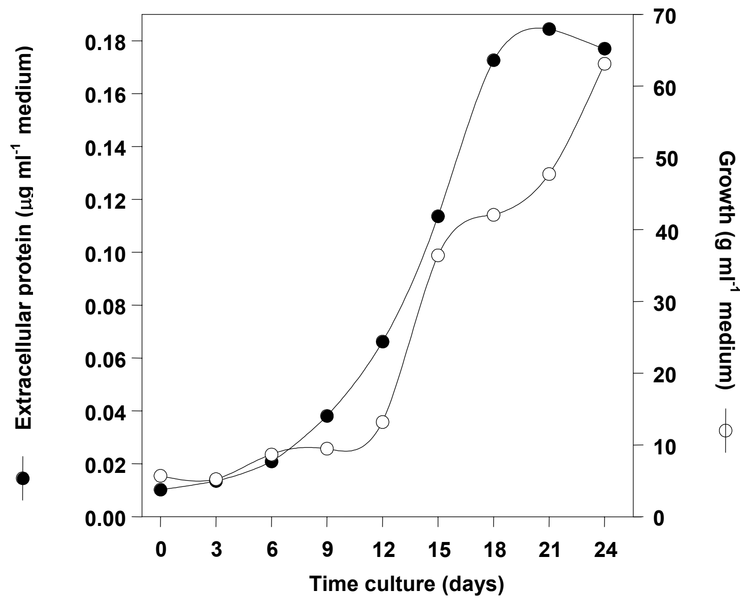

Figure 1. Protein accumulation in culture medium and cell growth of cell suspension culture of *C. arabica*

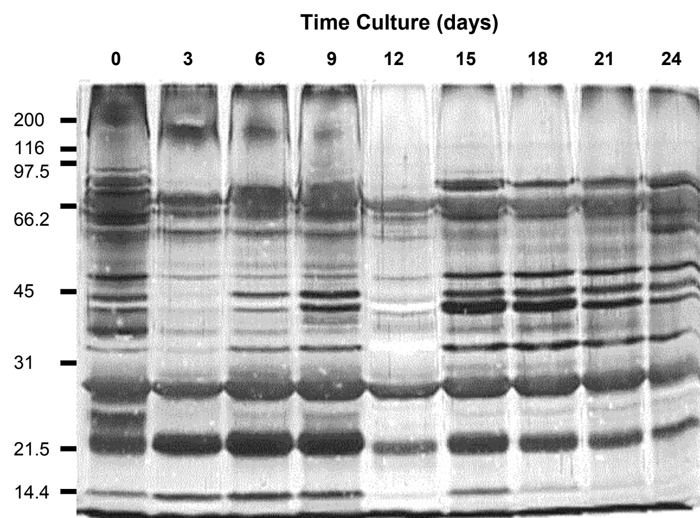

Figure 2. Electrophoretic pattern of extracellular proteins from *C. arabica* suspension culture sampled every three days during a 24 day culture cycle. Molecular weight is indicated on the left. Numbers on each line indicate day after subculture. Five micrograms of protein was loaded by lane and silver stained. SDS-PAGE 10% was used

At the beginning of the growth cycle there was high activity (4.2 U ml^{-1}) which decreased until day 6. It then increased to 8.9 U ml^{-1} by day 18 (Figure 4). Peroxidase activity was not detected from the intracellular soluble fraction, but it was detected in cell wall debris (data not shown).

The isoenzyme peroxidase pattern obtained by isoelectric focusing is shown in Figure 5. Twelve isoenzymes were detected between pH 3 and 7.9. During the first part of the cycle (days 0-6) slight activity was detected after which the activity increased steadily up to high levels in the final days. One major band at pH 7.1 was observed to show the same trend as the

(overall) activity performance. The results showed a direct relationship between growth, peroxidase activity and isoenzymatic patterns in coffee cell suspension cultures.

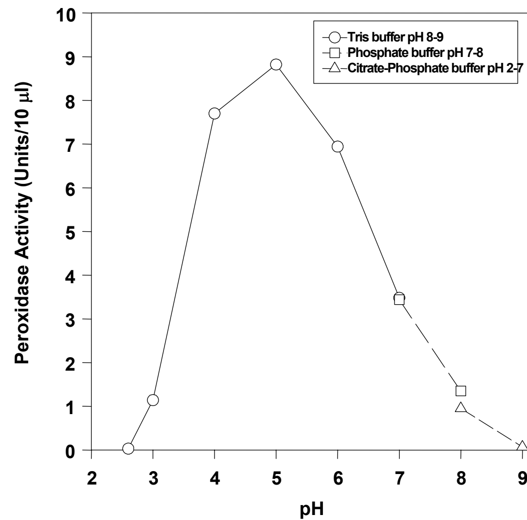

Figure 3. Total peroxidase activity in the culture medium at different values of pH. Peroxidase activity is expressed as units per 10 µl aliquot

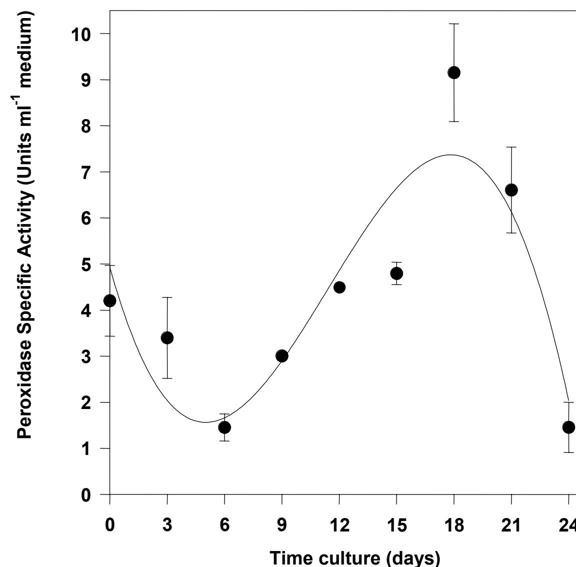

Figure 4. Total peroxidade activity in culture medium during 24 days growth cycle sampled every three days. Peroxidase activity is expressed as units per ml of medium

Somatic embryogenesis represents an attractive model system for the study of early events in plant cell differentiation. Several authors have reported that compounds secreted by the cells into culture media are involved in somatic embryogenesis (Quiroz-Figueroa et al., 2000). In *D. carota*, somatic embryogenesis can be blocked by the addition of tunicamycin, a glycosylation inhibitor. This inhibition can be removed by the addition of some of the secreted glycoproteins present in the embryogenic culture medium (De Vries et al., 1988a; De Vries et al., 1988b). In this work, extracellular peroxidases having a broad pH range (acidic to basic) were detected, and a major peroxidase isoenzyme, with a pH 7.1 was observed.

ACKNOWLEDGMENTS

The authors thank to M. Méndez-Zeel for technical assistance on tissue culture. FQF (116916) acknowledge his PhD scholarships from CONACyT. National Council for Science and Technology (CONACyT), grants Nos. 4123P-N and 31816-N. supported this work.

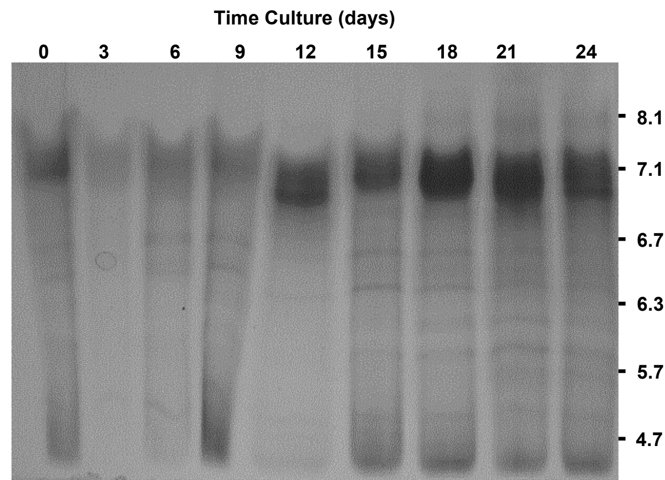

Figure 5. Isoelectric focusing (pH 3.5 to 10) of extracellular peroxidase isoenzymes during the growth cycle. pH is indicated on the right. Numbers on each lane indicate day after subculture. Forty micrograms of protein were loaded per lane

REFERENCES

Bredemeijer G. M. M., Burg H. C. J., Sree Ramulu K., Dijkhuis P. Release of peroxidases by cultured potato cells. Acta Bot. Need. **34**: 325-335; 1985.

Carlberg I., Jonsson L., Bergenstrahle A., Soderhall K. Purification of a trypsin inhibitor secreted by embryogenic carrot cells. Plant Physiol. **84**: 197-290; 1987.

Chibbar R. N., Van Huystee R. B. Characterization of peroxidase in plant cells. Plant Physiol. **75**: 956-958; 1984.

Ciarrocchi G., Cella R., Nielsen E. Release of nucleotide-cleaving acid phosphatase from carrot cells grown in suspension culture. Physiol. Plant. **53**: 375-377; 1981.

Cordewener J., Booij H., Van der Zandt H., Van Engelen F. A., Van Kammen, A., De Vries S. C., Tunicamycin-inhibited carrot somatic embryogenesis can be restored by secreted cationic peroxidase isoenzymes. Planta. **184**: 478-486; 1991.

De Vries S. C., Booij H., Janssens R., Vogels R., Saris L., Lo Schiavo F., Terzi M., Van Kammen A. Carrot somatic embryogenesis depends on the phytohormone-controlled presence of correctly glycosylated extracellular proteins. Devel. Genet. **2**: 462-476; 1988a.

De Vries S. C., Booij H., Meyerink P., Huisman G., Wilde H. D., Thomas T. L., Van Kammen A. Acquisition of embryogenic potential in carrot cell-suspension cultures. Planta. **176**: 196-204; 1988b.

Egertsdotter U., Mo L. H., Von Arnold S. Extracellular proteins in embryogenic suspension cultures of norway spruce (*Picea abies*). Physiol. Plant. **88**: 315-321; 1993.

Esaka M., Enoki K., Kouchi B., Sasaki T. Purification and characterization of abundant secreted protein in suspension-cultured pumpkin cells. Abundant secreted proteini may be a chitinase. Plant Physiol. **93**: 1037-1041; 1990.

Fincher G. B., Stone B. A., Clarke A. E. Arabinogalactan-proteins: structure, biosynthesis and function. Ann. Rev. Plant Physiol. **34**: 47-70; 1983.

Fry S. C. Gibberellin-controlled pectinic acid and protein secretion in growing cells. Phytochem. **19**: 735-740; 1980.

Gavish H., Vardi A., Fluhr R. Extracellular proteins and early embryo development in *Citrus* nucellar cell cultures. Physiol. Plant. **82**: 606-616; 1991.

Hari V. Effect of cell density changes and conditionated media on carrot cell embryogenesis. Z. Pflanzenphysiol. **96**: 227-231; 1980.

Helleboid S., Bauw G., Belingheri L., Vasseur J., Hilbert J. L. Extracellular β-1,3-glucanases are induced during early somatic embryogenesis in Cichorium. Planta. **205**: 56-63; 1998.

Huchín-May S.M.M. Efecto del pH inicial y la fuente de carbono en la embriogénesis somática i*n vitro* de *Coffea arabica* variedad caturra amarello.(2000) 1-62

Kawasaki S. Extensin secreted into the culture medium by tobacco cells I. Purification and some properties. Plant Cell Physiol. **30**: 259-265; 1989.

Kragh K. M., Hendriks T., De Jong A. J., Lo Schiavo F., Bucherna N., Hojrup P., Mikkelsen J. D., De Vries S. C. Characterization of chitinases able to rescue somatic embryos of the temperature-sensitive carrot variant *ts11*. Plant Mol. Biol. **31**: 631-645; 1996.

Kreuger M., Van Holst G. J. Arabinogalactan proteins are essential in somatic embryogenesis of *Daucus carota* L. Planta. **189**: 243-248; 1993.

Kunze I., Kunze G., Bröker M., Manteuffel R., Meins F., Jr., Müntz K. Evidence for secretion of vacuolar -mannosidase, class I chitinase, and class I β-1,3-glucanase in suspension cultures of tobacco cells. Planta. **205**: 92-99; 1998.

Mellon J. E. Some characteristics of peroxidase secreted by cotton ovule cultures. Plant Cell Rep. **5**: 338-341; 1986.

Moreno-Valenzuela O. A., Vazquez-Duhalt R., Ochoa J. L. Peroxidase activity in calluses and cell suspension cultures of radish *Raphanus sativus* var. Cherry Bell. Plant Cell Tissue Org. Culture. **18**: 321-327; 1989.

Moreno O. A., Vazquez-Duhalt R., Nolasco H. Extracellular accumulation of high specific-activity peroxidase be cell suspension cultures of cowpea. Plant Cell Rep. **9**: 147-150; 1990.

Nielsen K. A., Hansen I. B. Appearance of extracellular proteins associated with somatic embryogenesis in suspension cultures of barley (*Hordeum vulgare* L.). J. Plant Physiol. **139**: 489-497; 1992.

Peterson G. L. A simplification of protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. **83**: 346-356; 1977.

Quiroz-Figueroa F., Rojas-Herrera R., Sánchez-Teyer F., Loyola-Vargas V. M. Compuestos excretados por los ctv y su papel en la embriogénesis somática. In: Bernal-Lugo, I.; Loza, H., eds. Simposia académico en honor de la Dra. Estela Sánchez Quintanar. México: Facultad de Química, UNAM; 2000: 9-19.

Robertson E. F., Dannelly H. K., Malloy P. J., Reeves H. C. Rapid isoelectric focusing in a vertical polyacrilamide minigel system. Anal. Biochem. 167: 290-294; 1987.

Satoh S., Nojiri T., Gotoh Y. An extracellular insoluble protein in cultures and seeds of carrot. Plant Cell Physiol. **36**: 313-320; 1995.

Shetty K., Bothra D., Crawford D. L., Korus R. A. Extracellular peroxidases as indicators of growth in plant cell suspension cultures. Appl. Biochem. Biotechnol. **24/25**: 213-221; 1990.

Sterk P., Booij H., Schellekens G. A., Van Kammen A., De Vries S. C. Cell-specific expression of the carrot EP2 lipid transfer protein gene. The Plant Cell. **3**: 907-921; 1991.

Stirn S., Jacobsen H.-J. Marker proteins for embryogenic differentiation patterns in pea callus. Plant Cell Rep. **6**: 50-54; 1987.

Toonen M. A. J., Schmidt E. D. L., Van Kammen A., De Vries S. C. Promotive and inhibitory effects of diverse arabinogalactan proteins on *Daucus carota* L. somatic embryogenesis. Planta. **203**: 188-195; 1997.

Van den Berg B. M., Van Huystee R. B. Rapid isolation of plant peroxidase. Purification of peroxidase a from *Petunia*. Physiol. Plant. **60**: 299-304; 1984.

Efficient Use of Coffee Genetic Resources: Molecular Analyses of Genome Interactions in the Arabusta Hybrid (*Coffea arabica* x *C. canephora*)

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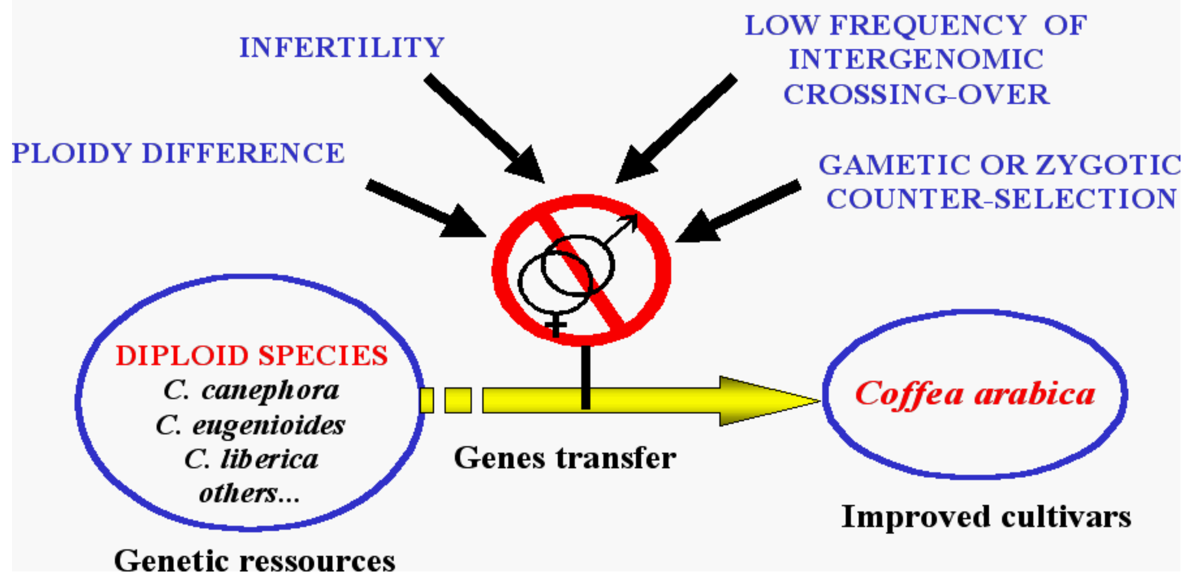
INTRODUCTION

Only a minimum fraction of the genetic ressources has been exploited so far in coffee. Selection of improved cultivars and breeding work have been restricted to the original plant material (Typica and Bourbon, mainly) from which coffee had been stablished, and to occasional new introductions (Carvalho, 1988). Wild relative *Coffea* species constitute the most valuable gene reservoir for breeders, but its utilisation requires more knowledge about inherents problems regarding interspecific hybridization in coffee. Molecular markers are invaluable tools for genome analysis of polyploid species and have resulted in a better understanding of polyploid evolution and genetics (Da Silva and Sorrells, 1996). Particularly, factors affecting genetic exchange between parental genomes in polyploid hybrids could be adressed by marker analysis in segregating populations or from patterns of introgression (Garcia et al*.*, 1995; Parkin and Lydiate, 1997). Recent molecular analyses in coffee showed that particular chromosomes of *C. arabica* only pair homogenetically in spite of the minor differentiation among its two constitutive genomes. Accordingly, a diploid-like segregation has been observed. Unlike *C. arabica*, in the interspecific tetraploid hybrid arabusta (*C. arabica x C. canephora* 4x) the four sets of chromosomes not display any preferential pairing (Lashermes et al*.*, 1999; Lashermes et al., 2000). Actually we are interested to study the behaviour of the *C. canephora* genome and its interaction with the *C. arabica* genome in the context of the tetraploid arabusta hybrid. Therefore, the purpose of this study was to analyse the allele segregation and chromosome recombination in a population of BC_1 individuals derived from tetraploid arabusta hybrids. The results are discussed in relation to the mechanism of introgression into *C. arabica* and the efficient use of genetic ressources in arabica breeding.

MATERIAL AND METHODS

Plant material

Plant material consisted of two BC_1 populations resulting from the backcross of two interspecific arabusta tetraploid F_1 plants (Et 30 x IF 181T) to *C. arabica* (accession Et30). The tetraploid plant of *C. canephora* (IF 181T) was previously obtained following colchicine treatment of the clone IF 181 (Figure 1).



Molecular marker assay and data scoring

Segregation and cosegregation of both restriction fragments (RFLP) and microsatellite polymorphic markers were studied in the BC_1 populations. Part of the analysed RFLP and microsatellite loci have been previously mapped in *C. canephora* and are distributed on 7 of the 11 identified linkage groups (Lashermes et al., 2001). Restriction fragments (i.e. RFLP locus) as well as PCR-amplified products (i.e. microsatellite locus) of different sizes were identified and easily interpreted as either canephora or arabica specific markers by comparing the parental accessions. When two different canephora specific markers were identified in the arabusta hybrid, the markers (i.e. RFLP or microsatellites) were interpreted as alleles of the considered locus and designed arbitrary by the letters C_1 and C_2 (Figure 2). Allelic interpretation of microsatellite loci was undertaken only when two different canephora specific markers were presents in the arabusta hybrids. In contrast, for RFLP loci, variations in banding intensity within the same lane were considered to represent differences in allele copy number and were designed by the letter C in single or double dose. Statistical analysis compared observed vs expected segregation frequencies of canephora alleles assuming random chromosome segregation in the hybrid.

RESULTS AND DISCUSSION

Analysing segregation patterns of 24 polymorphic loci (11 RFLP and 13 microsatellites) we scored for the presence of the specific canephora markers in the BC1 plants. Comparison between the two BC1 populations (i.e. P1 and P2) showed almost equal frequencies of plants with canephora markers. Overall analyses including both populations revealed that proportion of plants with canephora markers were consistent ($p<0.05$) with the expected proportion (i.e. 0.83) assuming random chromosome segregation.

On the other hand, and for almost all loci analysed, segregation of canephora alleles transmitted by the arabusta hybrids conformed to the expected ratio (i.e. 0.25) assuming random chromosome segregation and the absence of selection (Figure 3). Recombination fractions were analysed for seven marker intervals on four different linkage groups. Only two of the seven intervals analysed, exhibited a significant difference in recombination frequency

(Table 1). Although local differences in recombination may exist, these results suggest that overall recombination in the arabusta hybrid is not significantly restricted by genetic differentiation between chromosomes belonging to the different constitutive genomes (i.e. *C. arabica* and *C. canephora* genomes).

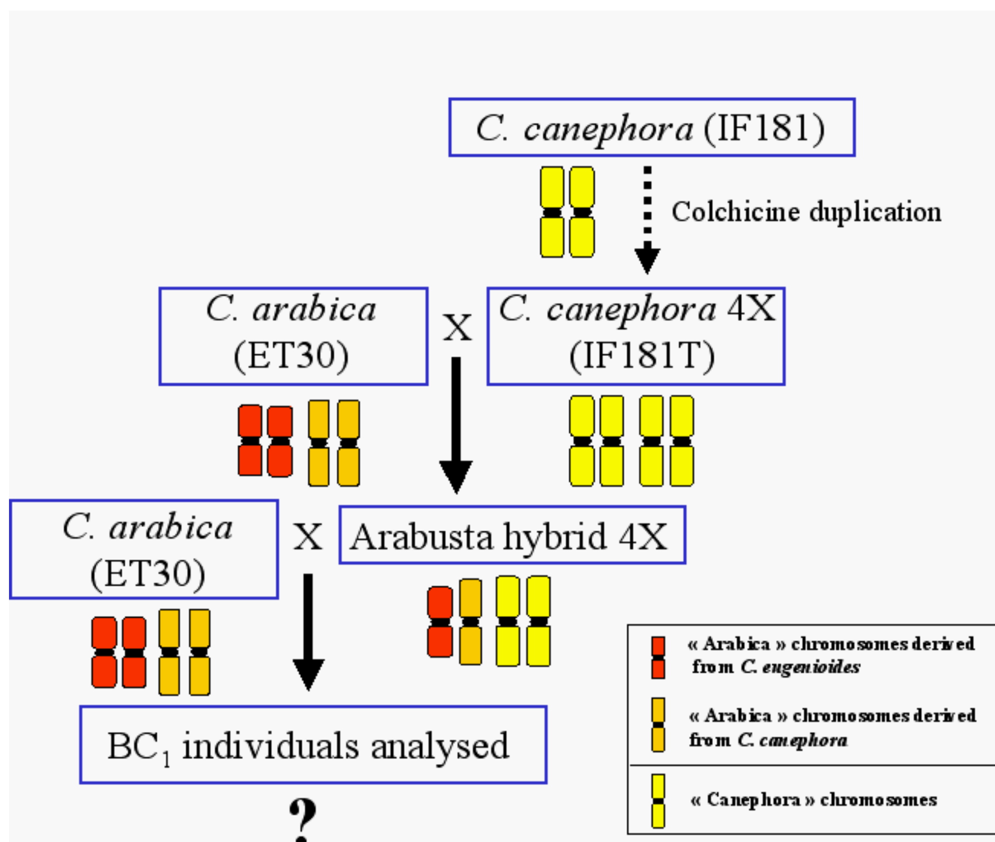


Figure 1. Origin of the surveyed populations BC_1

CONCLUSIONS

Enlarging the genetic base and improvement of arabica cultivars have become a priority for coffee breeders. Likewise, understanding of introgression mechanism could provide new perspectives to develop suitable strategies of field selection. In this report we present new evidence about the particulary favorable disposition of the arabusta hybrid (*C. arabica* x *C. canephora* 4x) to the introgression. Our results suggest that gene transfer from *C. canephora* (and probably from other diploid related species) to the cultivated *C. arabica*, should not be limited by differences either in sequence homology or in chromosome structure.

These preliminar results provides an optimistic view on major utilisation of coffee genetic ressources in coffee breeding programs. Although further investigations about genome interactions between *C. arabica* and others diploid related species are needed, monitoring of gene introgression using molecular tools, represent a first step towards real implementation of marker-assited selection in coffee.

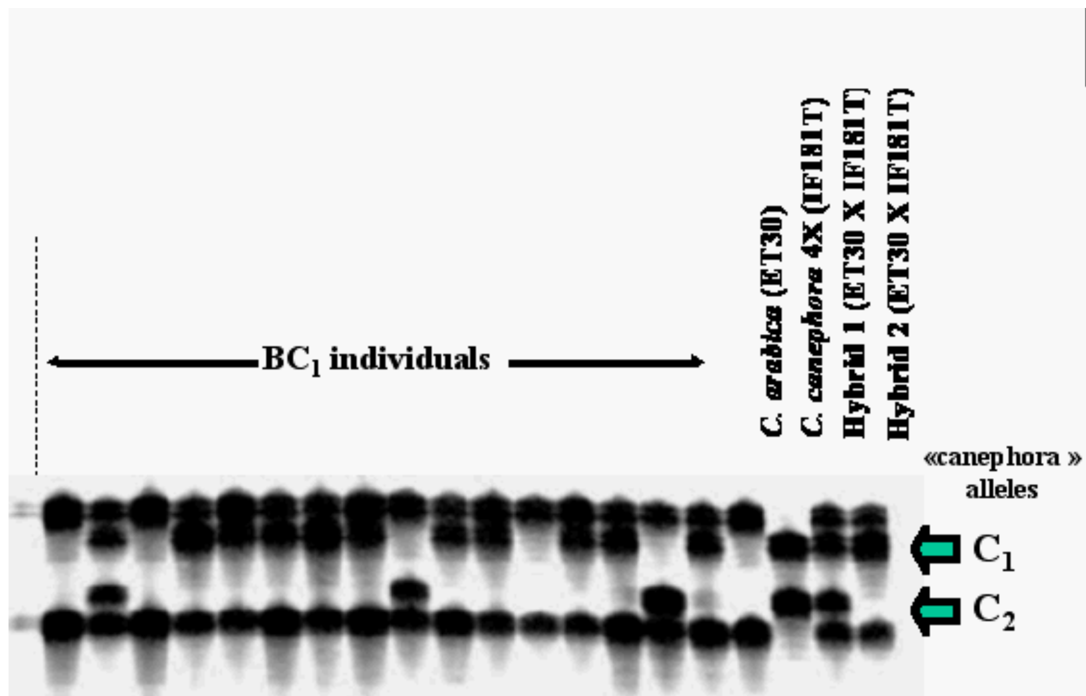


Figure 2. DNA marker analysis of introgression: Example of a microsatellite locus showing "canephora" allele segregation among BC$_1$ individuals resulting from the backcross of arabusta hybrids to *C. arabica*

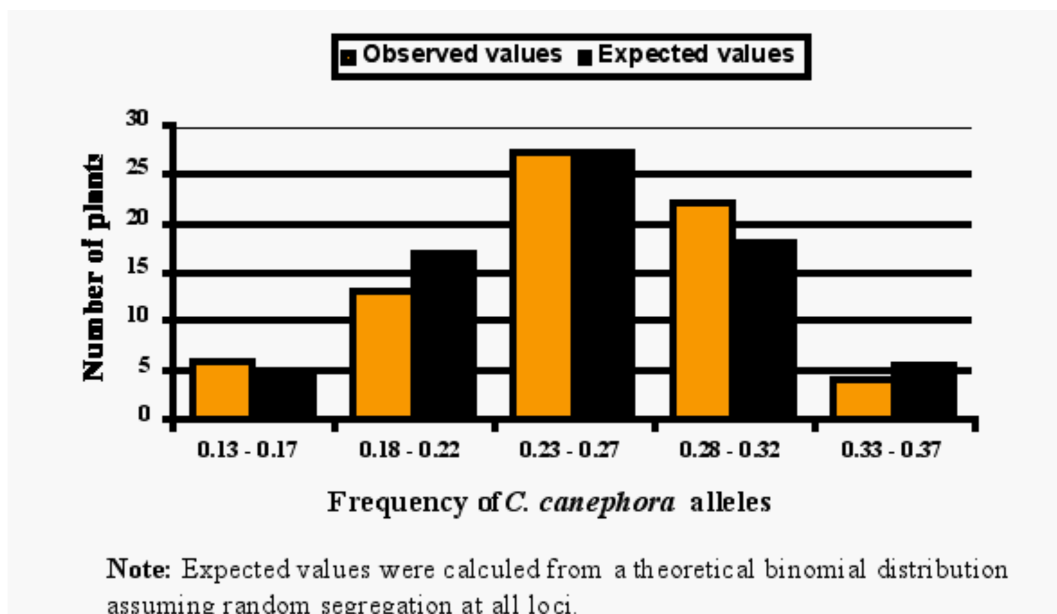


Figure 3. Frequency distribution of *C. canephora* alleles: Histograms of the number of BC$_1$ individuals in which particular frequency of *C. Canephora* alleles were detected

Table 1. Genome recombination: Comparison for different chromosome segments of the recombination frequencies estimated in the arabusta hybrid an in *C. canephora*

Linkage group of the canephora map	Marker Intervals	Recombination fractions	
		Arabusta	*C. canephora*
3	M41-gA71	0.24	0.31
	gA71-M157	0.37	0.32
	M157-M42	0.13 *	0.04
	M41-M42	0.27	0.40
4	M47-cR167	0.10	0.07
7	gA72-gA61	0.37 **	0.20
9	gA1-gA19	0.35	0.49

* , ** indicate statistical significant differences in the proportion of parental and recombinant gametes at $P < 0.05$ and $P < 0.01$, respectively, as indicated by the chi-square test.

REFERENCES

Carvalho A. (1988). In: Clarke RJ, Macrae R (eds). Coffee, vol V/4 ; Agronomy. Elsevier Applied Science, London, pp. 129-165.

Da Silva J.A.G., Sorrells M.E. (1996) In: Jahuar PP (ed). Methods of genome analysis in plants. CRC Press. Boca Raton USA, pp 211-228.

Garcia G.M., Stalker H.T., Kochert G. (1995) Genome, **38**: 166-176.

Lashermes P., Combes M.C., Robert J., Trouslot P., D'hont A., Anthony F., Charrier A. (1999) Mol. Gen. Genet. **261**: 259-266.

Lashermes P., Paczek V., Trouslot P., Combes M.C., Couturon E., Charrier A. (2000) J. Heredity, **91** (1): 81-85.

Lashermes P., Combes M.C., Prakash N.S., Trouslot P., Lorieux M., Charrier A. (2001) Genome (in press).

Parkin I.A.P., Lydiate D.J. (1997) Genome **40**: 496-504.

Identification of Repeated Sequences in *Coffea* Genomes

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INTRODUCTION

The genus *Coffea* originating from Africa and the Mascarene Islands contains over 80 species and taxons. All, but one, species are diploid with 2n=2x=22.

In order to take advantages of the genetic resources existing in the genus, inter-specific crosses have been realized involving wild species. Some combinations, mainly those involving East African X West African species, gave high levels of sterility in the offspring. This sterility is induced by the presence of univalent chromosomes during meiosis.

Nuclear DNA content varies between diploid species from 0.9 pg to 1.8 pg and East African species have generally a lower DNA content than West and Central African ones (Table 1). As all the species have the same basic genome, differences in DNA amount can be explained by variable abundance of repeated sequences.

As meiotic sterility of F1 hybrids is related to the genome size difference between parental species increases, repeated sequences could be at the origin of pairing difficulties.

Table 1. DNA content per nucleus for some coffee species

Species	Ploidy	Geographic repartition	DNA content (pg/nucleus)
C. arabica	4x	East Africa	2.61±0.2
C. humilis	2x	Guinea-Congo	1.78±0.33
C. sp. *moloundou*	2x	Guinea-Congo	1.69±0.25
C. liberica	2x	Guinea-Congo	1.68±0.29
C. congensis	2x	Guinea-Congo	1.62±0.19
C brevipes	2x	Guinea-Congo	1.55±0.18
C. canephora	2x	Guinea-Congo	1.54±0.22
C. eugenioides	2x	East Africa	1.39±0.12
C. stenophylla	2x	Guinea-Congo	1.35±0.12
C. sp F.	2x	East Africa	1.33±0.02
C. pseudozanguebariae	2x	East Africa	1.09±0.13
C. sessiflora	2x	East Africa	1.04±0.16
C.racemosa	2x	East Africa	0.95±0.13

In: Cros *et al.* Can. J. Bot. Vol. 73 (1995)

MATERIAL AND METHODS

A genomic library enriched in repeated sequences and containing 193 clones, was established from total DNA isolated from F1 plants issued from a cross between *C. pseudozanguebariae* and *C. liberica* var *Dewevrei*. 36 clones were sequenced on one strand and sequence comparisons with GenBank data were made using Blast.

Figure 1. Coffee center of origin and possible spread across the African continent

Table 2. Identification of some coffee sequences present in an enriched repeated sequences library

Seq #	Length bp.	%AT	Comments, similitude
12	513 (568)	61 (60)	Rep Seq, (AS=cytochrome?)
6	569	58	H.s. ?
33	563	61	Rep Seq
55	528	64	H. sapiens
36	527	63	?, Rep Seq
40	466	60	H. sapiens, Rep Seq
54	448	61	Rep Seq
38	444	65	cp DNA
4	266	64	Copia-like, gag-pol polyprotein
35	265	68	*L. esculentum* Rep Seq
10	186	63	Ty3/gypsy like, Integrase
11b	185	53	Athila like?
12b	185	60	Spacer rDNA
7a	153	64	Prot CLB1 (L. esc) Cal.B (A. t.)
21d	143	59	URF?
21c	132	70	Ty3/gypsy like, gag-pol polyprotéine
11a	53	60	transcription factor
12d	35	57	cp DNA rDNA spacer + ARNt Ile et Ala

CONCLUSION AND PERSPECTIVE

Coffee genome appears to be rich in repeated sequences. On a small sample, different types were identified including several kinds of retrotransposons. Further work will include finishing the sequencing of the repeated sequence library. Presence of the different repeated sequences will be investigated in different species. Number of repeats will be estimated through DNA blotting and/or quantitative PCR. Their chromosomal location will be observed using fluorescent *in situ* hybridization. (FISH). Relationships between repeated sequences presence; copy number, location and biogeographic repartition of coffee species will be studied and their role in coffee evolution will be evaluated.

Gene Flows and Evolutionary Process of *Coffea arabica* through the Natural Hybrids *C. arabica* x *C. canephora* in New Caledonia

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SUMMARY

The genomic affinities between the two cultivated species *C. arabica* (2n=4x=44 chr.) and *C. canephora* (2n=2x=22) permit the realization of their interspecific hybrids. At F1 stage, two types are artificially obtained: triploid, or tetraploid (called arabusta). These hybrids are generally vigorous, but they differentiate from each other by their imperfect fertility level. The 3x are nearly sterile, whereas the 4x are half-fertile.

In the New Caledonian neglected plantations sheltering the two species, such F1 hybrids are created naturally, as well as their backcrosses with *C. arabica*. The two parent species were introduced, respectively, in 1856 and during the1910's. The *C. arabica* trees devastated by rust (*Hemileia vastatrix*) were advantageously substituted by *C. canephora*. This second species, more rustic, was mixed planted in arabica plantations without previous pulling up of the latter.

Out of the 400 natural hybrids collected, observations *in situ*, flow cytometry and molecular analyses indicate a very important hybrid diversity. All genetic combinations varying from diploid to hexaploid are present. The hybrids are being evolving toward new resistant introgressed arabica genotypes. Thus, the evolutionary process *in situ* of *C. arabica*, in contact with the natural hybrids, allowed to isolate a Le Roy coffee tree genotype, which accumulates the recessive mutation *laurina* into a homozygous state (lr/lr) and the introgressed resistance brought by a part of *C. canephora* genome.

The genes flows create new original genetic resources in the New Caledonian neglected coffee plantations. Would they constitute a secondary center of diversification to *C. arabica*?

RÉSUMÉ

Les affinités génomiques entre les deux espèces cultivées *C. arabica* (2n=4x=44 chr.) et *C. canephora* (2n=2x=22) permettent la réalisation de leurs hybrides interspécifiques. Au stade F1, deux types ont été obtenus artificiellement: triploïdes, ou tétraploïdes (appelés arabusta). Les hybrides sont généralement vigoureux, mais se différencient par leur degré de fertilité imparfaite. Les 3x sont quasi-stériles alors que les 4x sont semi-fertiles.

Dans les plantations abandonnées de Nouvelle-Calédonie abritant les deux espèces, de tels hybrides F1 se sont créés naturellement, ainsi que leurs rétrocroisements avec *C. arabica*. Les deux espèces parentes avaient été introduites respectivement en 1856 et au cours des années 1910. L'espèce *C. arabica* dévastée par la rouille orangée (*Hemileia vastatrix*) fut remplacée en grande partie par *C. canephora*. Les caféiers de cette deuxième espèce, plus rustiques ont été alors plantés en mélange dans les plantations d'arabica sans arrachage préalable de ces dernières.

Sur les 400 hybrides naturels collectés, les observations sur le terrain, les analyses en cytométrie de flux et moléculaires indiquent une très importante diversité des hybrides. Toutes les combinaisons génétiques allant de la diploïdie à l'hexaploïdie sont présentes. Les hybrides sont en train d'évoluer vers de nouveaux génotypes arabica introgressés résistants. Ainsi, le processus évolutif *in situ* de *C. arabica*, en contact avec les hybrides naturels, a permis d'isoler un génotype de caféier Le Roy qui cumule la mutation récessive *laurina* à l'état homozygote (lr/lr) et la résistance apportée par l'introgression d'une partie du génome *C. canephora*.

Les flux de gènes créent des ressources génétiques nouvelles originales dans les caféières abandonnées de Nouvelle-Calédonie. Constitueraient-elles un centre secondaire de diversification de *C. arabica*?

INTRODUCTION

The hybridization between the two cultivated species *C. arabica* and *C. canephora* has for main objective to improve organoleptic qualities of the low altitude coffees or to bring resistance to the cultivated arabica. Their tetraploid F1 hybrids (arabusta) are half-fertile, while their triploid F1 are nearly sterile (Le Pierrès, 1995).

Such hybrids can occur naturally in areas where the two parent species coexist. It is the case in the mixed coffee neglected plantations in New Caledonia. The hybrid population, constituted of successive generations, has the particularity to be very variable and to produce some special types of arabica resistant to rust.

The succession of backcrossing with *C. arabica* gives new genotypes in which a large part of the *C. canephora* genome has been eliminated. Only parts of the genome that procure a selective advantage to hybrids that carry them, in particular these concerning the resistance to diseases, remain in the backcrossing. Such natural introgression case is well known: that is the Timor hybrid, which is extensively used to transfer the resistance to rust (and to other diseases) to *C. arabica* with its derivatives (catimor, Sarchimor, etc.).

The objective of our work is to find new genotypes efficient to replace advantageously the Timor hybrid.

MATERIAL AND METHODS

The natural hybrids have been known for the past 40 years in New Caledonia. Two hybrid collecting missions have been done in the 1990's (Charmetant and Le Pierrès, 1991; Le Pierrès, 1999). More than 400 natural hybrids have been collected from forty sites. Most of the genotypes are resistant to rust.

The genetic diversity of the parent species is very unequal: *C. canephora* is very variable, while *C. arabica* is essentially composed of related varieties: *Typica*, *Bourbon* and *Laurina*.
The genetic variability analyses have been done by molecular techniques (RAPD and AFLP), while the ploidy levels (Figure 1) are valued by the flow cytometry (Dolezel et al., 1989; Barre, 1997).

Some complementary verifications were also realized by chromosomal counting.

RESULTS

The favourable factors to the natural hybridization are the neglected plantations, the conditions of low temperatures and permanent rainy weather, the resistance to rust of hybrids, and the interest of planters for these hybrids.

The hybrid population is divided in two major compartments (Figure 1):

- triploid, intermediate between *C. canephora* and *C. arabica*;
- tétraploid, closed to *C. arabica* which has 44 chromosomes.

The 3x hybrids are more numerous than the 4x. The qDNA classes distribution, going from the diploidy (2x=22 chromosomes) to the hexaploidy (6x=66), shows many aneuploid hybrids.

The natural hybrid progenies are heterogeneous, what proves the genetic instability of their hybrid parents. The obtained genotypes have variable genome sizes or chomosomal numbers. The progenies of an hybrid 4x ("A") can be classified in two groups. The first major group from the normal beans is composed of genotypes quite homogeneous of the 4x type, as their mother. On the other hand, the second group, appeared from the badly formed seeds, shows a heterogeneous progeny similar to the one of the hybrid 3x progeny ("B"), with nuclear DNA quantities between those of 3x hybrids and the arabica. The cold weather, at meiosis, induces a great proportion of no-reduced gametes which produce, by fertilization, bigger genome sizes until 5x and 6x levels ("C"). This process does not reduce the progeny variability. Indeed, reporting to Veilleux's studies (1985), two types of gametes formation mechanisms explain their 5x or 6x variability: by FDR (first division restitution) or by SDR (second division restitution). The FDR contributes to increase the hybrid variability by varying the genetic polyploid combinations. Finally, multiple dependent factors, such genotypes, environments, and their interactions, promote the enlargement of the genetic diversification and the natural selection.

The whole of studies achieved on the hybrid material permitted to select some genotypes of interest for the improvement.

An original genotype, introgressed resistant *Laurina*, has been identified. It accumulates the recessive mutation *laurina* at the homozygous state (lr/lr), the resistance to rust brought by the introgression of a *C. canephora* genome part, and a balanced number of chromosomes (44).

> Remember that the *Laurina* mutation at the homozygous state is carried by the "Le Roy" arabica variety, obtained from the *Bourbon* variety. Its cup qualities are reputed the top-of-the-range by the taste and the aroma. This mutation expression makes half-size of the arabica caffeine content (Sondahl et al., 1995)

CONCLUSION

The genes flows between the two cultivated species and the genetic mixing of natural hybrids conduct to new *C. arabica* genotypes.

The natural hybrids contribute to the creation of a secondary center of *C. arabica* diversification in evolution at the New Caledonian old coffee plantations (Figure 2). This

new original natural genetic resource is required to enlarge the current genetic basis of the cultivated arabica and for improvements of the coffee quality.

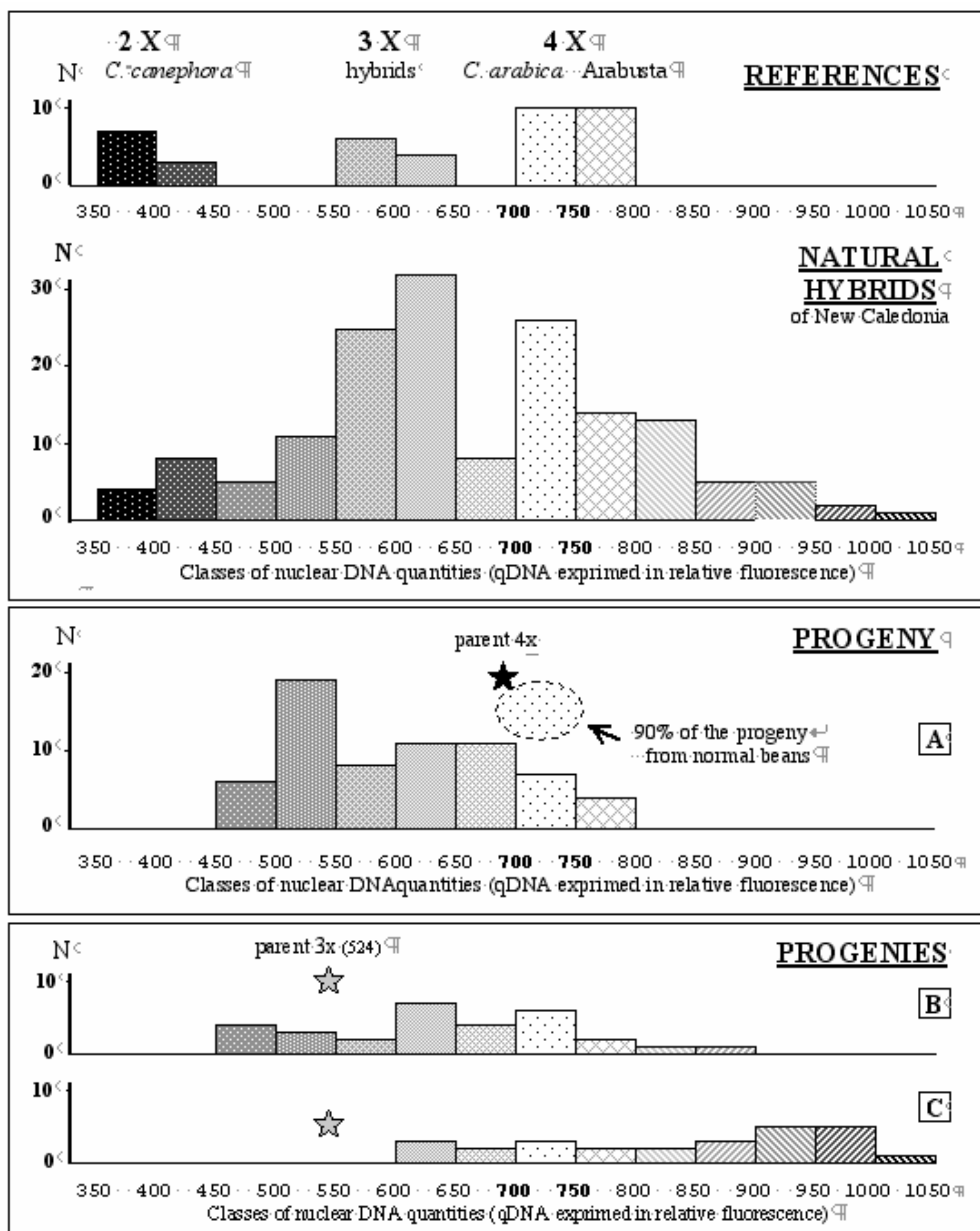

Figure 1. Nuclear DNA quantities (qDNA) of the natural hybrid coffee trees. Comparison with their progenies ("A" from a 4x, "B" from a 3x, and "C" from the same 3x under cold weather)

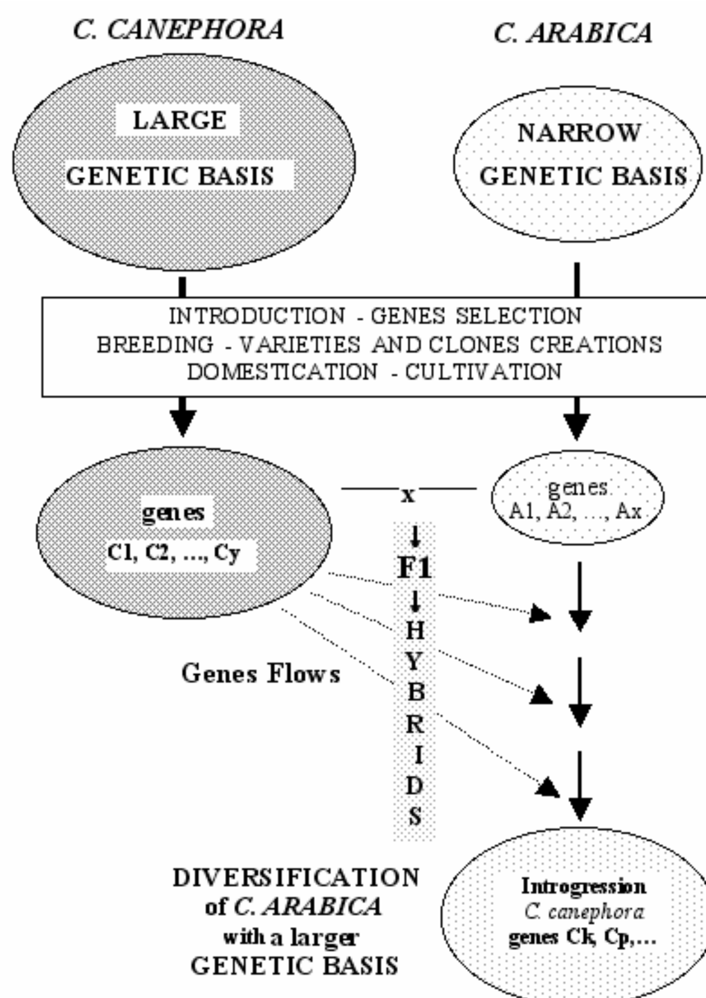

Figure 2. Formation of a secondary center of diversification for *C. arabica* in New Caledonia and increasing variability by *C. canephora* genes introgressions through the natural hybrids

REFERENCES

Barre Ph., 1997 – Les transferts de gènes entre espèces de caféiers diploïdes: Etude des hybrides F1 et BC1 entre *C. pseudozanguebariae* Bridson et *C. liberica* Bull ex. Hiern (*C. devewrei* De Wild et Th.Dur). Thèse de doctorat, ENSA de Montpellier, 117 pages et 5 annexes.

Charmetant P., Le Pierrès D., 1991 – Rapport de mission en Nouvelle-Calédonie: prospection et collecte d'hybrides naturels de caféiers cultivés. Multigr. IRCC/CIRAD – ORSTOM, 25 pages.

Dolezel J.P., Binarova P., Lucretti S., 1989 – Analysis of nuclear DNA content in plants cells by flow cytometry. Biology Plantarum (Prahia), **31**, 113-120.

Le Pierrès D., 1995 – Etude des hybrides interspécifiques tétraploïdes de première génération entre *Coffea arabica* L. et les caféiers diploïdes. Thèse doctorat, Univ. d'Orsay (Paris XI), 298 pages.

Le Pierrès D., 1999 – Survey of new caledonian natural hybrids *Coffea arabica* x *C. canephora*. IRD, Nouméa, 44 p..

Sondalh M.R., Romig W.R., Bragin A., 1995 – Induction and selection of somaclonal variation in Coffee. US Patent Number **5** 436 395.

Veilleux J., 1985 – Diploid and polyploid gametes in crop plants: mechanisms of formation and utilization in plant breeding. Theor. Appl. Genet., **59**: 95-100.

Relationship between Parental Chromosomic Contribution and Nuclear DNA Content in the Coffee Interspecific Hybrid: *C. pseudozanguebariae* x *C. liberica* var. *dewevrei**

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INTRODUCTION

F1 and backcross hybrids were obtained between two diploid coffee species (2n = 22) differing by their nuclear DNA contents (*C. pseudozanguebariae* (PSE) 2C = 1.13 pg and *C. liberica* var. *dewevrei* (DEW) 2C = 1.42 pg). Genomic *in situ* hybridisation (GISH) and flow cytometry were used on 6 F1 hybrids and 7 backcross hybrids in order to determine their parental chromosomic contribution and their nuclear DNA content (qDNA), respectively.

GISH AND FLOW CYTOMETRY RESULTS IN THE TWO SPECIES AND THEIR INTERSPECIFIC HYBRIDS

1. GISH efficiently identified chromosomes from both species.
2. In PSE and DEW, two 18S-5.8S-25S rDNA sites were localised on terminal segments of two chromosomes (Photo).
3. F1 hybrids had DNA content intermediate between the parental species and contained 11 chromosomes from each species as expected.
4. There was a linear relationship between the number of PSE chromosomes and the nuclear DNA content (Figure 1).

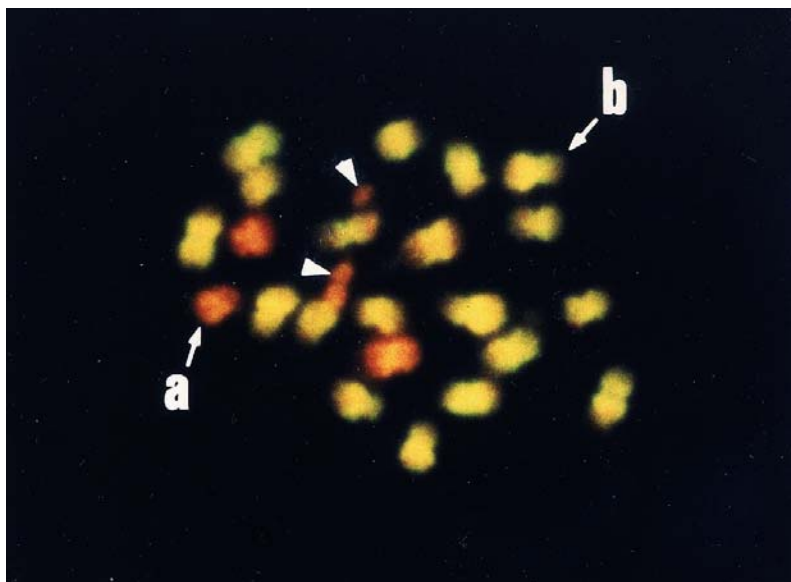

Photo 1. a: PSE chromosome b: DEW chromosome triangles: rDNA sites

CONCLUSIONS

Flow cytometry allows to sort hybrids with nuclear DNA content and DEW chromosome number close to the cultivated species.

In introgression programmes, the aim is to produce plants with the interesting wild trait, but conserving the back-ground of the cultivated species. This is usually done by backcrossing the hybrids having the favourable trait on the cultivated species and this process have to be carried out on about ten generations. This is time-consuming, especially for coffee trees which yield after four years. The use of molecular markers and flow cytometry for early selection could accelerate this pro-cess.

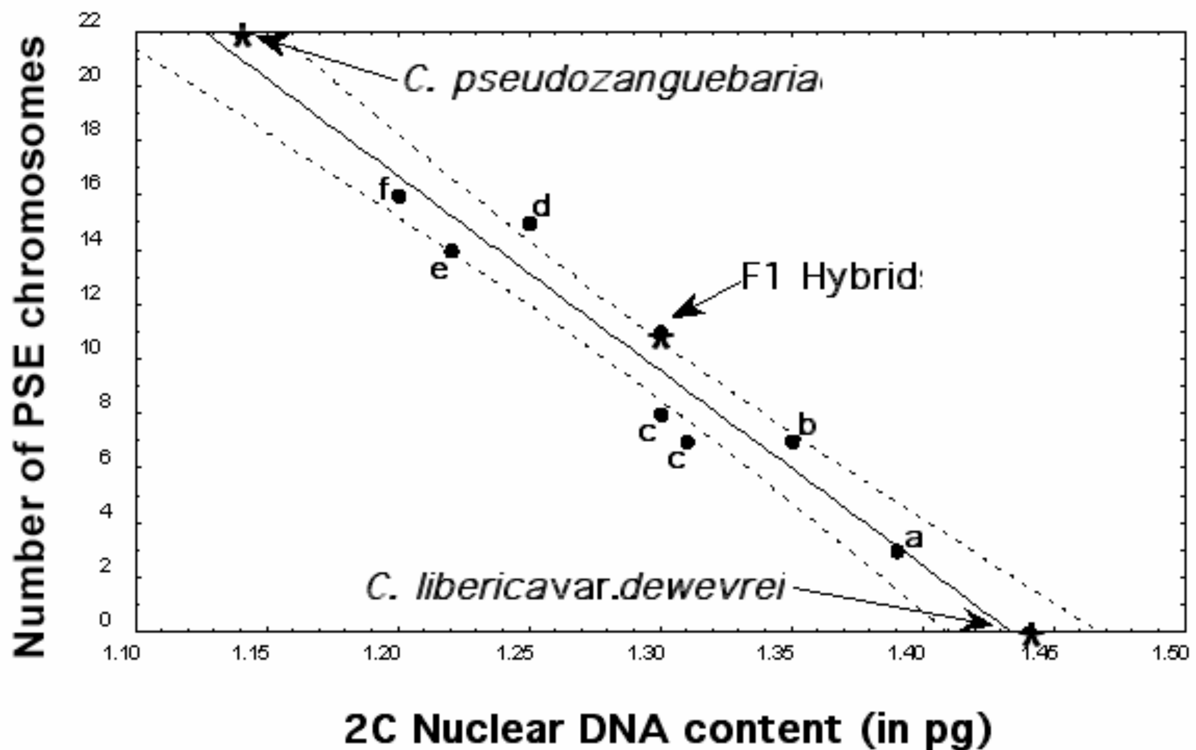

Figure 1. Linear relationship (y = -71.41 x + 102.42 r = 0.98) between the nuclear DNA content and the number of chromosomes on DEW and PSE, F1 and G2 hybrids. Results of the Newmann & Keuls test on qDNA are indicated with letters

Application of DNA Marker Technologies in Characterizing Genome Diversity of Selected Coffee Varieties and Accessions from India

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SUMMARY

Assessment of genome diversity in a genepool of any particular species offers great promise for better exploitation and management of genetic resources. The development of molecular markers in coffee trees (*Coffea* L.) has opened new perspectives for analysing genetic relationships and in marker assisted breeding. In this study, we report the successful use of two efficient PCR based DNA marker technologies, Amplified Fragment Length Polymorphism (AFLPs) and Simple Sequence Repeats (SSRs) for generating DNA profiles of the selected *C. arabica* accessions under cultivation and genebank collections of *C. canephora* available in India. Eighteen arabica genotypes covering the commercial accessions S.288, S.795 and S.1934 were subjected to AFLP analysis using 36 different primer combinations. Analysis of the DNA fingerprints revealed 18 to 45 amplified products depending on the genotype and primer set. In all, a total of 115 polymorphic bands were scored. The high polymorphism is mainly attributed to the introgression of *C. liberica* genetic material as these commercial lines were derived from S.26, a putative natural interspecific hybrid (*C. arabica* x *C. liberica*) and thus the molecular data established the natural hybrid lineage. Further, 39 accessions of *C. canephora* representing indigenous as well as exotic genebank collections were analyzed for genetic diversity in comparison with 16 IRD collections from different geographical regions of the world, using 15 AFLP primer combinations and 12 SSR primer sets. A total of 205 polymorphic bands were scored from the AFLP marker data while 72 alleles were identified with 12 SSR primers. Analysis of data for genetic relationships revealed the structure of Indian germplasm and the outcome is almost similar with both marker approaches. The potential use of AFLPs and SSRs for evaluating genetic diversity and in other molecular genetic applications are discussed.

INTRODUCTION

Coffee is one of the most important plantation crops of tropics including India. Although the genus *Coffea* is reported to comprise over 80 species (Bridson and Verdcourt, 1988), commercial coffee production mainly relies on two species *Coffea arabica* (arabica) and *C. canephora* (robusta). The cultivated arabicas show a homogeneous agronomic behaviour characterized by high susceptibility to many pests and diseases. On the other hand, considerable variability was reported among diploid coffee species and some of these species form valuable gene reservoir, for different breeding purposes (Berthaud and Charrier, 1988).

In the Indian context, leaf rust caused by *Hemileia vastatrix* is a serious disease of concern for arabica causing substantial crop losses. In early coffee breeding programmes of India, S.26 a putative natural interspecific hybrid between *C. arabica* and a diploid coffee species was used as main source for rust resistance and two commercial strains viz. S.288 and S.795 were evolved (Anonymous, 1985). S.795 was under commercial cultivation since 1947 because of its good vegetative vigour, high productivity, superior quality and field tolerance to leaf rust especially races I and II (Srinivasan and Narasimhaswamy, 1975; Ramachandran and Srinivasan, 1979) prevalent in Indian coffee tracts. These early Indian selections carry SH3 resistance factor for coffee rust, only known to be present in *Coffea liberica* (Wagner and Bettencourt, 1965). Hence, it has been assumed that *C. liberica* was likely involved in origin of this natural hybrid. An insight at molecular level would be particularly useful for understanding the genetic constitution of these populations, for a better exploitation of available variability.

The conventional breeding efforts towards improved cultivars especially in arabica coffee are often faced with constraints of low genetic diversity, long pre bearing periods and difference in ploidy level between potential donors and recipient species. Further, difficulties in enlarging the genetic base is also a limitation for cultivar development. Recent advancements in molecular marker technologies however opened up new possibilities to overcome some of these limitations and provide new tools for enhanced use of available genetic resources. The potential use of DNA marker technologies in coffee genetics have been demonstrated (Lashermes et al., 1996; Paillard et al., 1996; Lashermes et al., 1997; Agwanda et al., 1997; Combes et al., 2000; Lashermes et al., 1999a; Lashermes et al., 2000a; Lashermes et al., 2000b, Prakash et al., 2001). The present study is therefore aimed at successful application of two PCR based DNA marker technologies, Amplified Fragment Length Polymorphism (AFLPs) and Simple Sequence Repeats (SSRs) for characterizing the genome diversity in early Indian cultivars and robusta coffee genepool available in India.

MATERIALS AND METHODS

Plant material

Eighteen *C. arabica* genotypes covering the commercial accessions S.288, S.795 and S.1934 were subjected to AFLP analysis using 36 different primer combinations. S.795 and S.1934 represent F2 and F4 respectively, derived from the cross between S.288 (selfed offspring of S.26, a putative natural hybrid between *C. arabica* and *C. liberica*) and x 'Kent', a pure arabica selection. Besides the parents (S.288 and Kent), five representative accessions each of *C. arabica* and *C. liberica* species were also included in the analysis.

Further, 39 accessions of *C. canephora* representing indigenous and exotic gene bank collections available in India were subjected to genetic diversity analysis. For comparison, 16 accessions (13 robusta and three *C. congensis*) representative of the genetic diversity collected in different geographical regions of the world, available at IRD (ex ORSTOM), Montpellier, France were also included. DNA profiles of these selected materials were generated using 15 AFLP primer combinations and 12 SSR primer sets.

DNA isolation

Genomic DNA was isolated from lyophilised leaves of arabica materials using QIAGEN DNeasy plant mini kit-1999, as specified by the manufacturer. Genomic DNA of the robusta materials was extracted from the lyophilized leaves through a nuclei isolation step as described by Agwanda et al. (1997).

AFLP assay

AFLP analysis was performed essentially as described by Vos et al. (1995) with minor modifications (Lashermes et al., 2000a). The primers used are detailed in Table 1.

SSR polymorphism

The 12 SSR primers (Table 1) evaluated in the present study were developed jointly by Department of Biology, Trieste University, Italy and GeneTrop, IRD, Montpellier, France under INCO-DC project (Contract No.ERBIC 18CT 970181) of European Community.

Table 1. AFLP and SSR primers used for analysis

AFLP primers used for analysis		AFLP primers used for analysis		Microsatellites used
E + 3	M + 3	E + 3	M + 3	
AAC	CAG	AAC*	CAA*	Sat11
AAC	CTA	AAC*	CAC*	Sat25
AAC	CTC	AAC*	CTG*	Sat27
AAC	CTT	AAG*	CTA*	Sat29
AAG	CTT	AAG*	CTG*	Sat42
ACG	CAA	ACG*	CTA*	Sat157
ACG	CTC	ACG*	CAT*	Sat158
ACG	CTG	ACT*	CAT*	Sat161
ACG	CTT	ACT*	CTG*	Sat162
AGC	CAC	AGC*	CTG*	Sat166
AGC	CAG	ACA*	CAG*	Sat167
AGG	CAC	ACA*	CTT*	Sat177
AGG	CTA	AGG*	CTC*	
AGC	CTC	ACA*	CAC*	
ACT	CAC	ACA*	CAT*	
ACT	CTA			
ACT	CTT			
ACA	CAA			
ACA	CTC			
ACA	CTG			
AGG	CTT			

* *Primers used for AFLP analysis of both arabica and robusta materials*

The methodology followed for preparation of genomic libraries, enrichment, sequencing of positive clones and primer design has already been reported elsewhere (Vascotto et al., 1999; Rovelli et al., 2000). The PCR assays of SSR primers were carried out as reported by Combes et al. (2000).

Electrophoresis

Amplification products were electrophoresed on 6% denaturing polyacrylamide gel with 8 M urea and 1 X TBE. The dried gels were exposed to Kodak Biomax X-ray film.

Data analysis

The AFLP amplification products were designated in order of decreasing fragment size and according to the restriction enzymes as well as primer combinations used. Only clear polymorphic bands were scored for each genotype as present (1) or absent (0). SSR loci were scored individually and different alleles at each loci were recorded. Presence of single bands per sample was considered as presence of two identical alleles and two bands as two different alleles. Data analysis was carried out separately for AFLP and SSR data using TREECON (version 1.1) package (Van der Peer and De Wachter, 1994) as described elsewhere (Prakash et al., 2001)

RESULTS AND DISCUSSION

AFLP profiles of early Indian strains and extent of polymorphism

Analysis of AFLP fingerprints revealed that the number of clearly amplified products per sample ranged from 18 to 45, based on the genotype and primer combination used. A total of 137 polymorphic bands were scored in all the 29 accessions analysed (Figure 1). The number of polymorphic fragments within arabica accessions analysed was only 35. Among the natural hybrid derived genotypes i.e.; S.288, (offspring of the natural hybrid) and subsequent F2 (S.795) and F4 (S.1934) lines, 115 polymorphic bands were observed. Of these 115 bands, 13 were invariably present in 'Kent' but not in any other arabica accessions indicating that these

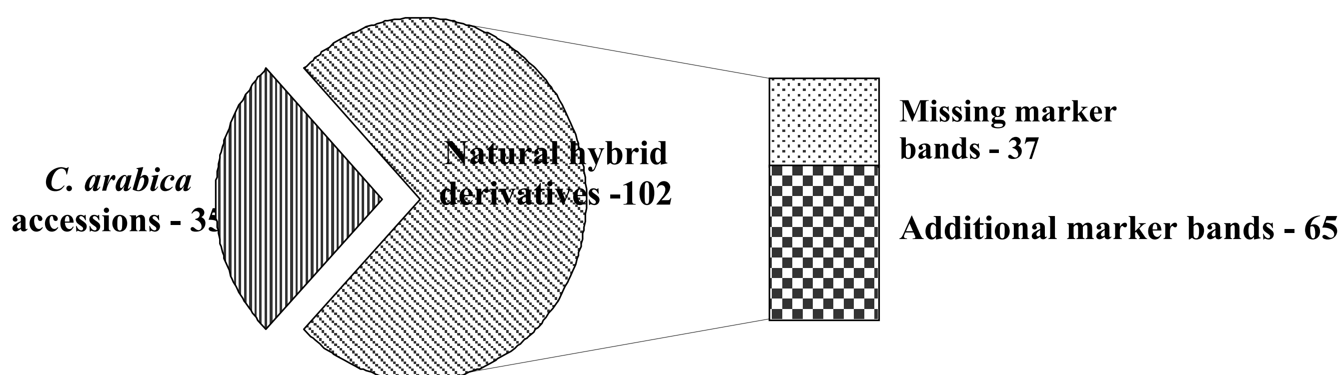

Figure 1. Pie chart depicting the number of polymorphic bands (AFLP) observed among individuals of each group i.e. accessions of *C. arabica* and natural hybrid derivatives. The introgressed markers associated with additional bands and missing bands were distinguished

marker bands were specific to 'Kent' parent. Of the remaining 102 marker bands, 63 were observed in S.288 parent and also seen in at least one of the *C. liberica* accessions analysed. None of these markers were seen either in 'Kent' or in other arabica accessions and were therefore considered as markers introgressed from *C. liberica*. Interestingly, only two introgression bands seen in some introgression lines were found absent in S.288 parent. The remaining 37 markers identified in introgression lines corresponds to the missing bands. In most of the cases, the marker bands due to band absence (missing bands) were also found absent in at least one of the five *C. liberica* accessions analysed and always present in *C. arabica* accessions including 'Kent'. It may be rather difficult to explain precisely the origin of these markers in introgressed hybrid derivatives. However, as opined by Lashermes et al., (2000a), these markers could be considered as 'related to introgression process'.

Further, the number of additional bands representing introgression varied from 10 to 56 among the 17 introgression genotypes as against 63 additional marker bands in introgressed parent S.288. The number of additional introgressed bands ranged between 10 to 56 in F_2 (S.795) and 18 to 56 in F_4 (S.1934) lines analysed. No genotype among F_2 and F_4 lines contained all these 63 introgressed bands.

The limited number of introgressed markers in general indicated that the introgression was restricted to few chromosome segments. Further, relatively less variation observed in the number of introgressed bands between F_2 and F_4 and some genotypes showing almost as many bands as that of introgressed parent suggested that there was neither elimination of introgressed segments nor counter-selection during generation advancement.

Based on the results, it could be inferred that *C. liberica* was involved in origin of natural hybrid, S.26. It may be assumed that the natural hybrid S.26 might have originated either from a natural tetraploid F_1 progenitor (union of reduced gamete from tetraploid *C. arabica* and unreduced gamete from diploid *C. liberica*) or triploid F_1 progenitor (by union of reduced gametes). The polymorphism identified in these hybrid derivatives was therefore the consequences of introgressive hybridizations involving *C. liberica*. As stated in history of coffee cultivation in India (Anonymous, 1985), *C. arabica* was the only species under commercial cultivation until 1900. In order to check the ravages of leaf rust (*Hemileia vastatrix*) during later part of 18th century, other tolerant species like *C. liberica* and *C. canephora* were introduced. Cultivation of these species together with *C. arabica* might have offered the possibility for natural hybridization resulting in spontaneous interspecific hybrids. Thus, our results clearly established the molecular evidence on origin of natural hybrid between *C. arabica* and *C. liberica.*

Genetic diversity analysis of robusta genepool

SSR polymorphism

A total of 72 alleles were identified with 12 different primer pairs and the banding patterns resolved by each primer pair are in accordance with single locus variation. An example of SSR alleles as resolved with the PCR assay is illustrated in Figure 2. The number of alleles varied widely among these 12 loci and ranged from three (Sat 11) to 10 (Sat 157). On average six alleles per loci were detected. For comparison, the total population analysed was divided in to three groups i.e.; collections of indigenous gene bank, exotic gene bank and IRD gene bank. The total number of alleles representing each group were 44, 51 and 62 in Indigenous, exotic and IRD collections, respectively. Furthermore, 13 group specific alleles were detected in IRD collections as against two (exotic) and three (indigenous collections of India). This clearly indicates the high amount of diversity present in world genepool, which is not represented by Indian genepool. It is interesting to note that, in terms of specific alleles the three accessions of *C. congensis* originating from both Central African and Congo did not exhibit any significant diversity from other robusta accessions except having only one different allele. This supports the school of thought that *C. congensis* forms a biotype of robusta (*C. canephora*).

A cluster analysis (UPGMA) was performed using the similarity matrix based on the proportion of shared alleles across the 12 SSR loci. From the dendrogram it is apparent that there is a clustering of small sub-groups forming six groups. However, the associations between the groups were not strong. Broadly the collections from both indigenous and exotic gene banks of India grouped together with the robusta types identified as diversity group ‘E’ (Dussert et al., 1999).

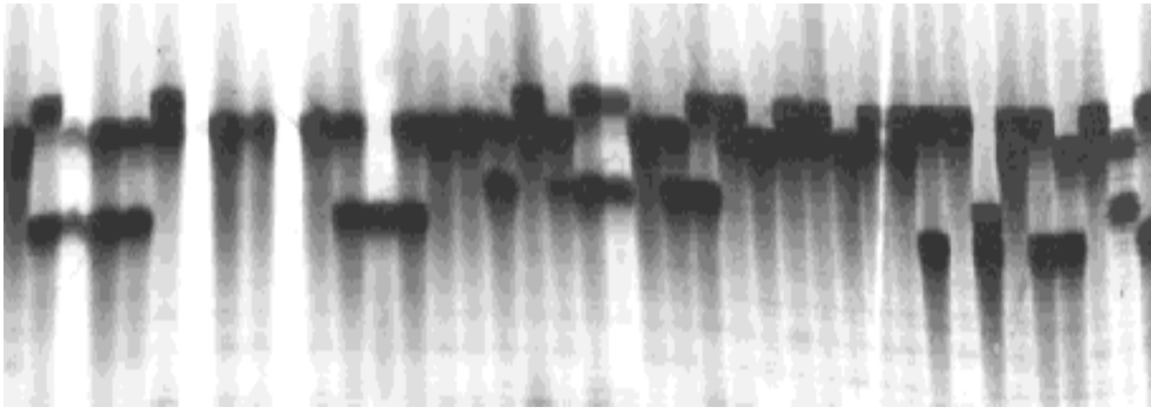

Figure 2. SSR polymorphism in robusta accessions with Sat.166

AFLP polymorphism in robusta collections

In total, 15 primer combinations were used in the study. The number of clearly amplified products per sample ranged from 25 to 45, depending on the genotype and primer combination. A total of 205 polymorphic bands were scored in all the 55 accessions analysed. The number of polymorphic bands varied from 51 to 94 and no single accession contained all polymorphic bands.

Group wise comparisons were made as in the case of SSR polymorphisms. The indigenous collections represented the low number of polymorphic bands (151) than exotic introductions (194) and IRD collections (189). The genetic diversity in indigenous collections reflected by AFLP marker data is in conformity with the results obtained with SSR markers. The grouping associations more or less followed the similar pattern (Figure 3) as seen with SSR markers.

Thus both AFLP and SSR techniques reflected similar results and revealed that the accessions originating from Indian gene banks tend to show good amount of genetic diversity and higher inter-accession differentiation. However, on comparison with the representative world collections from different geographic regions, it was found that the Indian robusta genepool mainly represents the diversity group E (Dussert et al., 1999) and by large other diversity groups (A,B, C and D) are not represented.

Further, as most of the commercially cultivated types in India forms the selections from indigenous collections, the exotic gene bank provide good source of additional variability for robusta breeding purposes.

CONCLUSIONS AND PROSPECTS

Germplasm characterization

The microsatellite and AFLP markers have been found useful in genotyping of germplasm, quantification of genetic diversity, establishing relationships with collections of different geographic origin and structure of genepool. In the Indian context, the preliminary findings obtained in present study provided opportunity towards detailed characterization of coffee germplasm. This helps in identifying core collections for easy management and efficient exploitation of genetic resources.

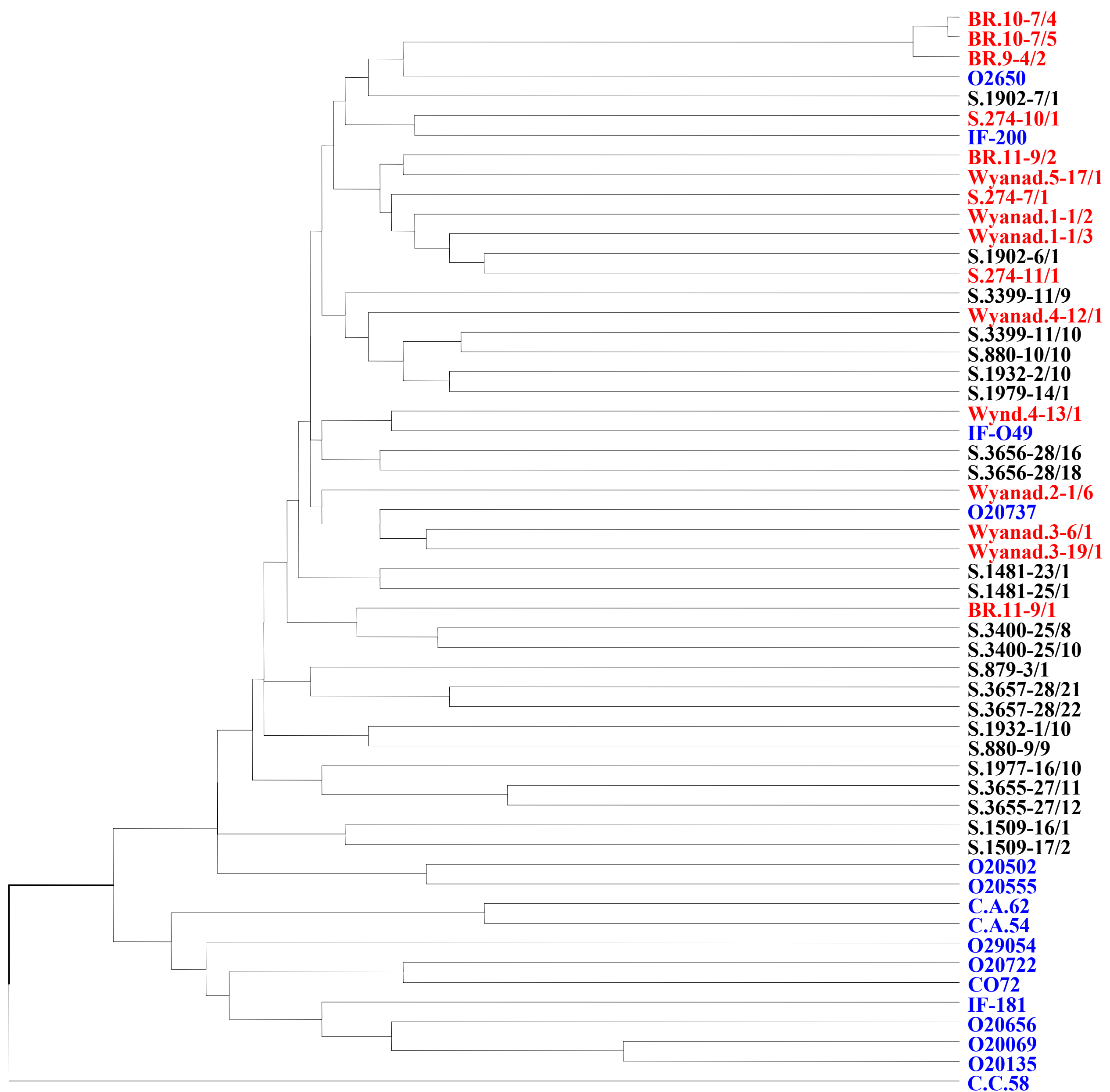

Figure 3. Dendrogram of the robusta accessions generated by group average clustering (UPGMA), using genetic distance based on AFLP polymorphism. Red, black and blue colours indicates collections from indigenous, exotic gene banks of India and IRD gene bank, respectively

Cultivar identification

Both SSR markers and AFLPs found are suitable for cultivar identification in coffee and it is possible to generate reliable genome fingerprints for establishing the plant proprietary rights.

Analysis of alien genome introgression

The efficiency of AFLP technology for polymorphism detection and analysis of alien genome introgression was demonstrated in Indian arabica coffee cultivars, there by offering wide scope of application in marker aided breeding programmes of coffee. The study established the uniqueness of these strains due to Liberica–introgression. The variability generated in

C. arabica due to introgressive hybridizations from *C. liberica* has potential implications especially in relation to leaf rust resistance sources. It is possible to exploit these introgressed lines for the purpose of gene pyramiding. Further, the molecular information and fingerprints generated on early Indian arabica strains could be utilised for claiming the national proprietary rights of the material. In similar lines, alien genome introgression in several other proven interspecific (Congensis x Robusta and Robusta x arabica) hybrids and promising genotypes derived from natural interspecific hybrids like 'Devamachy' could be analysed.

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REFERENCES

Agwanda C.O., Lashermes P., Trouslot P., Combes M.C. and Charrier A., 1997. Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae,* in arabica coffee. Euphytica **97**: 241-248.

Anonymous, 1985. Coffee Guide, Coffee Board, Bangalore, India,200p.

Bridson D.M. and Verdcourt B., 1988. *Coffea* & *Psilanthus*. In: Balkema (Ed.), Fl.Trop.East Africa: Rubiaceae (part 2), pp 703-727. R.M.Polhill, Rotterdam.

Berthaud J. and Charrier A., 1988. Genetics resources of *Coffea*. In: R.J. Clarke and R. Macrae (Eds), Coffee vol. **4**: Agronomy, pp 1-42. Elsevier Applied Science, London.

Combes M.C., Andrzejewski S., Anthony F., Bertrand B., Rovelli P., Graziosi G. and Lashermes P., 2000. Characterization of microsatellite loci in *Coffea arabica* and related coffee species. Molecular Ecology **9**: 1178-1180.

Dussert S., Lashermes P., Anthony F., Montagnon C., Trouslot P., Combes M.C., Berthaud J., Noirot M. and Hamon S., 1999. Le caféier, *Coffea canephora.* In Hamon P.,. Seguin M, Perrier X. and Glaszmann J.C. (Eds), Diversité génétique des plantes tropicales cultivées, CIRAD, Montpellier, France.

Lashermes P., Trouslot P., Anthony F., Combes M.C. and Charrier A., 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. Euphytica **87**: 59-64.

Lashermes P., Combes M.C., Trouslot P. and Charrier A., 1997. Phylogenetic relationships of coffee tree species *(Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. Theor Appl Genet **94**: 947-955.

Lashermes P., Combes M.C., Robert J., Trouslot P., D'Hont A., Anthony F. and Charrier A., 1999. Molecular characterisation and origin of the *Coffea arabica* L. genome. Molecular General Genetics **261**: 259-266.

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S., Graziosi G., Trouslot P. and Anthony F., 2000a. Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.). Theor Appl Genet **100**: 139-146.

Lashermes P., Paczek V., Trouslot P., Combes M.C., Couturon E. and Charrier A., 2000b. Single-locus inheritance in the allotetraploid *Coffea arabica* L. and interspecific hybrid *C. arabica* x *C. canephora.* J.Heredity **91** (1): 81-85.

Mettulio R., Rovelli P., Anthony F., Anzueto P., Lashermes P. and Graziosi G., 1999. Polymorphic microsatellites in *Coffea arabica*. 18th conference of ASIC, Helsinki, Finland. pp. 344-347.

Paillard M., Lashermes P. and Petiard V., 1996. Construction of a molecular linkage map in coffee. Theor Appl Genet **93**: 41-47.

Praksh N.S., Combes M.C., Naveen K.S. and Lashermes P., 2001. AFLP analysis of introgression in coffee cultivars of (*Coffea arabica* L.) derived from a natural interspecific hybrid. Euphytica (In press)

Ramachandran M. and Srinivasan C.S., 1979. Four generations of selection for resistance to race I of leaf rust in arabica cv. S.288 x 'Kents'. Indian Coffee **43** (6): 159 -161.

Rovelli P., Mettulio R., Anthony F., Anzueto F., Lashermes P. and Graziosi G., 2000. Microsatellites in *Coffea arabica* L. In Coffee Biotechnology and Quality. Sera, Soccol, Pandey and Roussos Eds., Kluwer Academic Publishers, Dordrecht., pp 123-133.

Srinivasan K.H. and Narasimhaswamy R.L., 1975. A review of coffee breeding work done at the Government coffee experiment station, Balehonnur. Indian coffee **34**: 311-321.

Vos P., Hogers R., Bleeker M., Reijans M., Van der Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. and Zabeau M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res **23**: 4407-4414.

Van de Peer Y., De Wachter R., 1994. TREECOM for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput Applic Biosci **10**: 569-570.

Wagner M. and Bettencourt A. J., 1965. Inheritance of reaction to *Hemileia vastatrix* Berk and Br. in *Coffea arabica* L. Progress report 1960-65, Coffee Rusts Research Center, Oeiras, Portugal.

AFLP Analysis of Genetic Diversity within and among *Coffea arabica* Varieties

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SUMMARY

Genetic diversity of *Coffea arabica* varieties was estimated using amplified fragment length polymorphism (AFLP) markers. The natural variation within arabica varieties is the source of variants of genes that control agronomic traits. The degree of this natural variation has direct impact on coffee variety improvement program. Fifty-seven arabica accessions representing five major arabica variety groups, including Typica, Bourbon, Catimor, Catuai, and Mokka hybrid, and two related *Coffea* species were analyzed with six *Eco*R I – *Mse* I primer combinations. A total of 274 informative AFLP markers was generated and scored as binary data. These data were analyzed using cluster methods in the software package NTSYSpc. The differences among varieties at the DNA level were small with an average genetic similarity of 0.933. Most accessions within a variety clustered together, although deviant samples occurred in all five varieties examined due to residue heterozygosity from the ancestral materials each variety was derived from. Among the five varieties fingerprinted, the highest level of genetic diversity was found within the variety Catimor, with an average genetic similarity of 0.880. The lowest level was found within Typica accessions with an average genetic similarity of 0.966. The genetic similarities of Bourbon, Catuai, and Mokka hybrid were 0.933, 0.942, and 0.944, respectively. We also compared the diversity between arabica and two other *Coffea* species, *C. canephora* and *C. liberica* with average genetic similarities of 0.540 and 0.413, respectively, indicating that *C. canephora* is more closely related to *C. arabica*. Although arabica varieties appear to have a very narrow genetic base, our results show that sufficient polymorphism can be found among some arabica varieties with genetic similarity as low as 0.767. AFLP appears to be applicable to genetic and QTL mapping in coffee, as it rapidly generates a large number of informative markers. This information is necessary as a first step in using marker-assisted selection for coffee breeding.

Genetic Linkage Map of a Backcross between *C. canephora* P. and *C. heterocalyx* and Autogamy Gene Location

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SUMMARY

We have constructed a genetic linkage map based on an interspecific cross between *C. canephora* P. and *C. heterocalyx*. In a population of 74 back-cross plants, self-compatibility trait and 207 AFLP markers segregations were analysed. Of these markers, 157 were assigned to 14 linkage groups covering total map length of 1404.8 centimorgans (cM). Plants self-compatibility was evaluated by means of fruit setting and pollen-tube behaviour using ultraviolet fluorescence microscopy. The results obtained showed the monofactotial control of self-compatibility trait which has been mapped in a linkage group.

INTRODUCTION

Moleculars markers techniques greatly facilitated construction of linkage maps, especially PCR-based markers including random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers (Vos et al., 1996). AFLP technique combines advantages of simultaneous identification of a large number of marker loci in a short time and requirement of small amount of DNA. It has been recently used to create create linkage maps in many plants species.

Self-compability is uncommon in coffee tree. Among more than 80 coffee taxa, only the tetraploid cultivated species *Coffea arabica* L. and two wild species: *C. heterocalyx* Stoff. (Louarn, 1992; Stoffelen, 1996) and *C. sp Moloundou* (Anthony, 1992) are self-fertile. In *C. canephora*, self-incompatibility is due to a gametophytic system controlled by a single multiallelic gene (Devreux et al., 1959; Berthaud, 1986; Lashermes et al., 1996) In the SI gametophytic system, incompatibility reaction results from the matching of pollen S allele with one of the two alleles of diploid pistil. *C. canephora* self-incompatibility represents a contrainst to overcome for its breeding and cultivation comparatively to *Coffea arabica.*

In the study presented here, backcross progeny originating from a cross between *C. canephora* and *C. heterocalyx* were used to construct a genetic linkage map using AFLP markers and to map self-compatibility trait.

MATERIALS AND METHODS

Plant material

Plant materials were maintained at the IRD (Institut de Recherche pour le Développement) coffee breeding station of Man in Côte-d'Ivoire. Interspecific F1 hybrids were obtained from a cross between CAN-IF182 (used as female) and the unique available *C. heterocalyx* genotype. The crossing procedure was made as described by Louarn (1992).

The F1 hybrid # 2C017, which flowered abundantly, was fertilised by CAN pollen, generating back-crosses. A subset of 74 plants was used as segregating population

DNA extraction

Lyophilised leaves were crushed in a ball mill. About 1 g of the powder obtained was mixed with 100 mL of slightly modified lysis buffer of Dolezel (1989) in an Erlenmeyer flask The Erlenmeyer flask was shaked for 2 h. The suspension was centrifuged for 20 min at 3000 g. The pellets were resuspended with MATAB lysis buffer. The nuclei suspension is incubated at 65°C for 4 h and then centrifuged at 3000 g for 10 mn. The supernatant is mixed with chloroform-isoamyl alcohol (24/1 : v/v) and then briefly agitated. The emulsion is centrifuged for 10 min at 3000 g. This step is repeated. RNA is suppressed from the supernatant by incubation for 30 mn at 37°C with Rnase solution. DNA is precipitated with isopropyl alcohol and then centrifuged with at 3000g for 10 min. The pellet is resuspended in TE (Tris-HCl, 1 mM EDTA, pH 8). DNA is reprecipitated with isopropyl alcohol and 3 M sodium acetate into 1.5 ml tube. The mix is centrifuged at 12000 g for 10 min. The pellet is washed in 70% ethanol and the mix centrifuged at 12000g for 10min. The pellet is dried and re-suspended in TE. The DNA was quantified in agarose gels by comparison with standard lambda DNA.

AFLP analysis

The protocol for the AFLP was carried out as described by Zabeau and Vos (1993) with minor modifications. Genomic DNA (250 ng) were digested with the restriction enzymes EcoRI and MseI for 2 h at 37°C. After ligation with EcoRI and MseI adaptors, preamplifications were performed with primers carrying one selective nucleotide at the 3'end. The selective amplification reaction was performed using primers EcoRI and MseI with 3 additional selective nucleotides at the 3' end of each primer. The EcoRI primers were labeled by phosphorylating the 5'end with [γ^{-33} P] ATP for fragment detection. The reaction products were separated on 6% denaturing polyacrylamide sequencing gels and autoradiographed.

Scoring AFLP markers and map construction

Clearly readable AFLP bands specific to HET were scored as dominant genetic markers from top to bottom of the sequencing gels. HET specific bands were detected by comparison with 10 trees of CAN. The bands were designated with two triplets of nucleotides followed by a number. The two triplets correspond respectively to the three selective nucleotides of EcoRI and MseI primers which generated the band. The number designated the position of the band, with smaller numbers representing fragments of greater sizes. Markers were recorded as present or absent.

The software program MAPMAKER/Exp version 3.0b was used to determine linkage groups and to order loci. Analysis were performed with a LOD score threshold of 5.0 and a maximum recombination value of 30% for grouping and ordering markers. Markers order was confirmed with the ripple command. Map distances were calculated using Kosambi's mapping function (Kosambi, 1944). The software MapDisto v.1.2 was used to estimate segregation distortion and draw map. Distorted markers and non-distorted markers were mapped separately in order to avoid false linkages. Then, linkage groups of the two types were regrouped at LOD score threshold of 3 and maximum distance of 30 cM.

Self-compatibility evaluation

The day before flowering, two branches (with at least 100 closed flowers) per tree were bagged. Next day, these branches were shake to allow self-pollination (SP). Two days later, bags were removed. All new floral buds were removed in order to avoid a second flowering. For each plant, two branches – the control - were also observed in open pollination (OP). Fruit-set (fruit number/flower number) was estimated 10 months later for each branch. A tree is classified self-compatible when fruit-set occured on SP branches or self-incompatible when there is absence of fruit.

Pollen tube growth

Pollen–pistil interactions were observed in self-pollination by ultraviolet fluorescence microscopy as described by Martin (1959). About 36 hours after pollination, pistils were fixed in a FAA solution [formaldehyde at 40%, pure acetic acid and ethanol (at 95%) in a 1:1:8 ratio]. Following first wash in water, the pistils were softened by submerging in a 1 N NaOH solution for 24 hours. They were thereafter washed again before being stained during 12 hours at least with 1% aniline blue prepared in 0.1 M K_2PO_4. Pistils were observed by fluorescence microscopy in ultra-violet lighting at 350-400 nm. Five pistils were observed per branch. Pollen germination on stigma and penetration level of pollen tubes into style were observed.

RESULTS

DNA marker generation

A total of 57 primer pairs were tested on HET plant and 10 CAN plants in order to determine which ones produced maximum of HET specific clearly detectable bands. Twelve primer pairs were selected which produced an average of 17 polymorphic bands per primer combination ranging from 11 to 25 (Table 1). Finally 207 markers were scored on the 74 backcrosses progeny. Of this, deviation from the expected ratio (1:1) was significant at $P<0.01$ for 66 markers. Among those distorted markers, 14 were skewed towards HET alleles and the remaining 52 were skewed towards CAN alleles.

Linkage analysis and map construction

Among non-distorted markers 117 markers were assigned to 12 linkages groups while 13 markers were unlinked and 5 pairs of linked markers were detected. Among distorted markers, 19 markers were unlinked while 4 linkages groups containing at least 4 markers and 4 small-sized linkage groups, containing 3 markers at the most, were identified. The step of regrouping linkage groups at LOD score 3 led to a final map of 14 linkage groups covering 1353,3 cM. The average distance between adjacent markers was 8.9 cM. Linkage groups were named based upon their cM length, from the longest to the shortest. Linkage group 1 displayed the longest genetic distance but not the largest number of markers. Linkage group 14 had the shortest genetic distance and the lowest number of matkers. Markers skewed towards HET alleles were gathered in linkage group 3. About half of markers skewed towards CAN alleles formed linkage group 1 while the other half were located at the end of 3 linkage groups. Five clusters were observed.

Table 1. Number of polymorphic bands generated by 12 AFLP primer combinations. Eco + 3 : 3' end selective nucleotides complementary to the Eco and MseI adapters, respectively

Primer combinations		Number of polymorphic bands
EcoRI + 3	MseI + 3	
AAC	CAA	15
AAC	CAG	19
AAC	CAT	18
AAC	CTA	15
AAG	CAA	21
AAG	CAC	25
AAG	CAG	22
AAG	CTG	21
AAG	CTT	11
ACA	CAA	13
ACC	CTT	14
ACT	CAA	13

Mapping of self-compatibility gene

In pistils of self-incompatible plants detected, pollen tubes were short with vesicle formation at the tip. In pistils of self-compatible plants, mainly long pollen tubes run through the length transmitting tract of style towards ovary. In this latter situation, we could also observe inhibited pollen tubes in stigma region. Three plants could not be classified for their branches broke or pollen did not germinate on stigma or pollen tubes were very thin and thus hardly observable.

In total, within a population of 29 back-crossed plants segregating for self-fertility, 12 were self-compatible and 17 were self-incompatible (Table 2).

This segregation did not deviate significantly from the 1:1 ratio at $p<0.01$ in the hypotheses of a monogenic control of the trait. For mapping, self-compatibility was scored as qualitative trait. Linkage analysis place the corresponding gene at the end of the linkage group 11.

DISCUSSION

The number of linkage groups is higher than the 11 chromosome pairs of diploid coffee. This indicates that gaps or breakpoints still remain in this map. It will be necessary to increase the number of molecular markers and the size of the segregating population to saturate this linkage map and bridge the gaps.

Third of markers did not follow Mendelian segregation. Segregation distortion of molecular markers has commonly been reported in mapping population of several annual and perennial crops. Irregular meiosis had been reported for F1 hybrid (Louarn, 1992) generating segregating population. One could think that the skewed segregation here observed is due to gametic, zygotic or/and post-zygotic selection. It could also be explained simply by sampling error or linked lethal genes.

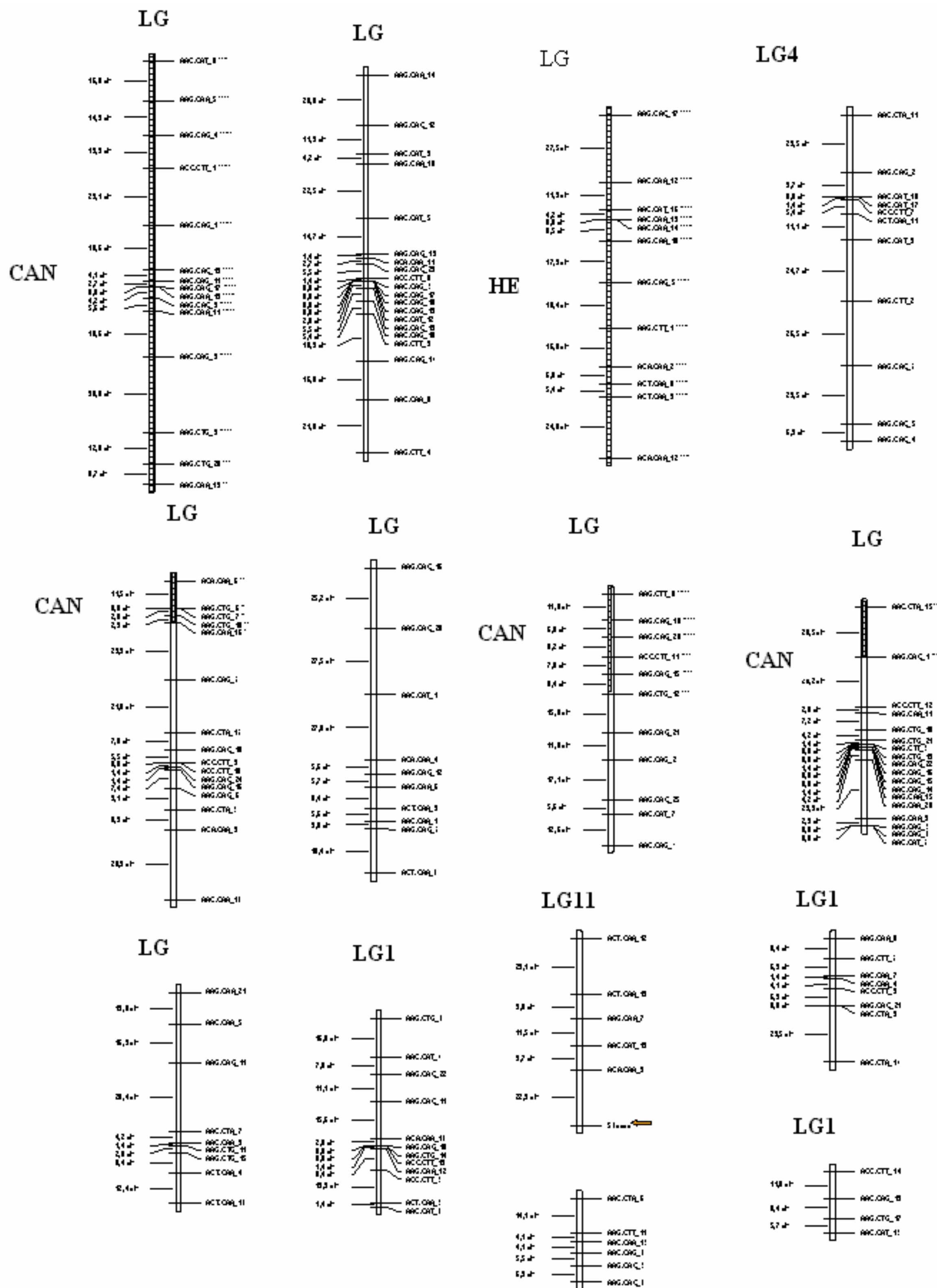

Figure 1. Genetic linkage map and S locus. Map was constructed from 74 backcross hybrids deived from the interspecific cross (CAN xHET) x CAN showing AFLP loci. Recombination distances are given in cM (Kosambi) on the left side of each linkage group and markers names are given on the right side. Loci marked **, *, ****, ***** deviated significantly from 1:1 ration at p < 0.01, p < 0.001, p < 0.01, p < 0.0001. The hachured segments correspond to distortion towards CAN or HET alleles. An arrow indicates the S locus location on LG 11**

Table 2. Linkage groups main characteristics

Linkage groups	Length (cM)	Number of markers	Means distances
1	172,6	15	11,5
2	155,1	20	7,7
3	140,3	12	11,7
4	132,8	11	12,1
5	131,7	16	8,2
6	126,5	10	12,6
7	103,4	11	9,4
8	90,0	18	5
9	86,8	9	9,6
10	77,5	12	6,5
11	77,1	6	12,8
12	51,2	8	6,4
13	34,7	6	5,8
14	25,1	4	6,3

Table 3. Self-compatibility segregation and pollen behavior

	Fruit-set (%)	Pollen tube growth
Self-incompatible plants (17)	0	-
Self-compatible plants (12)	16 (6-45)	+ -

+ compatible pollen - incompatible pollen

Self-compatibility segregated following a 1-1 ratio. These results are consistent with the assumption of monofactorial control of self-incompatibility (Devreux et al., 1959; Berthaud, 1986; Lashermes et al., 1996). ABF experiments showed that back-crossed self-compatible plants produced simultaneously self-compatible pollen and self-incompatible pollen. The first set of pollen have the S_0 allele inherited from HET and the second set of pollen have S_n alleles inherited from CAN. S_0 allele originating from *C. heterocalyx* have no inhibitor effect on self-pollen (Koyama et al., 1994; Socias i Company et al., 1995), allowing self-fertilisation.

REFERENCES

Anthony F., 1992. Les ressources génétiques des caféiers. Collecte, gestion d'un conservatoire et évaluation de la diversité génétique. Travaux et Documents de l'ORSTOM, **81**, 320 p.

Berthaud J., 1986. Les ressources génétiques pour l'amélioration des caféiers africains diploïdes: évaluation de la richesse génétique des populations sylvestres et de ses mécanismes organisateurs. Conséquences pour l'application. Travaux et documents de l'ORSTOM, **188**, 372 p.

Devreux M., Vallayes G., Pochet P., Gilles A., (1959) Recherches sur l'autofertilité du caféier robusta (C. canephora Pierre). Publication INEAC, Série scientifique **78**

Dolezel J., Binarova P., Lucretti S. 1989. Analysis of nuclear DNA content in plant cells by flow cytometry. Biol. Plant. **32**: 113-120.

Grassias M., 1980. Etude de la fertilité et du comportement méiotique des hybrides interspécifiques Arabusta Coffea arabica x C. canephora. Thèse Doct. 3è cycle Paris XI (Orsay) 108 pp.

Kosambi D.D., 1944. The estimation of map distances recombination values. Ann. Eugen. **12**: 172-175.

Koyama Y., Kunz C., Lewis I., Newbigin E., Clarke A.E., Anderson M.A. (1994) Self-compatibility in a Lycopersicon peruvianum variant (LA2157) is associated with a lack of style S-Rnase activity. TAG **88**: 859-864

Lashermes P., Couturon E., Moreau N., Paillard M., Louarn J., 1996. Inheritance and genetic mapping of self-incompatibility in *Coffea canephora* Pierre. Theoretical Applied Genetics, **93**: 458-462

Louarn J., 1992. La fertilité des hybrides interspécifiques et les relations génomiques entre caféiers diploïdes d'origine Africaine (Genre *Coffea L.* Sous-genre *Coffea).* Thèse d'état, Paris XI (Orsay), 200 p.

Martin FW (1959) Staining and observing pollen tubes in the style by means of fluorescence. Stain Technol **34** 125-128

Paillard M., Lashermes P., Pétiard V., 1996. Construction of a molecular linkage map in coffee. Theoretical Applied Genetics, **93**: 41-47

Socias i Company R, Felipe AJ, Gomez AprisiJ (1995) Capacidad de autogamia en algunas seleeciones de almendro (P. amygdalus Batsch). In: Sixth Congr Soc Espanola Ciencias Horticolas p.103

Stoffelen P., Robbrecht E., Smets E., 1996. Coffea (Rubiaceae) in Cameroun : a new species and a nomen recognized as a species. Belg. Journ. Bot. **129**: 71-76

Vos P., Hogers R., Bleeker M., Reijans M., Van de Lee, T., Hornes, M., Frijters, A., Pot J., Peleman J., Kuiper, M., Zabeau M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Reseach. **23**: 4407-4414.

Zabeau M. and Vos P. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application number. 92402629. 7, Publication number 0 534 858 A1.

Genetic Diversity and Introgression Analyses in Coffee (*Coffea arabica* L.) Using Molecular Markers

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SUMMARY

DNA markers (AFLP, RAPD, RFLP, SSR) were recently used to assess the genetic diversity among wild and cultivated *C. arabica* accessions, and to detect introgressions from *C. canephora* and *C. liberica* into *C. arabica* genome. The results allowed for the definition of breeding strategies using the whole genetic diversity that are conserved in field genebanks and for the control of alien gene transfer to improve arabica cultivars.

Almost all polymorphism was generated by the Ethiopian material. The southwestern Ethiopian accessions were grouped separately from the southeastern Ethiopian accessions. The cultivars were classified according to their genetic origin (i.e. Typica or Bourbon). The Yemen cultivars were grouped with the Typica-derived accessions, confirming the Yemen origin of the coffee plant cultivated in Amsterdam and Paris at the beginning of the 18th century and later known as Typica.

Introgressions of *C. canephora* and *C. liberica* were identified in derivatives from natural interspecific hybrids (i.e. Timor Hybrid and S.26). The introgressed genotypes were distinguished from the *C. arabica* accessions by additional bands (i.e. introgressed markers) and missing bands (i.e. markers related to introgression process). The missing bands might be associated with the stabilization process of introgressed fragments over the generations.

Segregation of the *C. canephora* genome in the tetraploid interspecific hybrid (*C. arabica* x *C. canephora*) was studied using a complete linkage map of *C. canephora*. The chromosomes segregated at random in the tetraploid hybrid, indicating the absence of preferential pairing of the four sets of chromosomes. Recombination in the tetraploid hybrid was not significantly restricted by the genetic differentiation of chromosomes belonging to the different genomes.

RÉSUMÉ

Des marqueurs de l'ADN (AFLP, RAPD, RFLP, SSR) furent récemment utilisés pour évaluer la diversité génétique présente chez les caféiers (*C. arabica*) sauvages et cultivés, et pour détecter les introgressions de *C. canephora* et *C. liberica* dans le génome *C. arabica*. Les résultats permettent de définir des stratégies d'amélioration qui utilisent l'ensemble de la diversité génétique conservée dans les collections en champ et de contrôler les transferts de gènes pour améliorer les cultivars *C. arabica*.

Presque tout le polymorphisme fut généré par le matériel d'Ethiopie. Les accessions du sud ouest de l'Ethiopie se sont classées séparément des accessions du sud est. Les cultivars se sont

regroupés selon leur origine génétique (Typica ou Bourbon). Les cultivars du Yémen furent associés avec les accessions dérivées du Typica, ce qui confirme l'origine yéménite du caféier cultivé à Amsterdam et Paris au début du XVIIIe siècle et connu plus tard comme Typica.

Les introgressions de *C. canephora* et *C. liberica* furent identifiées dans des descendances d'hybrides interspécifiques naturels (Hybride de Timor et S26). Les génotypes introgressés se sont distingués des accessions *C. arabica* par la présence de bandes additionnelles (marqueurs introgressés) et l'absence de bandes (marqueurs liés au processus d'introgression). Les bandes manquantes pourraient être associées au processus de stabilisation des fragments introgressés au cours des générations.

Les ségrégations du génome *C. canephora* furent étudiées chez l'hybride interspécifique tétraploïde (*C. arabica* x *C. canephora*), en utilisant une carte de liaison de *C. canephora*. Les chromosomes ségrègent au hasard chez l'hybride tétraploïde, ce qui indique l'absence d'appariements préférentiels des quatre ensembles de chromosomes. Les recombinaisons chez l'hybride tétraploïde ne sont pas significativement limitées par la différentiation génétique des chromosomes appartenant aux différents génomes.

INTRODUCTION

Coffea arabica L. is an amphidiploid species (2n=4x=44) (Lashermes et al., 1999) native to the highlands of South West Ethiopia (Sylvain, 1955), the Boma Plateau of Sudan (Thomas, 1942) and Mount Marsabit of Kenya (Anthony et al., 1987). It is the only polyploid coffee species and is self-fertile at approximately 90% (Carvalho et al., 1991) while other coffee species are generally self-incompatible. Arabica coffee has been cultivated in Yemen for at least five centuries but spread to South East Asia about 1700. In the early 18th century, progenies of a single plant from Indonesia, cultivated in Amsterdam and Paris, were spread to Latin America (Chevalier and Dagron, 1928). Other introductions followed in the late 18th century from Yemen to Brazil, via Bourbon Island (now Réunion) (Haarer, 1956). These base populations gave rise to many cultivars and were described as two distinct varieties, respectively *C. arabica* var. *arabica*, usually called *C. arabica* var. *typica* Cramer, and *C. arabica* var. *bourbon* (B. Rodr.) Choussy, commonly called Typica and Bourbon respectively (Krug et al., 1939; Carvalho et al., 1969). The cultivars present an homogeneous agronomic behaviour, characterised by a high susceptibility to many pests and diseases (Bertrand et al., 1999).

Enlarging the genetic base and improvement of arabica cultivars have become priorities. Spontaneous accessions collected in the primary centre of diversity as well as wild relative *Coffea* species constitute a valuable gene reservoir for breeding purposes (Anthony et al., 1999). Genes from diploid species can be transfer into *C. arabica* cultivars exploiting natural and controlled interspecific hybrids. However, transferring various resistance genes without reducing coffee quality appears as a very difficult task in an acceptable time-frame through traditional breeding approaches.

In recent years, DNA-based genetic markers have gained widespread applications in many fields of plant genetics and breeding. Several results related to *C. arabica* genetics and based on molecular markers utilisation have been already reported in ASIC conferences on: genetic diversity and phylogenetic relationships in *Coffea* (Cros et al., 1993), the origin of *C. arabica* genome (Lashermes et al., 1995), the use of molecular markers for assisting selection (Lashermes et al., 1997a) and the development of microsatellite markers (Mettulio et al., 1999) which constitute powerful markers for breeding and genetic mapping. The results presented here concern the genetic diversity available in wild and cultivated *C. arabica*

accessions, and an analysis of introgressions from *C. canephora* and C. *liberica* into *C. arabica* genome.

GENETIC DIVERSITY ANALYSIS

Genetic diversity of wild coffee

The genetic diversity was studied using Random Amplified Polymorphic DNA (RAPD) markers among 119 coffee individuals representing 88 accessions derived from spontaneous and subspontaneous trees in Ethiopia, 6 cultivars grown locally in Ethiopia and 2 Typica- and Bourbon-derived accessions (Anthony et al., 2001). The sampling could be considered representative of the FAO (1968) and ORSTOM (Guillaumet and Hallé, 1978) material conserved in the CATIE field genebank. Only 16 of the 150 10-mer oligonucleotides used in the study (10.7%) detected polymorphism between accessions. This result confirmed the low polymorphism observed in the species *C. arabica* at the level of the rDNA (Lashermes et al., 1997b) and cpDNA (Cros et al., 1998). The Ethiopian accessions tended to form groups according to their origin (Figure 1). Ethiopian 1 was composed of 78 accessions and 2 Ethiopian cultivars (Anfilo, Dalle). It comprised all accessions from Gojjam, Ilubabor and Shoa provinces, all accessions except three from Kefa, one from Harerge and two from Sidamo. Except for 1 accession from Kefa province, all accessions classified in the other groups (Ethiopian 2, 3, 4) originated from Harerge province in the South East and Sidamo province in the South. Most of the detected diversity was found in accessions classified as Ethiopian 1. They presented 28 of the 29 identified markers whereas the accessions classified in other groups presented only 5 to 16 markers. The Typica- and Bourbon-derived accessions presented only 3 and 7 markers, respectively. This should increase interest in spontaneous and subspontaneous coffee for enlarging the genetic base of cultivars.

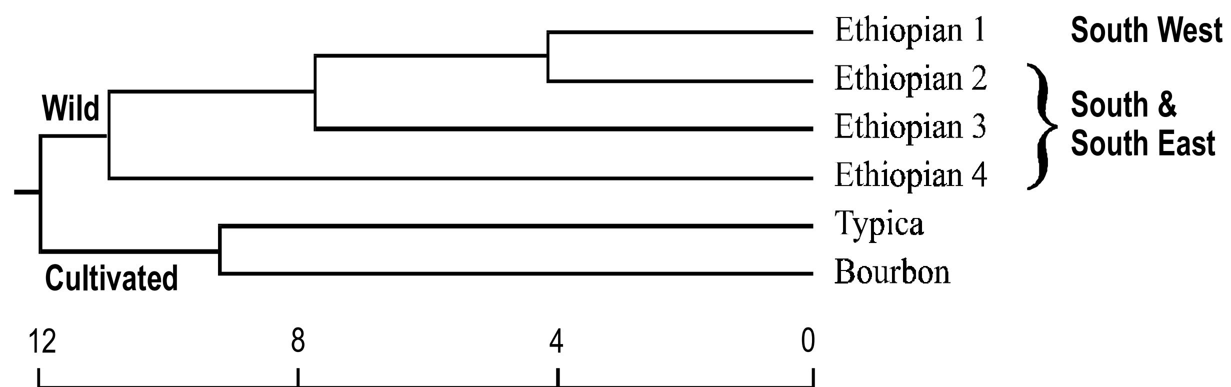

Figure 1. Structure of the genetic diversity in spontaneous and subspontaneous coffee (*C. arabica*) collected in Ethiopia, using RAPD markers (Anthony et al., 2001)

The distinction between the southwestern and southern Ethiopian coffee trees is not a consequence of their genetic isolation due to the presence of the tectonic break “the Great Rift Valley”, which crosses Ethiopia from North East to South West, since the molecular characterisation of *C. arabica* genome suggested a recent origin of the species (Lashermes et al., 1999). The genetic distance estimated by RAPD markers showed that southern and southeastern coffee trees presented a low differentiation from southwestern coffee trees. This supports the hypothesis that southern and southeastern coffee trees were not selected from wild coffee growing locally but introduced from the South West where Lejeune (1958) situated the first cultivation of coffee. Moreover, no references mention the existence of wild coffee on the east side of the tectonic break.

Genetic diversity of cultivated coffee

Amplified Fragment Length Polymorphism (AFLP) markers were used to assess polymorphism among 8 accessions derived from the Typica and Bourbon genetic bases, 2 accessions of cv. Catuai ((Typica x Bourbon) x Bourbon), 4 cultivars growing in the Popular Democratic Republic of Yemen (Eskes, 1989) and 11 subspontaneous-derived accessions (Anthony et al., in press). A total of 107 AFLP polymorphic markers were used to construct a dendrogram using genetic distances between accession pairs (Figure 2). The Typica- and Bourbon-derived accessions were classified in 2 distinct groups according to their genetic origin, each group being supported by a high bootstrap value (89%). The 2 accessions of cv. Catuai were classified closer to the Typica-derived accessions than to the Bourbon-derived accessions. The subspontaneous accessions were clearly separated from the cultivated accessions, confirming the classification based on RAPD data. They did not constitute a structured group, but rather chains, with poor bootstrap values. The subspontaneous accession from South Ethiopia (E-238), which was classified in the Ethiopian 4 group by RAPD data, was separated from the southwestern Ethiopian and Sudan accessions. The 4 Yemen cultivars were associated with the Typica-derived accessions. This result was in accordance with the historical data about the diffusion of the Typica genetic base from Yemen to Amsterdam, via Java. The estimation of the genetic distances showed that the cultivars were closer to the subspontaneous coffee of the west side of the Great Rift Valley than to the east side. If the coffee of Yemen came from seeds harvested in the Harar region, as affirmed by Wellman (1961), it was an intermediate step in the introduction to Yemen.

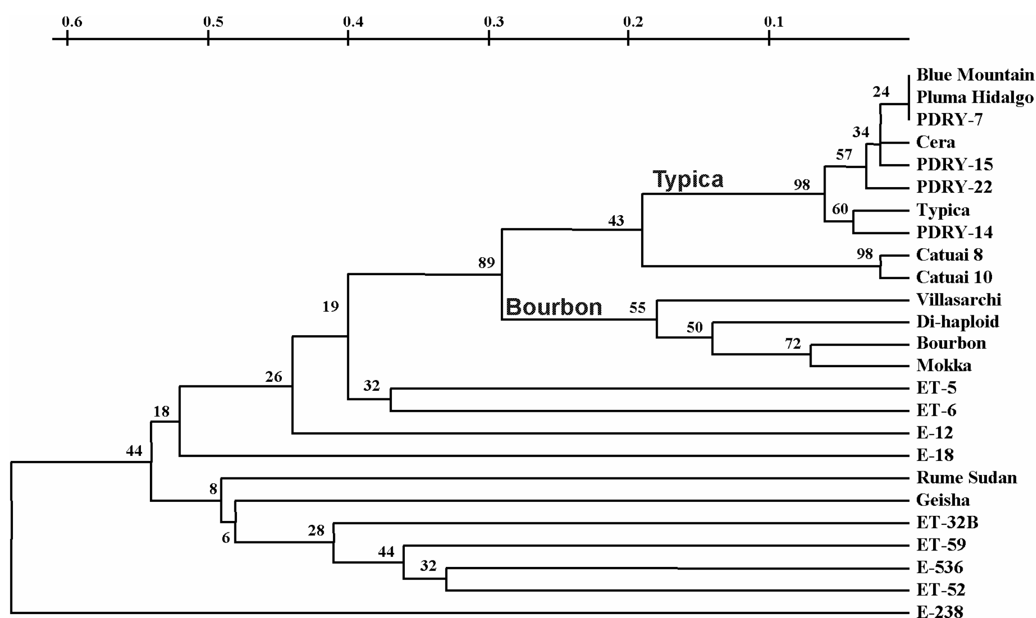

Figure 2. Dendrogram generated after UPGMA using AFLP-based genetic distance. Numbers on the branches are bootstrap values (%) obtained from 200 replicate analyses (Anthony et al., in press)

The number of polymorphic markers varied significantly within the cultivated and wild accessions. Whereas 94 AFLP markers were polymorphic within wild coffee, only 7 and 14 were respectively polymorphic within the Typica-derived accessions and the Yemen cultivars, and within the Bourbon-derived accessions. The mode of diffusion of coffee and the selection that followed have strongly reduced the genetic diversity present in the wild coffee. The higher polymorphism observed in the Bourbon group indicated that the genetic base of Bourbon was constituted by the descendants of several individuals and not from one single

individual as for the genetic base of Typica. This result confirmed the historical data given by Haarer (1956) in which several introductions took place from Yemen to the Reunion Island.

INTROGRESSION ANALYSIS

Introgression from *C. canephora*

Twenty-one Timor Hybrid-derived accessions were analysed for the introgression of *C. canephora* genetic material using AFLP markers (Lashermes et al., 2000). They were compared to 23 *C. arabica* accessions and 8 *C. canephora* accessions. The Timor Hybrid-derived accessions were distinguished from the *C. arabica* accessions by 178 markers consisting of 109 additional bands and 69 missing bands. The additional bands corresponded to introgressed fragments whereas a part of the missing bands might be associated with the stabilization process of introgressed fragments over the generations. The number of additional and missing bands varied respectively from 18 to 59 and from 0 to 32 among the Timor Hybrid-derived accessions (Figure 3). The introgressed fragments were estimated to represent from 8% to 27% of the *C. canephora* genome. Assuming a unique genotype of *C. canephora* was involved in the formation of the Timor Hybrid, the overall 109 introgressed fragments identified in the Timor Hybrid-derived accessions were estimated to represent 51% of the *C. canephora* genome. Most of the introgressed chromosome segments were not eliminated or counter-selected during the process of selfing and selection. These results should justify the development of adapted breeding strategies.

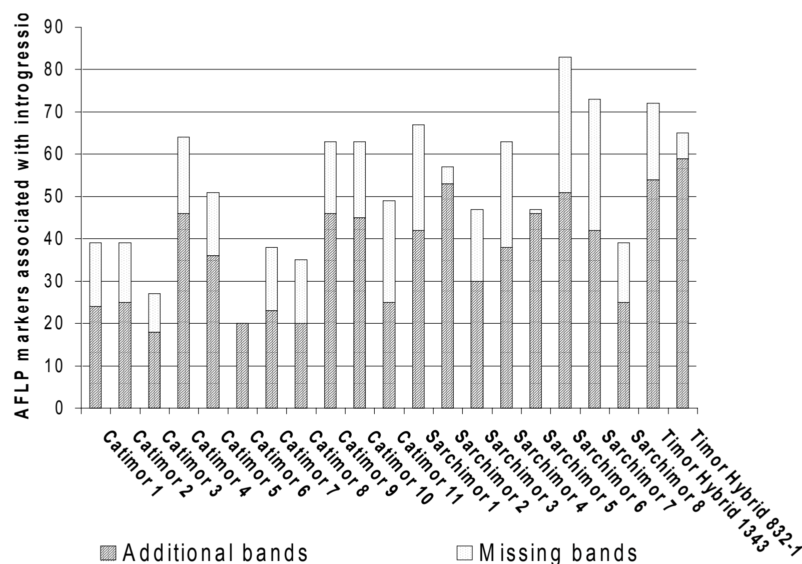

Figure 3. Number of AFLP polymorphic bands attributable to introgression detected in Timor Hybrid-derived accessions (Lashermes et al., 2000)

Behaviour of the *C. canephora* genome and its interaction with the *C. arabica* genome were investigated in tetraploid hybrids (*C. arabica* x *C. canephora* 4x) called arabusta hybrids (Herrera et al., in press). Segregation and co-segregation of Restriction Fragment Length Polymorphism (RFLP) and microsatellite loci-markers were studied in two back-cross (BC1) populations of 28 and 45 individuals. The presence of specific *C. canephora* markers were scored for 11 RFLP and 13 microsatellite loci, distributed on at least 7 of the 11 linkage groups identified in *C. canephora* by Lashermes et al. (in press). The segregation of *C. canephora* alleles in the BC1 plants conformed to the expected values of a theoretical binomial distribution assuming a random chromosome segregation (Figure 4). The recombination rate of *C. canephora* chromosome segments estimated in the arabusta hybrids

was found to be similar to the recombination rate observed in *C. canephora*. The recombination in the tetraploid hybrids appeared therefore not to be affected significantly by the genetic differentiation between chromosomes belonging to the different genomes. The arabusta hybrids appeared to be particularly favourable to intergenomic recombinations. Genes of *C. canephora* might be more readily introgressed into *C. arabica* genome than originally believed.

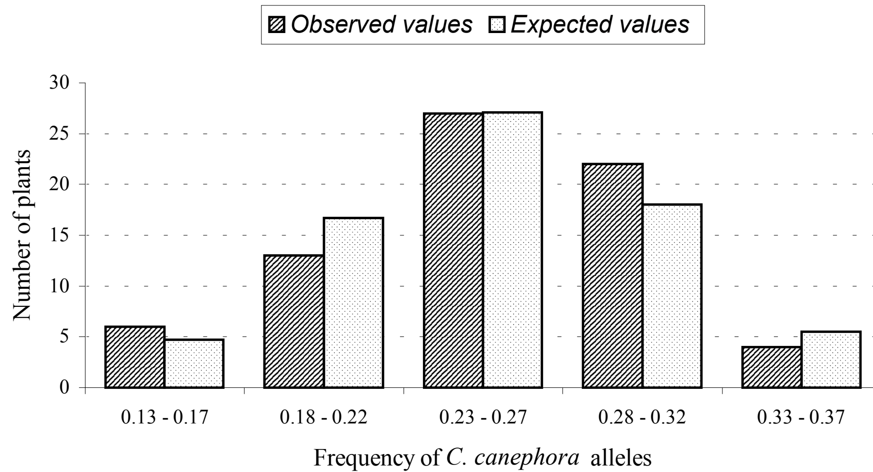

Figure 4. Frequencies of *C. canephora* alleles observed in BC_1 hybrids (*C. arabica* x *C. canephora*) and expected values for a theoretical binomial distribution assuming a random segregation at all loci (Herrera et al., in press)

Introgression from *C. liberica*

The offspring S.288 of a putative spontaneous hybrid (*C. arabica* x *C. liberica*), and 17 introgression lines derived from the cross (S.288 x Kent) were evaluated for introgression of

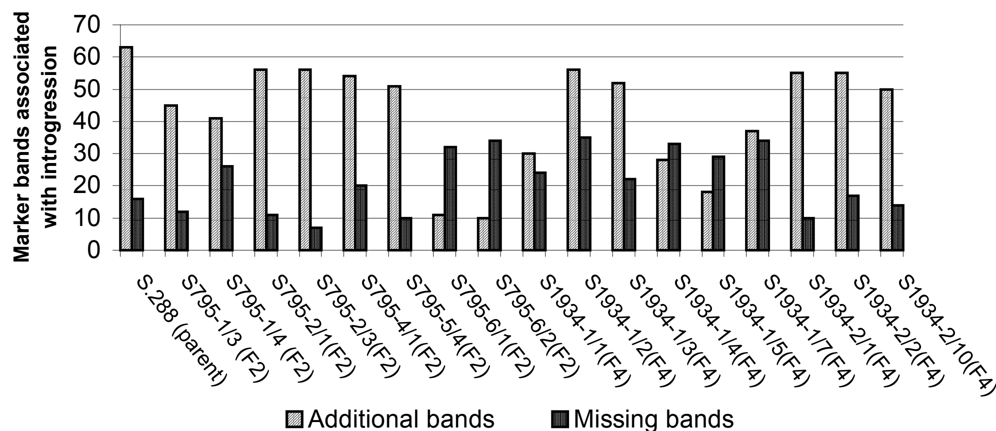

Figure 5. Numbers of AFLP polymorphic bands attributable to introgression in S.288 parent and introgressed lines (Prakash et al., in press)

C. liberica genetic material, using AFLP markers (Prakash et al., in press). The AFLP profiles of introgression lines were compared to 5 accessions each of *C. arabica* and *C. liberica*. The introgression lines were distinguished from the *C. arabica* accessions by 102 markers consisting of 65 additional bands and 37 missing bands. Large variation was observed in the number of additional bands (10 to 56) and missing bands (7 to 35) among the introgression lines (Figure 5). The differences in the level of introgression between introgressed parents, F2

and F4 progenies was not pronounced. The alien genetic material appeared to be fixed and not eliminated or counter-selected over generations. The limited number of introgressed markers in general indicated that the introgression was restricted to few chromosome segments. Considering the 36 AFLP primer combinations common to this study and to the analysis of *C. canephora* introgression (Lashermes et al., 2000), the number of polymorphic bands attributed to introgression was found less in the *C. liberica* introgressed lines than in the Timor Hybrid-derived lines.

CONCLUSION

Molecular markers appeared particularly relevant to fingerprint coffee accessions, to reveal the structure of genetic diversity present in wild and cultivated accessions, and to detect chromosome segments introgressed from diploid relative species. They were also used successfully for characterising mechanisms of introgression in interspecific hybrids between *C. arabica* and diploid relative species. The results allow for the definition of breeding strategies using the whole genetic diversity that are conserved in field genebanks. Wild genitors can be chosen based on the diversity structure revealed by molecular markers and on field characterisation data.

Efforts should now be concentrated on the identification and localisation of resistance genes available in the genetic resources for *C. arabica* breeding. The development of a genetic map would constitute a powerful tool for a molecular control of alien gene transfer. The development of a method of selection assisted by molecular markers would increase the efficiency of coffee breeding programs by 1) allowing for selection at early stage and on a large number of breeding lines, 2) reducing the number of backcross cycles required to restore the quality of the Typica and Bourbon cultivars, and 3) selecting in one-step for various traits or resistance genes.

ACKNOWLEDGMENTS

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REFERENCES

Anthony F., Berthaud J., Guillaumet J.L. and Lourd M., 1987. Collecting wild *Coffea* species in Kenya and Tanzania. Plant Genet. Resources Newsl. **69**: 23-29.

Anthony F., Astorga C. and Berthaud J., 1999. Los recursos genéticos: las bases de una solución genética a los problemas de la caficultura latinoamericana. In: B. Bertrand & B. Rapidel (Eds.), Desafíos de la caficultura centroamericana, pp. 369-406. IICA, San José, Costa Rica.

Anthony F., Bertrand B., Quiros O., Lashermes P., Berthaud J. and Charrier A., 2001. Genetic diversity of wild coffee (*Coffea arabica* L.) using molecular markers. Euphytica **118**: 53-65.

Anthony F., Combes M.C., Astorga C., Bertrand B., Graziosi G. & Lashermes P., in press. The origin of cultivated coffee (*Coffea arabica* L.) revealed by molecular markers. Theor. Appl. Genet.

Bertrand B., Aguilar G., Santacreo R. and Anzueto F., 1999. El mejoramiento genético en América Central. In: B. Bertrand & B. Rapidel (Eds.), Desafíos de la caficultura centroamericana, pp. 407-456. IICA, San José, Costa Rica.

Carvalho A., Ferwerda F.P., Frahm-Leliveld J.A., Medina P.M., Mendes A.J.T. and Monaco L.C., 1969. Coffee. In: Ferwerda F.P. and Wit F. (Eds.), Outlines of perennial crop breeding in the Tropics, pp. 189-241. Veenman and Zonen NV, Wageningen.

Carvalho A., Medina Filho H.P., Fazuoli L.C., Guerreiro Filho O. and Lima M.M.A., 1991. Aspectos genéticos do cafeeiro. Rev. Brasil. Genet. **14**: 135-183.

Chevalier A. and Dagron M., 1928. Recherches historiques sur les débuts de la culture du caféier en Amérique. Communications et Actes de l'Académie des Sciences Coloniales, Paris.

Cros J., Lashermes P., Marmey P., Anthony F., Hamon S. and Charrier A., 1993. Molecular analysis of genetic diversity and phylogenetic relationships in *Coffea*. 15th international conference on coffee science, 6-11 June 1993, Montpellier, ASIC, pp. 41-46.

Cros J., Combes M.C., Trouslot P., Anthony F., Hamon S., Charrier A. and Lashermes P., 1998. Phylogenetic relationships of *Coffea* species: new evidence based on the chloroplast DNA variation analysis. Mol. Phylogenet. Evol. **9**: 109-117.

Eskes A.B., 1989 Identification, description and collection of coffee types in P.D.R. Yemen. IPGRI, Rome.

FAO, 1968. FAO coffee mission to Ethiopia 1964 - 1965. FAO, Rome.

Guillaumet J.L. and Hallé F., 1978. Echantillonnage du matériel récolté en Ethiopie. Bull. IFCC **14**: 13-18.

Haarer A.E., 1956. Modern coffee production. Leonard Hill (books) Limited, London.

Herrera J.C., Combes M.C., Anthony F., Charrier A. and Lashermes P., in press. Introgression into the allotetraploid coffee (*Coffea arabica* L.): segregation and recombination of the *C. canephora* genome in the tetraploid interspecific hybrid (*C. arabica* x *C. canephora*). Theor. Appl. Genet.

Krug C.A., Mendes J.E.T. and Carvalho A., 1939. Taxonomia de *Coffea arabica* L. Bolétim Técnico nº 62. Instituto Agronômico do Estado, Campinas, Brazil.

Lashermes P., Combes M.C., Cros J., Trouslot P. and Anthony F., 1995. Origin and genetic diversity of *Coffea arabica* L. based on DNA molecular markers. 16th international conference on coffee science, 10-14 April 1995, Kyoto, ASIC, pp. 528-536.

Lashermes P., Agwanda C.O., Anthony F., Combes M.C., Trouslot P. and Charrier A., 1997a. Molecular marker-assisted selection: a powerful approach for coffee improvement. 17th international conference on coffee science, 21-25 July 1997, Nairobi, ASIC, pp. 474-480.

Lashermes P., Combes M.C., Trouslot P. & Charrier A., 1997b. Phylogenetic relationships of coffee tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. Theor. Appl. Genet. **94**: 947-955.

Lashermes P., Combes M.C., Robert J., Trouslot P., D'Hont A., Anthony F. and Charrier A., 1999. Molecular characterisation and origin of the *Coffea arabica* L. genome. Mol. Gen. Genet. 261 (2): 259-266.

Lashermes P., Andrzejewski S., Bertrand B., Combes M.C., Dussert S., Graziosi G., Trouslot P. and Anthony F., 2000. Molecular analysis of introgressive breeding in coffee (*Coffea arabica* L.). Theor. Appl. Genet. **100** (1): 139-146.

Lashermes P., Combes M.C., Prakash N.S., Trouslot P., Lorieux M. and Charrier A., in press. Genetic linkage map of *Coffea canephora*: effect of segregation distorsion and analysis of recombination rate in male and female meiosis. Genome.

Lejeune J.B.H., 1958. Rapport au Gouvernement Impérial d'Ethiopie sur la production caféière. FAO, Rome.

Mettulio R., Rovelli P., Anthony F., Anzueto A., Lashermes P. and Graziosi G., 1999. Polymorphic microsatellites in *Coffea arabica*. 18th international conference on coffee science, 2-6 August 1999, Helsinki, ASIC, pp. 344-347.

Montagnon C. and Bouharmont P., 1996. Multivariate analysis of phenotypic diversity of *Coffea arabica*. Genet. Res. Crop Evol. **43**: 221-227.

Prakash N.S., Combes M.C., Somanna N. & Lashermes P., in press. AFLP analysis of introgression in coffee cultivars (*Coffea arabica* L.) derived from a natural interspecific hybrid. Euphytica.

Sylvain P.G., 1955. Some observations on *Coffea arabica* L. in Ethiopia. Turrialba **5**: 37-53.

Thomas A.S., 1942. The wild arabica coffee on the Boma Plateau, Anglo-Egyptian Sudan. Empire J. Expt. Agric. **10**: 207-212.

Wellman F.L., 1961. Coffee: botany, cultivation and utilization. Leonard Hill Books, London.

Premières observations sur la résistance au champ de plantes de *Coffea canephora* génétiquement modifiées contre la mineuse des feuilles *Perileucoptera coffeella* Guérin-Méneville

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SUMMARY

58 transformed clones of the *Coffea canephora* 126 genotype that were obtained with the bacterias *Agrobacterium tumefaciens* and *A. rhizogenes* have been planted in a field trial in french Guiana. Introduced Bt genes are for the synthesis of cry1Ac Lepidopteran-specific toxiprotein. One purpose is to observe the agronomical disturbances caused by the transformation. A second purpose is to assess the crylAc accumulation in the transformed clones by testing their resistance toward the caterpillars of *Perileucoptera coffeella* (a leafminer). The control is the untransformed *C. canephora* 126. After the release of 12000 cocoons of the insect, the count of damages gave the first datas. The majority of the clones, less than one year old, are strongly resistant. Future prospects regarding the trial are discussed.

RÉSUMÉ

58 clones transfonnés à l'aide des bactéries *Agrobacterium tumefaciens* et *A. rhizogenes* du génotype 126 de *C. canephora* ont été plantés dans un essai en Guyane française. Les gènes introduits sont des gènes Bt codant pour la toxiprotéine crylAc spécifique des Lépidoptères. Un but de l'essai est d'évaluer les modifications du comportement agronomique des clones. L'autre est d'évaluer l'accumulation de crylAc par les clones transfonnés en testant leur résistance aux chenilles de *Perileucoptera coffeella* (mineuse des feuilles). Des *C. canephora* 126 non transformés sont utilisés comme témoins. Après un lâcher de 12000 cocons de l'insecte sur la parcelle, le comptage des mines a fourni les premières indications de résistance aux chenilles. La majorité des clones sont très résistants moins de un an après la plantation. Les suites à donner à l'essai sont discutées.

INTRODUCTION

Le caféier a fait l'objet de plusieurs années de recherche en transformation génétique *C.canephora* peut être transformé aussi bien au moyen de la bactérie *Agrobacterium rhizogenes* (Spiral et Pétiard, 1993; Spiral et al., 1993) qu'au moyen de la bactérie *A. tumefaciens* (Leroy et al., 1997; Spiral et al., 1999). La transformation par *A. rhizogenes* conduit souvent à l'obtention de clones souffrant d'une anomalie phénotypique "**hairy root**". Ceci amène à privilégier la transformation à l'aide de *A. tumefaciens* (Leroy et al., 2000).

Les travaux du CIRAD ont porté jusqu'à présent sur la mise au point de la technique et sur l'introduction de gènes codant pour la protéine insecticide de Bacillus thuringiensis (B.t.) CrylAc. Elle est spécifiquement toxique pour les chenilles des insectes de l'ordre des Lépidoptères (papillons) et considérée non toxique pour les mammifères.

En ce qui concerne le caféier, la finalité pratique de l'utilisation de ce gène est la lutte contre les très nuisibles lépidoptères mineurs des feuilles qui attaquent surtout *C. arabica*.

Après l'importante étape de laboratoire concernant *C. canephora*, le CIRAD a décidé de poursuivre par une étape de terrain comportant la plantation d'un essai, le premier essai au champ de caféiers transgéniques (Leroy et al., 1999). Le but est d'évaluer au cours du temps l'expression des gènes introduits et d'observer les perturbations causées par la transformation sur le comportement agronomique de la plante. Grâce à cet essai, on pourra également étudier certains effets potentiels des caféiers génétiquement modifiés pour l'environnement et la santé humaine: déplacement du pollen des caféiers transformés, effets sur les abeilles et autres insectes, concentration en protéines insecticides dans les plantes. Le choix du lieu de l'essai s'est porté sur la Guyane française. Les réglementations concernant les essais de plantes transformées en vigueur sur le territoire français obligent à prendre des mesures de précaution rigoureuses. Des observations préliminaires ont confirmé la présence de la mineuse sud américaine des feuilles du caféier *Perileucoptera coffeella* Guérin-Méneville sur de vieilles plantations et collections de *C. canephora*. Cet insecte pouvait donc être utilisé pour étudier la résistance de *C. canephora* aux chenilles de Lépidoptères conférée par le gène cryIAc introduit.

Cette présentation expose les premiers résultats sur la résistance au champ contre *P. coffeella* exprimée par les plantes de *C. canephora* transformées.

MATÉRIELS ET MÉTHODES

L'essai de caféiers génétiquement modifiés a été implanté après autorisation par la Commission du Génie Biomoléculaire française (CGB) pour une durée de 5 ans. La parcelle a été plantée dans les conditions suivantes :

- éloignement de toute culture de caféiers,
- absence de plante du genre Coffea de la flore spontanée d'Amérique du sud,
- cordon forestier très large entourant la parcelle, plantation d'arbres à croissance rapide autour de la parcelle (Acacia sp.),
- plantation des caféiers sous un léger ombrage d'hévéas préexistant pour limiter la dissémination du pollen,
- éloignement par rapport à toute ruche.

Toute la récolte devra être détruite par incinération après mesures et analyses. Après la fin de l'essai, tous les caféiers devront être coupés et brûlés, et le sol laissé en jachère durant un an puis labouré. L'exécution du cahier des charges est sous le contrôle du service départementale de la Protection des Végétaux. Matériel végétal On parle de «clone transformé» pour désigner les plantes issues d'un évènement de transformation indépendant obtenu. Dans le cadre de l'essai, un seul génotype a été transformé: le génotype *C. canephora* 126, qui est un génotype hybride congolais X guinéen.

Cinquante clones de C. canephora 126 transformés par la bactérie **C3/LBA** ont été plantés. La construction génétique **C3** comporte un gène B.t. *cry*lAc synthétique (pour la synthèse de la protéine CrylAc active contre les Lépidoptères), le gène *csr*l-l (gène de résistance à l'herbicide chlorsulfuron pour le tri des plants transformés), le gène *uid*A (gène GUS marqueur de la transformation). La construction génétique a été introduite par le plasmide pBin19 de la souche désarmée **LBA 4404** d'*Agrobacterium tumefaciens*. Par analyse moléculaire de type Southem blotting, on connaît sur 37 clones le nombre de copies de la construction insérées dans le génome (Leroy et al., 2000).

Huit clones transformés ont été obtenus à l'aide d'une souche **A4** d'*A.rhizogenes* ont aussi été plantés. Quatre présentent l'anomalie «**hairy root**», due à la transformation avec cette souche sauvage d'*Agrobacterium* qui confère à la plante transformée ce faciès particulier, avec comme conséquences un phénotype modifié et une croissance perturbée. Il y a 3 clones transformés par la bactérie **C3/A4**, comportant la construction génétique **C3**. Il y a 2 clones transformés par la bactérie **C2/A4** dont la construction génétique **C2** contient un gène B.t. *cry*lAc naturel (dit "natif") et le gène *csr*1-1. Il y a 3 clones transformés par la bactérie **B2/A4** dont la construction génétique **B2** contient le gène B.t. *cry*lAc naturel et un gène de résistance à un autre herbicide.

Dispositif expérimental

L'essai a été planté en mai 2000. Quatre lignes élémentaires de cinq plants ont été plantées pour chaque clone transformé (soit 20 plants par clone), sauf pour les quatre clones «hairy root» pour lesquels il y a moins de plants. Seize lignes élémentaires du génotype 126 non transformé ont été installées comme témoins (soit 80 plants). Les emplacements des lignes élémentaires ont été tirés au sort. Entre deux lignes élémentaires sur les lignes de plantation se trouve un plant de *C. canephora* tout venant pour assurer la pollinisation, car les génotypes de *C. canephora* sont auto-stériles. Au total, l'essai comporte 1955 caféiers répartis sur 1,79 hectares (140 m X 128 m): 1115 caféiers pour les 58 clones transformés du génotype 126, 80 témoins du génotype 126, 760 *C. canephora* pollinisateurs.

Photo 1. Vue d'ensemble de l'essai

Notations agronomiques

La mortalité en pépinière et au champ, le diamètre au collet et le nombre de rameaux ont été notés pied par pied. L'aspect général des plants a été évalué sur des arbres âgés de neuf mois.

Elevage et lâchers de Perileucoptera coffeella

Perileucoptera coffeella est un très petit Lépidoptère Lyonetiidae, la longueur maximale d'un papillon est 2,5 mm. Il s'élève mieux sur *C. arabica* que sur *C. canephora*. Sur plants de *C. arabica* (lignées Guinée Pita et Catuaï), on a observé à Kourou pendant les mois les plus chauds et secs (septembre-novembre) un taux de multiplication supérieur à 4 d'une génération à l'autre dans des cages de 60 cm X 60 cm X 80 cm placées sous abris extérieurs.

Pendant les mois pluvieux, le taux de multiplication est proche de 2. La durée d'une génération est d'environ 20 jours en période sèche et de 30 jours en période pluvieuse. Des cocons sont placés dans la cage avec des plants de *C. arabica* de 30-60 cm de haut. Après la

ponte des papillons on sort les plants. Le développement larvaire à l'intérieur des feuilles a lieu. Avant la fin du développement, une poche de polyéthylène transparent est mise autour des plants pour que les chenilles qui sortent puissent s'y laisser tomber et tisser leur cocon de nymphose. Le cocon adhère bien sur ce support. Des carrés de plastique sont ensuite découpés autour des cocons, afin de pouvoir introduire de nouveau des cocons en cage ou bien pour les introduire sur la parcelle.

Photo 2. Vue de 2 lignes de caféiers en expérimentation

Dans la parcelle, les cocons sont répartis uniformément, placés sous de petits abris ou bien sous les feuilles du *Pueraria phaseolides* (liane de couverture) poussant dans les andains. Ils y sont bien protégés du soleil, de la pluie, et des fourmis, avant l'émergence des papillons.

Notation des développements larvaires

Les oeufs sont pondus à la face supérieure des feuilles. Les chenilles pénètrent dans l'épaisseur de la feuille et se développent dans le parenchyme pendant 10 à 20 jours selon la température, causant la formation de galeries et de plaques appelées "mines". Au fur et à mesure des lâchers de cocons et des pontes, les mines apparaissent sur le feuillage des plants sensibles aux chenilles. On procède à une notation des développements de mines quand il y en a un nombre significatif sur les plants témoins. On ne peut pas savoir si le développement larvaire a été complet dans une mine. La variabilité d'attaque entre lignes élémentaires peut être forte.

RÉSULTATS

Données agronomiques

Neuf clones sur 50 plantés obtenus avec *A. tumefaciens* sont peu vigoureux à 6 mois; ils ont présenté une mortalité significative en pépinière. Pour le moment, on ne constate pas de phénotypes ayant un aspect différant de manière évidente du génotype 126 témoin non transformé. Les caféiers issus de transformation avec *A. rhizogenes* présentent des développements très différents, selon qu'ils présentent ou non l'anomalie phénotypique «Hairy root»: les trois clones transformés avec cette bactérie qui ne présentent pas cette

anomalie phénotypique ont un développement similaire à celui des plantes transformés avec *A. tumefaciens*, alors que les autres se développent très peu.

Lâchers de *P. coffeella*

12.000 cocons ont été introduits d'octobre 2000 à janvier 2001, tout au long d'une période ensoleillée, chaude, peu pluvieuse. Après chaque introduction des développements de mines de première génération ont été constatés. Les papillons semblent mieux se disperser dans la direction des vents dominants. En mars 2001, une recherche de mines jeunes a été effectuée pour évaluer l'éventuelle installation d'une population résidente de mineuses dans la parcelle. Aucune mine jeune n'a été trouvée, la population d'insectes de deuxième génération est donc encore très peu importante malgré un apport initial de 12 000 cocons. Le faible ombrage de cette jeune parcelle située sur la côte guyanaise (2500 heures de soleil par an) semble prépondérant pour expliquer le phénomène.

Résistance des clones transformés aux chenilles de *P. leucoptera*

Les résultats sont présentés dans le Tableau 1.

Tableau 1. Résistance aux chenilles de mineuse des clones transformés

	Témoin 126	**Catégorie**				
		C3/LBA		**C2/A4**	**B2/A4**	**C3/A4**
		Sensibles	Résistants	Sensibles	Sensibles	Résistants
		7 clones/47	40 clones/47	1 clone/1	2 clones/2	1 clone/1
Mines/ligne élémentaire (moy.)	9,5	3,5 – 6,5	0 – 0,5	9	9,5	0

Trois clones **C3/LBA** ne peuvent être classés car seul un plant du clone présente des mines en abondance.

Il s'avère que 40 clones **C3/LBA** sur 47 classés paraissent résistants, avec moins de 0.5 mine observée par ligne de 5 plantes. 7 clones **C3/LBA** sur 47 sont non différentiables du témoin. Il s'agit donc de clones sensibles, qui présentent en moyenne 5 mines observées par ligne.

Un seul des 4 clones complets transformés avec A. rhizogenes apparaît résistant, les autres sont aussi sensibles que le témoin, avec 9 mines observées en moyenne sur chaque ligne. Ces clones contiennent les constructions génétiques **C2** et **B2** au lieu de **C3**. Cette mauvaise performance est due soit à la bactérie transformante soit aux constructions.

DISCUSSION

Le but principal des observations entomologiques sur l'essai est la compréhension de l'accumulation de la protéine Cry1Ac par les clones transformés et par conséquent la preuve et la compréhension de l'expression du gène insecticide.

Clones sensibles

Le fait de ne pas pouvoir différencier les clones sensibles des témoins peut-être lié au faible nombre de mines. On ne sait pas non plus si le développement larvaire est complet et la

nymphose normale. Certains de ces clones présentent peut-être une sensibilité inférieure au témoin, avec une concentration significative en Cry1Ac. Une sensibilité intermédiaire peut être suffisante pour lutter contre un ravageur (en favorisant cependant l'apparition de résistances à la protéine chez celui-ci). L'obtention d'une densité de mines plus élevée, des mesures de mines et des dosages de protéine Cry1Ac seront donc indispensables.

Photo 3. Clone sensible

Photo 4. Clone résistant

Clones résistants

Ceux-ci ne permettent pas le développement larvaire de *P. coffeella.* Pour l'interprétation, il est important de prendre en compte le phénomène de mauvaise adaptation des chenilles à l'alimentation offerte par les feuilles de *C. canephora* non transformés. La mortalité larvaire est supérieure à ce qu'elle est sur *C. arabica*, les cocons et les adultes provenant des chenilles y sont plus petits. Il est par conséquent possible que des concentrations faibles en protéine cry1Ac soient suffisantes pour expliquer les résistances constatées. Des dosages de protéine B.t. permettront une interprétation plus précise.

PERSPECTIVES A COURT TERME

Avec plus de répétitions de lâchers de *P. coffeella* suivis de comptages et de mesures de mines, les résultats sur les résistances des plantes au développement des chenilles devraient être plus précis et permettre de différencier plus de catégories. Ils permettront aussi de suivre l'évolution de la résistance aux chenilles au cours de la croissance des plants. Une population

résidente de l'insecte, si elle s'installait sur les caféiers pollinisateurs, faciliterait le bon déroulement de l'essai.

D'autre part la mise en ceuvre d'une méthode de dosage de la protéine cry1Ac augmentera aussi la précision des résultats. Une connaissance de la corrélation entre niveaux d'attaque et concentration en protéine insecticide pourra alors permettre de conclure sur l'accumulation de la protéine dans les clones. Ces connaissances faciliteront le troisième volet de la présente étude, c'est-à-dire l'évaluation des risques potentiels des protéines de B.t. sur l'environnement.

CONCLUSION

Les clones transformés de C. *canephora* 126 ne présentent pas de nettes modifications d'aspect un an après leur plantation. Ils sont, à cet âge, résistants aux chenilles de la mineuse sud-américaine des feuilles dans leur large majorité. Il conviendra de suivre l'évolution de cette résistance au cours du temps et de doser la protéine insecticide présente dans les plantes. L'essai servira aussi à évaluer les effets des transformations sur le phénotype, l'impact sur les abeilles, le déplacement du pollen. Les prochaines expériences porteront sur l'introduction de nouvelles constructions génétiques dans *C. canephora* et *C. arabica*. Dans un premier type de constructions, deux gènes de B.t. seront combinés pour conférer une résistance aux mineuses des feuilles; d'autres constructions génétiques seront réalisées avec des gènes B.t. conférant la résistance à un coléoptère ravageur, le scolyte des baies.

RÉFÉRENCES BIBLIOGRAPHIQUES

Genetically modified coffee trees for resistance to coffee leaf miner. Analysis of gene expression, resistance to insects and agronomic value. ln: ASIC (eds), 18th colloquium, Helsinki (Fin), pp 332-338. ASIC, Paris (France).

Leroy T., Henry A.M., Royer M., Altosaar I., Frutos R., Duris D., Philippe R., 2000. Genetically modified coffee plants expressing the *Bacillus thuringiensis* crylAc gene for resistance to leafminer. Plant Cell Reports, **19**: 382-389

Leroy T., Philippe R., Royer M., Frutos R., Duris D., Dufour M., Jourdan I., Lacombe C., Fenouillet C., 1999.

Leroy T., Royer M., Paillard M., Berthouly M., Spiral J., Tessereau S., Legavre T., Altosaar 1.,1997. Introduction de gènes d'intérêt agronomique dans l'espèce *Coffea canephora* Pierre par transformation avec *Agrobacterium* sp. ln: ASIC (eds.), 17 colloquium, Nairobi (Ken), 20-25 July 1997 pp 439-446. ASIC, Paris (France).

Spiral J., Leroy T., Paillard M., Pétiard V., 1999. Transgenic Coffee (Co.lfea Species). ln: Y.P.S. Bajaj (scientific ed.), Biotechnology in Agriculture and Forestry, Vol. **44**, pp. 55-76. Springer-Verlag, Berlin.

Spiral J., Pétiard V., 1993. Obtention de plantules de *Coffea canephora* Pierre (Robusta) transformées par *Agrobacterium rhizogenes*. C.R. Acad Sci Paris (Ser 3), **316** : 1-6.

Temporary Immersion: A Technique for Mass Propagation of Heterozygous *Coffea spp.* Genotypes through Somatic Embryogenesis

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SUMMARY

In many countries *Coffea arabica* breeding creates and selects hybrids that combine vigour, high yields, cup quality and genes of resistance to the major diseases and pests. Somatic embryogenesis has been considered to evaluate these heterozygous genetic structures on a large scale, and later for mass propagation. Somatic embryogenesis may also replace horticultural cuttings for *Coffea canephora*. Finally this technique would allow fast establishment of clonal seed gardens. The mass propagation technique through somatic embryogenesis developed by CIRAD is based on the use of temporary immersion bioreactors and on direct sowing *ex vitro* of the embryos. This process was successfully used in various situations: a) mass propagation of selected hybrid genotypes in Central America and Tanzania, b) propagation of the progenitors of the Nemaya variety (rootstock resistant to nematodes in Central America). A large-scale multilocational trial and Nemaya seed gardens have been established in five Central American countries, totalling 50,000 plants. About 200,000 plants produced with this technique should be established in Tanzania and Central America within two years. This work allowed us to optimise the various phases of the process and to evaluate on a large scale the conformity of the plants obtained.

RÉSUMÉ

L'amélioration variétale de *Coffea arabica* s'oriente, dans de nombreux pays, vers la production et la sélection d'hybrides vigoureux, productifs, donnant un produit de qualité et possédant des gènes de résistance aux principales maladies et ravageurs.

Pour évaluer en grandeur nature ces structures hétérozygotes et les propager à grande échelle, le CIRAD a développé un procédé de propagation de masse par embryogenèse somatique basé sur l'utilisation de bio réacteurs à immersion temporaire et le semis direct des embryons *ex vitro*. Ce procédé a été utilisé avec succès dans trois situations différentes: i) multiplication des géniteurs de la variété porte-greffe 'Nemaya' tolérante aux nématodes en Amérique Centrale; ii) propagation d'hybrides sélectionnés en Amérique Centrale et en Tanzanie. 50.000 plantes – champs semenciers et réseau d'essais d'évaluation multilocale – ont été mis en place dans cinq pays centre-américains. 200.000 plantes issues de ce procédé doivent mises au champ en Amérique Centrale et en Tanzanie. Ces productions ont permis d'optimiser les différentes phases du procédé et d'évaluer à grande échelle la conformité génétique du matériel régénéré.

INTRODUCTION

Coffea arabica traditional breeding aiming at resistance, productivity and quality uses genealogic selection, and ends up with the release of fixed varieties that are multiplied and dispatched through seeds.

Recent Coffee breeding programmes (Montagnon et al., 2000; Sondhal et al., 1984), especially for *Coffea arabica* use more and more the creation and selection of vigorous, high yielding hybrids that produce quality coffee and that show some resistance to the major pests and diseases (Nyange et al., 2000).

Selected individual trees can only be multiplied and disseminated through vegetative propagation. Therefore one major objective associated with the breeding strategy is to elaborate efficient and economical processes to propagate selected individual trees of the cultivated species (Aitken-Christie and Davies, 1998; Etienne et al., 1997b; Lorenzo et al., 1998).

Cirad developped a mass propagation process using Somatic Embryogenesis based on the use of temporary immersion bioreactors (Alavard et al., 1993; Berthouly et al., 1995; Teisson et al., 1999) and direct sowing of embryos obtained.

Work and achievements presented below are the result of a multi-organisation collaboration between CIRAD and IICA/Promecafe, CATIE, Tanzania Coffee Research now TACRI. They represent two practical examples of the use of this technique.

These results allow considering new breeding strategies making an enhanced use of the genetic variability and giving faster answers to parasitic risks by developing F1 hybrids that can be easily multiplied (Etienne et al., 1997a).

MATERIAL AND METHODS

From leaf explants a highly embryogenic tissue can be easily obtained on a solid medium (Berthouly, 1996). Then this tissue can be amplified or multiplied by cell suspension (Van Boxtel and Berthouly, 1996) either in a liquid medium, or by temporary immersion (Berthouly et al., 1995; Etienne et al., 1997a); Cirad has been developing the latter technique for ten years.

This technique is used for regeneration and for germination (Photos a, b). It allows direct acclimatisation of the germinated embryos (Etienne and Berthouly, 2001; Berthouly and Etienne, 1999). Results presented below were obtained by using this technique for pilot productions of 100,000 embryos in both Central America (CATIE) and France (CIRAD), the latter for multiplying Tanzanian coffee hybrids.

In Central America Somatic Embryogenesis was used to multiply two types of material, *Coffea canephora* and *Coffea arabica*.

Coffea canephora

A hybrid variety was selected for its resistance to nematodes *Meloidogyne* and *Pratylenchus*. It is used as rootstock for *C. arabica*. T3751 (1-2) et T 3561 (2-1), the parent clones of this variety had to be multiplied rapidly in order to establish seed gardens in the regions affected by nematodes. The somatic embryos were regenerated directly from the high frequency

embryogenic callus, without amplification in liquid medium. Germinated embryos were acclimatised directly.

Coffea arabica

Many F1 hybrid progenies have been created between commercial varieties, Ethiopians accessions, and Catimor lines. Twenty high yielding individual trees were selected for yield, vigour, and cup quality. They were multiplied using somatic Embryogenesis in order to validate the technique, and to establish multilocational clone trials.

Photo a.

Photo b.

After culturing leaf explants using the method developed by CIRAD (Berthouly and Michaux-Ferrière, 1996) high frequency embryogenic callus was produced on solid medium. This callus was then

- either amplified in liquid medium by cell suspensions in erlens for 5 months. Somatic embryos were regenerated every 3 months in 600 bioreactors. The effect of the culture duration on the frequencies and types of variants could thus be studied;
- or regenerated directly without amplification, so as to limit the apparition of variants. Pre-germinated embryos obtained by this method were dispatched directly to 5 Central American countries for direct sowing, acclimatisation and hardening.

In both cases regeneration and germination (Photos a, b) took place in temporary immersion devices (RITA). The germinated embryos were transferred into simple trays for acclimatisation (Photo c) until plantlets develop. The latter were transplanted into normal nurseries for six-month hardening (Photo e).

Photo c.

Tanzania / CIRAD

Based on the selection within selected progenies of twelve hybrid trees (Table 1), leaf explants were cultured from the beginning of 1999 on Lyamungu Research Station (Tanzania), and transferred to Cirad (France) for multiplication. They belong to various, more or less complex, crosses between commercial lines susceptible to Leaf Rust and to CBD, and progenitors like Rume Sudan and Hybrid of Timor, that are resistant to either or both diseases (Kilmambo et al., 2000; Nyange et al., 2000). The transfer of fresh leaves from Tanzania to Montpellier for culturing was not successful.

After 6 months on solid medium high frequency embryogenic callus (Berthouly and Michaux-Ferrière, 1996) was amplified in liquid medium using temporary immersion (Berthouly et al.,

1995). Regeneration medium (Van Boxtel and Berthouly, 1996) was used for 2 to 3 cycles of 6 weeks. Unlike at CATIE regeneration did not use cell suspension (Van Boxtel and Berthouly, 1996).

Then the embryos were dispatched into more RITAs in order to get a density suitable for good development and germination (Photos a, b). Embryos at the right stage were then stored in sterile plastic containers at 20°C before being sent to Tanzania for acclimatisation (Photo c), and after six weeks transferred in nurseries (Photo d).

Photo d.

They were dispatched in 4 batches:

- March 2000: 43,000 germinated embryos
- July 2000: 39,000 germinated embryos
- October 2000: 8,000 germinated embryos
- February 2001: 22,000 germinated embryos

These various batches will allow to study the effect of the duration of the culture on somaclonal variation.

RESULTS

Central America

C. canephora

Over 20,000 plants of the parent clones of the hybrid variety have been produced. No variants have been detected up to now. The plants are being established in seed gardens in various countries in Central America.

C. arabica

Somaclonal variation was assessed on these plants issued from cell suspensions in a trial field (Photo f). Indeed it is well known that the culture duration influences the apparition, type (Photo g) and proportions of variants.

The types of variants observed in this material are described in Table 2 show some of the variants.

Table 1. Mother trees used for multiplication, Tanzania

Clone	Yield kg gb/ha (1)	Availability of leaves (2)	Stem Diameter (mm)	Parentage
1	1630	++	108	(N39 x Hdt) x RS
2	2170	+	125	(N39 x Hdt) x RS
3	1988	++	115	Kaffa x (‘N39 x Geisha) x Hdt)
4	2464	+++	117	(N39 x kaffa) x (RS x Hdt)
5	1963	+++	110	(N39 x kaffa) x (RS x Hdt)
6	1979	++	107	((N39 x OP729)xHdt) x (H66 x Hdt)
7	1973	+++	118	((N39 x OP729)xHdt) x (H66 x Hdt)
8	2020	+++	129	(N39 x Hdt) x (N39x(N39 x Geisha) x Hdt)
9	2078	++	99	N39 x Hdt
10	2020	+	N/A	H66 x Hdt
11	2468	+	126	N39 x RS
12	2020	++	N/A	KP423 x Hdt

(1)Average yield (5 years) of the mother tree
(2)Presence of leaves at the right stage for culturing explants

Photo e.

Table 2. Description of variant types observed

Variants types	Phenotypes	Specific weight of leaves g^{-3} x m^{-4}	Stomates density No x m^{-6}	No. of chloroplasts/ Guard Cell
Thick Leaves	Thick leaves Large fruits Star flowers	9,5-10,3 (6,4-9,7)	176-199 (164,3-191,6)	16,4-20,3 (15,3-15,8)
Dwarf	Dwarf Small leaves Small fruit Low viguor	5,8-8,6 (8,7-9,7)	171-277 (172-192)	13,23-14,93 (15,8-16,97)
Dwarf with peaberries	Dwarf Small leaves Small fruit Low vigour >50% peaberries	6,4 (6,4-9,7)	278 (164-191,6)	13,8 (15,3-15,8)

However the results in Table 3 indicate that, although it may depend on the genotype, the percentage of variants as a whole is low.

Also, 100,000 plants have been produced using another process. High frequency callus was produced on solid medium (3) but regenerated directly by temporary immersion without the preliminary amplification phase, in order to minimise the apparition of variants. These plants will be established this year in multilocational trials for final selection. The effect of this process on the rate of variants will be assessed.

Tanzania

At Lyamungu the embryos were transferred to plastic trays for acclimatisation. Various substrates were tested at Lyamungu for acclimatisation; the best results were obtained using a 2:1 mixture of forest soil/rice husks. After six weeks normally plants with 2 to 3 pairs of leaves were transferred to the nursery. Some delays were experienced during the cold season, and fungus attacks were responsible for some mortality.

We expect these plants to be established in 2001 as a large-scale multilocational trial in Arusha and Kilimanjaro regions. This will allow us to assess their conformity thus the somaclonal variation but also their adaptation to various agro-ecological conditions and to confirm their resistance to CBD and to Leaf Rust.

Table 3. Types of variants and their frequency within plants derived from four *C. arabica* hybrid genotypes

Genotype (Clone)	Number of plants	Thick leaf	Dwarf	Dwarf/ Pea berries	Total frequency
1	199	1	2	0	0,5
2	136	0	1	0	0,7
3	161	8	1	1	6,2
4	148	0	0	0	0
Total	644	9	4	1	2,1

Photo f.

CONCLUSION AND PERSPECTIVES

One should notice that this technique, elaborated thanks to partnership between Cirad, Central America and Tanzania, might be applied to both cultivated coffee species, *C. arabica* and *C. canephora*, and to various genotypes. The results of the two pilot projects presented here confirm the feasibility and the industrial potential of this process. Indeed 100,000 germinated embryos could be produced in quite different conditions, Central America versus East Africa/France.

Software was developed in order to calculate production costs. It will allow the identification and optimisation of the bottleneck phases of the process.

Assessment of somaclonal variation goes on in Central America and should start soon in Tanzania before and after the plants are established in the field.

The next steps towards the complete validation of the technique for its use at industrial stage are:

- perfect control of somaclonal variation (less than 5%);
- development of routine industrial production;
- commercial valorisation of the hybrids in the middle term.

Photo g.

REFERENCES

Aitken-Christie J. and Davies H.E. (1998). Development of a semi automated micropropagation system. Acta Hortic. **230**:81-87

Alavard D., Cote F. and Teisson C. (1993). Comparison of methods of liquid medium culture for banana micropropagation. Effects of temporary immersion of explants. Plant Cell Tiss.Org. Cult. **32**: 55-60

Berthouly M. and Michaux-Ferrière N. (1996). High frequency somatic embryogenesis in *Coffea canephora*: Induction conditions and histological evolution. Plant Cell, Tissue and Organ Culture **44**: 169-176

Berthouly M., Dufour M., Alvard D., Carasco C., Alemano L. and Teisson C. (1995). Coffee micropropagation in a liquid medium using the temporary immersion technique. In: ASIC Publishers (eds) 16th International Scientific Colloquium on Coffee, Kyoto, Japon, p. 514-519

Berthouly M. and Etienne H. (1999). Somatic embryogenesis of Coffee .In: Jain S.M.,Gupta P.K. and Newton R.J. (eds) Somatic Embryogenesis in Woody Plants, Vol.5, p 259-288. Kluwer Academic Publishers, Dordrecht.

Etienne H., Bertrand Anthony F., Cote F., Berthouly M. (1997a). L'embryogenèse somatique: un outil pour l'amélioration génétique du caféier. In: ASIC Publishers (eds) 17th International Scientific Colloquium on Coffee, Nairobi, p. 457-465

Etienne H., Lartaud M., Michaux Ferrière N., Carronb M.P., Berthouly M. and Teisson C. (1997b). Improvement of somatic embryogenesis in Hevea Brasiliensis (Mull.Arg) usng the temporary immersion technique. In Vitro Cell. Dev. Biol. **33**: 81-87

Etienne-Barry D., Bertrand B., Vasquez N. and Etienne H. (1999). Direct sowing of *Coffea arabica* somatic embryos mass-produced in a bioreactor and regeneration of plants. Plant Cell Rep. **19**: 111-117

Etienne H. and Berthouly M. (2001). Temporary immersion systems in plant micropropagation.

Kilmambo D., Swai F.P., Nyange N.E., Kipokola Tp., Mtenga Dj. and Charmetant P. (2000): Techniques for screening resistance to Coffee Berry Disease (Colletotrichum kahawae Waller & bridge). In: Dix-huitième colloque Scientifique International sur le Café. Paris: Asic, 2000, p. 508-511

Lorenzo J.C., Gonzales B.L., Escalona M., Teisson C., Espinossa P. and Borroto C. (1998). Sugarcane shoot formationin an temporary immersion system. Plant Cell Tiss. Orga Cult. **54**: 197-200

Montagnon C., Cilas C., Leroy T., Yapo A. and Charmetant P. (2000). Genotype-location interactions for Coffea canephora yield in the Ivory Coast. In: Agronomie = ISSN 0249-5627, Vol. **20**: n°1, p.101-109

Noriega C. and Sondhal Mr. (1993). Arabica coffee micropropagation throygh somatic embryogenesis via bioreactors. In: ASIC Publishers (eds) 15th International Scientific Colloqium on Coffee, Montpellier, France, p.73-81

Nyange N.E., Kipola T.P., Mtenga D.J., Kilambo D.J., Swai F.P. and Charmetant P. (2000). Creation and selection of *Coffea arabica* hybrids in Tanzania. In: Dix-huitième colloque Scientifique International sur le Café. Paris: Asic, 2000/03, p. 356-362

Sondhal M.R., Nakamura T., Medina-Filho Hp., Carvalho A., Fazuoli L.C. and Costa W.M. (1984). Coffee. In: Ammirato P.V., Evans D.A., Sharp W.R. and Yamada Y. (eds) Handbook of plant cell culture, Vol III Crop species, p. 564-590. Mac Millan Publishing Company, New York.

Teisson C., Alvard D., Lartyaud M., Etienne H., Berthouly M., Escalona M. and Lorenzo J.C. (1999). Temporary immersion for plant tissue culture. In: Plant Biotechnology and In vitro Biology in the 21st Century, Proceedings of the Ixth International Congress of PlantTissue and Cell Culture, Section h: Novel micropropagation methods, p 629-632.

Van Boxtel J. and Berthouly M. (1996). High Frequency somatic embryogenesis from coffee leaves: factors influencing embryogenesis, and subsequent proliferation en regeneration in liquid medium. Plant Cell, Tissue and Organ Culture **44**: 7-17

Current Advances on Coffee Intercropping Systems

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SUMMARY

The paper gives an overview of coffee intercropping systems with annual crops, perennial crops and shade trees. Despite a possible coffee yield depression due to intercropping the overall economic net benefits is higher than in coffee monoculture. The intercrops do not affect the coffee bean quality characteristics or liquor. It concludes by indicating possibilities of intercropping coffee with a wide range of annual crops, perennial crops and shade trees.

INTRODUCTION

Coffee is mainly grown as a monocrop in most countries, the main reason being that coffee quality might be affected adversely if farmers ignore coffee in favour of intercrops. These could be due to competition for nutrient, water and light between coffee and the intercrops. However, the intercrops could also be beneficial to the farmer in terms of food provision, timber and overall total intercropping system economic benefit to the farmer. Intercropping systems may also playa role in pest management.

Table 1. Common intercrops in coffee

Country	Crops	Country	Crops
Kenya	Beans, Irish potatoes, Maize, Sweet potatoes, Macadamia, Mangoes, Avocado, Passion, Citrus, Pawpaw, Banana, Yam, Pyrethrum, Cutflowers (Njoroge and Kiomenia, 1993)	**Papua New Guinea**	Banana and other various food crops, Casuarina (Bourke, 1985; Allen, 1985)
Tanzani a	Taro, Yam, Banana (Fernades and al., 1984)	**Venezuela**	Citrus, Banana, Avocado (Escalante, 1985)
Uganda	Banana	**Colombia**	Banana (Dario, 1986)
India	Pepper, Citrus, Spices, Arecanut, Rubber, Coconut, Foot yam, Green chillies, Pulses, Cocoa (Reddy and Rao,1999; Awatramani, 1977)	**Côte d’Ivoire**	Upland rice, maize, Yam, Groundnuts (N’Goran and Snoeck, 1987)
Ethiopia	Maize, Yam, Sorghum, Legumes, Taro, Cabbages, Pepper, Spices, (Awoke, 1997; Teketay and Tegineh, 1991)		

In indigenous home of coffee, Ethiopia, coffee is mostly grown in multistorey cropping system with trees in the upper storey followed by coffee along with food crops such as maize,

sorghum and legumes e.g beans, peas and lentils while the ground floor is covered by vegetables e.g cabbages and peppers and spices such as ginger and cardamon (Awoke, 1997).

A wide range of intercrops is observed in other coffee growing countries like Kenya, Tanzania, Uganda, India, Papua New Guinea, Venezuela, Colombia, Brazil and Cote d'Ivoire (Table 1).

Since coffee occupies a substantial amount of the high potential land, available land for food crops planting is becoming limited and hence more intercropping is expected to occur in most countries as in Kenya. The paper attempts to highlight the current information available on intercropping and shading studies with special reference to Kenya.

COFFEE/ANNUAL CROPS INTERCROPPING SYSTEMS

Preliminary screening of grain legumes in newly established unshaded Arabica coffee cv. Catimor planted at 5,000 trees/ha (2 x 1m) at Ruiru, Kenya showed that dry beans (*Phaseolus vulgaris* L.), garden peas (*Pisum sativum*), green grams (*Vigna mungo*), cowpeas (*Vigna unguiculata*) and chick peas (*Cicer arientinum*) depressed the coffee yields by 31,29,39,50 and 30%, respectively (Njoroge and Mwakha, 1994). Further studies by Njoroge et al., 1993 and Njoroge and Kimemia, 1993,1995a and 1995b in newly established Arabica coffee hybrid cv Ruiru 11 showed that maize (*Zea mays* L.) and sweet potatoes (*Ipomea batatas*) affected the coffee growth adversely while tomatoes (*Lycopersicon esculentum* Mill), Irish potatoes (*Solanum tuberosum* L.) and dry beans did not (Table 2).

Table 2. Effects of food crop intercrops on the yield of young Arabica coffe cv. Ruiru 11 (t/ha), number of bearing primaries per tree and number of bearing nodes per primary branch during the first cycle after establishment

Food crops	Clean Coffee Yield	Primary per tree		Nodes per primary	
		9 january	24 april	9 january	24 july
Potatoes	0,44	17	18	10	3
Beans	0,25	16	17	10	6
Maize	0,16	9	10	5	2
Tomatoes	0,29	16	20	8	4
Maize+Beans	0,22	13	11	8	2
Potatoes+Beans	0,26	20	15	11	4
Tomatoes+Beans	0,34	20	19	9	3
Sole Coffee	0,62	20	20	11	6
SED (14 df)	0,06	3	4	2	1

Yields of clean coffee were reduced by the maize intercrop by 59-100%. The coffee bean sizes, raw, roast and liquor quality were not affected adversely. A non-significant coffee yield improvement was noted when the food crops were intercropped at alternate coffee inter-rows than in all the coffee inter-rows. All the intercrops gave positive net economic benefits per hectare per year (Table 3). Intercropping unshaded robusta coffee (*Coffea canephora*) at coffee establishment with sweet potatoes, cassava and maize affected adversely the coffee tree growth and yield as well as the cherry to buni ratio (Njoroge, unpublished). However, there was a possibility of intercropping robusta coffee with Irish potatoes, dry beans, carrots (*Daucus carota*), soya bean (*Glyzine max*), groundnuts (*Arachis hypogaea*), tomatoes, sorghum (*Sorghum bicolor*), millet (*Eleusine corocana*), kales (*Brassicae oleracea*), cowpeas, onions (*Allium cepa*) and simsim (*Sesamum indicum*). However, the vegetables like carrots, kales, tomatoes require good moisture at their establishment stages. Yields of food crops were

also noted to be higher in the intercrops system than in the pure plots. At tree conversion period, the intercropping system showed a similar trend as in young coffee seedlings (Njoroge, unpublished).

Table 3. Arabica coffee cv. Ruiru 11 men yield (t/ha), gross profit , total variable cost and net agronomic benefit (x 10^3 Ksh) of the intecrops and the initial depression in the gross profit from the coffe during the first production cycle after establishment

Food crops	Main Yield 1987-89	Gross profit	Variable costs	Net economic benefit	Depression in gross profit from coffe
Potatoes	118,2	236,3	37,5	198,8	5,4
Beans	5,0	30,0	12,5	17,5	11,3
Maize	28,5	71,2	12,8	58,4	13,8
Tomatoes	47,6	142,9	25,9	117,0	10,0
Maize+Beans	15,0 + 4,7	65,9	12,9	53,0	12,1
Potatoes+Beans	61,5 + 6,1	159,3	27,9	131,4	10,8
Tomatoes+Beans	54,5 + 6,5	202,3	23,4	178,8	8,6

Table 4. Effects of food crop intercrops on clean coffee bean yield during the second year of change of cycle period (1993)

Coffee + Food crops	Tree density				% yield depression
	1130	2660	5320	Mean	
Beans	764 ef	548 f	2199 ab	1171 a	16,7
Cowpeas	750 ef	681 ef	1895 a-d	1109 a	21,1
Irish potatoes	543 f	764 ef	2093 a-c	1133 a	19,4
Tomatoes	1119 def	798 ef	1512 b-c	1143 a	18,7
Sweet potatoes	787 ef	1130 c-f	1463 b-c	1127 a	19,8
Garden peas	742 ef	1130 c-f	2129 ab	1374 a	2,3
Sole coffee	678 ef	731 ef	2810 a	1406 a	
Mean	769 b	826 b	2014 a	1203	
SED tree density		134,7			
SED Food crop		1102,4			
SED interaction		177,4			
CV %		29,5			

Intercropping arabica coffee cv SL28 during the change of cycle phase with dry beans, cowpeas, irish potatoes, tomatoes, sweet potatoes and garden peas (Table 4) showed that the intercrops did not significantly affect coffee growth and coffee bean yield, size of beans and liquor quality (Kimemia, 1998).Yields of the intercrops were also not affected by coffee plants during the first year after change of cycle. However, during the second year, the yields were reduced due to the heavy canopy especially under high density.Intercropping peas, irish potatoes, dry beans and tomatoes in arabica coffee hybrid cv. Ruiru 11 at trees conversion period was also observed to have no adverse effects on coffee yields and quality (Njoroge, unpublished).

Intercropping bearing coffee with some crops in Brazil reduced coffee yields with millet being worst and beans best (Mendes, 1950). In the same country, Chaves and Guerreiro, 1989) noted that cotton, rice, bean, maize and soyabeans removed large quantities of nutrients but did not affect coffee yields. Taller type of crops affected the development and yield of

coffee in the same study. In a replacement series experiment in Papua New Guinea, Kanua (1997), showed that intercropping coffee with sweet potatoes was beneficial especially at 71:29 ratio of coffee to sweet potato, respectively. N'Goran and Snoek (1987) working in Cote d'Ivoire recommended upland rice, maize, yams (*Dioscorea* sp.) and groundnuts but maize depressed the first coffee crop which was in agreement with Njoroge (1992) in Kenya. Nayar (1976) noted that ginger (*Zingiber officinale*), yam in young Robusta coffee was a source of higher return per unit area/time, food and employment.

It is of general consensus that coffee can be intercropped successfully at early stages or at conversion period with short and early maturing crops (Blencowe, 1969, Von Hesmeer, 1970, Harwood and Price, 1976, Mwakha, 1987, Njoroge, 1992, Njoroge et al., 1993, Njoroge and Kimemia, 1995a and b, Kimemia, 1998). However, yield depression of annual crops from third year of tree establishment/conversion due to shading is evident (Maghembe and Redhead, 1982, Parmesh, 1987, Mwakha, 1987. Njoroge unpublished, Kimemia, 1998). Choice of an intercrop would be based on its economic value over relatively few years of intercropping. This would also help to sustain the coffee and farmers in periods of low coffee prices. Intercropping may also assist in protecting the soil from vagaries of soil erosion before the coffee canopy closes-up, better utilization of the sunlight energy and better weed management. However, more understanding of the intercrop systems is still needed especially on pests dynamics, nutrition dynamics, proportional replacement seriles systems, etc.

COFFEE/PERENNIAL CROPS INTERCROPPING SYSTEM

Coffee/fruit trees system

Studies by Kimemia (1998) on the effect of intercropping coffee plants, during the establishment phase, with papaws (*Carica papaya*), passion fruit (*Passiflora edulis*), apples (*Malus pumila*), oranges (*Citrus sinesis*), bananas (*Musa sapentium*), guava (*Psidium guajava*), avocadoes (*Perea americana*), loquats (*Eriobatrya japonica*) and Macadamia (*Macadamia ternifolia)* showed that banana and guava intercrops significantly depressed coffee growth and yield components. However, the depression did not significantly affect clean coffee yields and economic benefits (Table 5).

The other fruit tree intercrops did not significantly affect coffee tree growth or clean coffee yields or the coffee bean size, raw, roast and liquor. At coffee production phases, intercropping Arabica coffee cv. Ruiru 11 with guava significantly reduced the clean coffee yields at Ruiru site (Table 6) .The yields of cv SL28 were not significantly affected by the intercrops. At Kitale site, the various tree crops did not affect significantly the clean coffee yields (Table 6).The size of beans and the liquor quality were not affected by the intercrops. In India, it is an age old practice to train pepper vines on shade trees in coffee (Reddy and Rao, 1999). A long term study in India on coffee intercropping systems with bananas, oranges and pepper indicate that the income realised from coffee alone was not significantly different from the intercropping systems (Korikanthimath, 1999), the intercrops could also cushion farmers when coffee is not economical especially during drought years or when coffee price plummets. In Uganda, intercropping Robusta coffee with bananas and sweet potatoes showed that sweet potatoes reduced coffee yields (Oduol and Aluma, 1990) as in Kenya (Njoroge, unpublished).

Table 5. Effects of fruit tree intercrops on arabica coffee yields, fruit tree yields and net benefit during the third year of production after establishment (1993)

Coffee + Fruit trees	Clean coffee Yield (t/ha)			Fruit yield (kg/ha)	Increment benefit (x 1000 Ksh) 1993	
	Ruiru 11	SL 28	Mean		Ruiru 11	SL 28
Pawpaw	1785 a-e	1388 b-f	1586 ab	25131	145,55	167,46
Passion	2230 a	119 b-f	1715 ab	4749	160,98	119,50
Apple	1625 a-f	1643 a-f	1634 ab	348	17,62	81,22
Orange	1941 a-c	1515 a-f	1728 ab	1204	55,78	74,98
Banana	1429 a-f	1141 c-f	1285 bcf	21002	48,91	81,91
Guava	1097 def	1404 b-f	1251 bc	12154	35,15	57,35
Avocado	1408 b-f	1265 b-f	1337 bc	741	-2,18	46,12
Loquat	1859 a-d	1580 a-f	1719 bc	0	28,90	62,80
Macadamia	1978 ab	1920 a-d	1949 a	25	41,30	97,30
Sole Coffee	1570 a-f	952 f	1261 bc			
Mean	1692 a	1306 b	1499			
SED Coffee cultivar		77,1				
SED Fruit trees		238				
SED interaction		236				
CV%		27,8				

Table 6. Effects of intercropping mature arabica coffee with fruit trees on coffee yields, Ruiru 1996-99 and Kitale 1997-99

Coffee + Fruit trees	Ruiru (1996-99)			Kitalé (1997-99)		
	Ruiru 11	SL 28	Mean	Ruiru 11	SL 28	Mean
Pawpaw	1146 a	919 abc	1032 ab	1673 a	835 d	1254
Passion	1138 a	1023 abc	1081 ab	1469 a	841 d	1155
Apple	906 a	1029 abc	968 ab	1666 a	910 bcd	1288
Orange	1203 a	905 abc	1054 ab	1419 ab	632 d	1026
Custard apples				1697 a	825 d	1261
Banana	784 abc	769 abc	777 bc			
Guava	594 c	629 bc	612 c	1483 a	879 bcd	1181
Mulberry	1098 ab	769 abc	934 ab	1459 a	818 d	1139
Avocado	941 a	901 abc	921 ab	1459 a	717 d	1088
Loquat	1071 abc	631 bc	851 ab	1520 a	833 d	1177
Macadamia	1095 ab	1085 abc	1090 a	1732 a	863 cd	1298
Mango	1259 a	932 abc	1096 a			
Sole Coffee	1124 ab	894 abc	1009 ab	1288 abc	559 d	974
Mean	1030 a	874 a		1542 a	792 b	

Coffee/shade trees systems

Current studies on effects of shade trees planted at the same time with the coffee trees to coffee yields showed no adverse effects on clean coffee yields except *Mimosa scrabella* (Table 8). Other trees being evaluated are *Cordia abyssinica*, *Leucaena diversfolia, Grevillea robusta, Markhamia lutea, Albizia gamifella* and *Hakea saligna*.

Table 8. Effects of shade trees on yields of arabica coffee, Ruiru 11, clean coffee during the 1st cycle after establishment (1997-99) in Kitale

Shade trees	Yield (kg/ha)
Cordia abyssinica	1917 a
Leucena diversifolia	2320 a
Mimosa scrabella	1051 b
Grevillea robusta	2000 a
Markhamia lutea	1901 ab
Albizia gamifella	1903 ab
Hakea saligna	2007 a
Sole coffee	1938 a
Mean	1880

Several tree species have been grown in coffee mainly as shade trees or as wind break, such as *Cordia* spp., *Grevillea robusta*, *Albizia* spp., *Leucaena leucocephala* and *Cyperus* spp, (Njoroge and Kimemia, 1993). In Ethiopia, indigenous trees such as *Albizia gummifera, Allophylus abyssinica*, *Celtis africana, Cordia africana, Ekebergia capensis, Ficus sur, F. sycomorus, F. vasta, Milletia ferruginea, Macaranga kilimandscharica, Croton, Machrostachys* are left as shade trees (Awoke, 1997).

In India, coffee is commonly planted under forest trees or selected shade trees such as Grevillea spp and Dadap (Reddy and Rao, 1999). In parts of Colombia, Costa Rica, shade trees of guan, Dadaps (*Erythrena lithosperma*), Eucalyptus, bananas and Inga are found while in Guatemala, coffee is grown in temporary shades of bananas and Dadaps and permanent shades of Inga and Grevillea (Menan, 1999a and b). An experiment to investigate the effect of Leucaena leucacephala shade on Robusta coffee under the influence of different nitrogen rates was carried out in the Western part of Kenya (Njoroge, unpublished). He observed that although the shaded coffee trees enjoyed higher soil nutrients than the unshaded coffee, its buni production was lower. This could have been due to less photosynthetic radiation reaching the coffee trees irrespective of the amount of the nutrients supplied to it. This means that shade in coffee needs to be maintained at a lower density for good yield and soil fertility sustainability. A study in Ghana (Amoah, et al., 1997) showed a 37% lower yields in shaded Robusta coffee with Glyriaka maculata compared to unshaded coffee trees. In Togo, use of *Albizzia adianthifolia* as shaded tree in Robusta coffee maintained the longevity of the fruit bearing branches and stimulates production (Koudjega and Djiekpor, 1997).

Use of shade trees have been observed to help even out erratic yields caused by periodic overbearing, reduce crinkling of coffee leaves commonly known as "hot and cold" disease and reduce hail injury. Shade has also been shown to reduce infection of Bacterial Blight of coffee (*Pseudomonas syringae*) due to reduced hail injury on the coffee trees thereby reducing pesticide usage. Further research is needed into suitable trees of economic value and the effect of shade on coffee trees as the original introduction of shade trees in coffee was not preceded by such studies. It was taken that coffee needed shade being an understorey plant in the centre of origin. Apart from the above, shade trees help to recycle soil nutrients deep in the soil to the coffee rooting top soil through litterfall, leguminous trees fix atmospheric nitrogen, assist in controlling soil erosion, weed suppression and encourage rainfall. In a multistorey intercrop system, most nutrients are held in the vegetative mass which is returned to the soil as litterfall. This system may also utilize the soil more efficiently. Management of shade coffee system in terms of optimal shade, coffee nutrition requirements, management of pests, etc need further investigations. Njoroge (Unpublished) noted no significant Robusta lea-N between fertilized and unfertilized coffee under *Leucaena leucocephala* shade.

CONCLUSION

Intercropping or shading coffee is likely to depress coffee yields. However this is compensated by the total net income which is generally higher than for sole coffee. The intercrop system also may ensure food security. Income security is also ensured especially during the years when coffee prices plummet and during the coffee non productive stages. The indications are that coffee can be intercropped with a range of annual crops, perennial crops and shade trees with exception of creeping or tall crops such as sweet potatoes, cassava and maize especially in the young trees or young suckers.

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REFERENCES

Allen B.V. 1985. Dynamics of fallow successions and introduction of Robusta coffee in shifting cultivation areas in lowlands of Papua New Guinea:Agroforestry systems **3**: 227-238.

Amoath F.M., Osei-bonsu K. and Oppong F.K. 1997. The effect of shade; stem number and spacing in Robusta coffee yield in Ghana. In 17th International Scientific Colloquium on coffee (ASIC). 528-534.

Awatramani N.A. 1977. Multistoreyed Cropping patterns with coffee for maximising production. Indian coffee **41**: 7-8.

Awoke T.C. 1997. The culture of coffee in Ethiopia. Agroforestry today **9** (i) 19-21.

Blencowe J.W. 1969. Crop diversification in Malaysia Incorporated Society of Planters, Kuala Lumpur, Singapore.

Bourke M.R. 1985. Food, coffee and casuarina. An agroforestry systems from the Papua New Guinea highlands. Agroforestry systems **2**: 273-79.

Chaves J.C.O and Guerreiro E. 1989. Intercropping in coffee plantations. Pesquisa agropeculna Brasileira. **24**: 177-90

Dario A.J. 1986. El cultivo de planto en hilevas O "Barrera" estableciaminto y manejo. Cacaofero colombiano **9**: 31-44.

Escalante E.E. 1985. Promising Agroforestry systems in Venezuela. Agroforestry systems **3**: 209-21.

Fernandes E.C.M., Oktingati A. and Maghembe J.A. 1984. The chagga home gardens. A multistoried agroforestry cropping systems on Mt Kilimanjaro. Agroforestry systems **2**: 73-86.

Harwood R.R. and Price E.C. 1976. Multiple cropping in tropical Asia. In. Multiple cropping Eds. papendick R-1) American Society of Agronomy, Madison, Wisconsin USA. 11-40.

Kanua M. B. 1997. Intercropping effect of sweet potato on the growth, development and yield of Arabica coffee growth at Aiyura, Papua New Guinea. 17th International Scientific Colloquium on coffee, Nairobi, July 1997. 542-549.

Kaswani C.L. and Ndunauru B.J. Arid areas Ed. IDRC. Othawa, Canada. 43-49.

Kimemia J.K. 1998. Studies in green manure application and intercropping in *Coffea arabica* L. production PhD. Thesis. University of Nairobi, Kenya. 237pp. arid areas Ed. Kaswani, C.L. and Ndunauru, B.J.) IDRC. Othawa, Canada. 43-49.

Menan Sunalini N. 1999b. Report on visit to coffee producing countries in South and Central America. Indian Coffee. LXII (11) :14-17 & 40.

Mendes C.T. 1950. Osombreamento dos cafezais.Horticultural Abstracts 21: No. 4007.

Mwakha E. 1987. Interaction in intercropped *Coffea arabica* L. and *Phaseolus vulgaris* L. PhD. Thesis University of Nairobi. 262 pp.

N'Goran K. and Snoek J. 1987. Food crops associated with coffee trees in Cote d'Ivoire. Cafe' Cacao The' **31**: 121-134

Nayer T.V.R. 1976. Intercropping in young Robusta coffea Indian coffee 40: 70-71.

Njoroge J.M. and Kimemia J.K. 1995a. Economic benefits of intercropping young Arabica and Robusta coffee with food crops in Kenya. Outlook on Agriculture. **24**: 27- 34.

Njoroge J.M. and Kimemia J.K. 1995b. Effects of inter~ropping young compact Arabica coffee hybrid with foodcrops at three ecological zones on yields and coffee growth in Kenya. Experimental Agriculture **31**: 49 -55.

Njoroge J.M. 1992. Studies on fertilization, plant density pruning, replacement methods of established coffee and intercropping food crops with coffea arabica L. cv. Ruiru 11. PhD Thesis. University of Nairobi, Kenya, 337 pp.

Njoroge J.M., Waithaka K. and Chweya J.A. 1993. Effects of intercropping young plants of compact Arabica coffee hybrid cultivar Ruiru 11 with potatoes, tomatoes, beans and maize on coffee yields. Experimental Agriculture **29**: 373-377.

Njoroge J.M. and Kimemia J.K. 1993. Current intercropping observations and future trends in Arabica coffee, Kenya. Outlook in agriculture **22**: 43-48.

Njoroge J.M. and Mwakha E. 1994. Intercropping young arabica coffee cv. Catimor with grain legumes.Discovery and Innovations. **6**: 415-419.

Oduol P.A. and Aluma J.R.W. 1990. The banana (Musa spp.) – Coffee robusta traditional agroforestry systems of Uganda. Agroforestry systems. **11**: 213-6.

Parmesh S. 1987. Potential of agroforestry as land use technology for rural poor. IUFRO on agroforestry for rural need. New Delhi, India.

Reddy A.G.S. and Rao Ananda I.V. 1999. Coffee as an intercrop to coconut in the plains of Karnataka. India Coffee. LXIII: 3-6.

Teketay O. and Tegineh A. 1991. Traditional tree crop based agroforestry in coffee producing areas in Harenge Eastern Ethiopia Agroforestry systems **16**: 257-267.

Von Hesmeer H. 1970. Der Kombinerte Land-und forstwirschafliche anbau 11. Tropisches and subf tropisches. Asien EMST Kleh vorlaga stuttgovit. 219p.

Weed Flora and Weed Control Practices in Coffee (*Coffee arabica* L.) in Ethiopia. A Review

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SUMMARY

Ethiopia is the home of Arabica coffee (*Coffea arabica* L.) in Ethiopia coffee grows at various altitudes, ranging from 550-2750 meters above sea level and under four production systems namely, forest, semi-forest garden and plantation coffee accounting 10, 34, 35 and 21% of the total volume produced respectively. The coffee growing areas characterized with high rainfall and suitable temperatures and edaphic condition encourage the growth of diverse weed species ranging from abundant seed producing annuals, to hard-to-control rhizomatous and stoloniferous perennial grasses and sedges. As a result, coffee yield and quality is seriously reduced and weed control is one of the major cultural operations which entails high cost. Coffee can seriously suffer from weed competition and results obtained from loss assessment studies have revealed that yield loss can reach as high as 65 percent depending on the type and the frequency of weeding operations. According to surveys made hitherto, a total of over 52 species from 8 families was identified. In spite of the divergent ecologies and production systems under which coffee is growing weed control practices are more or less the same in all coffee growing areas and include manual slashing and digging, mulching, shading, cover cropping using leguminous crops and application of synthetic herbicides in coffee production. The advantages of coffee by-products such as coffee husk as mulch for weed control is now well recognized at the research level. Integrated Weed Management (IWM) is however, the most appreciated and recommended practice for controlling weeds in coffee, because it is environmentally sound, economically viable and socially acceptable practice for sustainable coffee production.

INTRODUCTION

Ethiopia is the home of Arabica Coffee (*Coffea arabica* L.). Coffee is the single most important commercial crop in the national economy of Ethiopia contributing more than 60 per cent of its foreign exchange earnings. In Ethiopia coffee grows at various altitudes ranging from 550-2750 meters above sea level and under four production systems namely, forest, semi-forest garden and plantation coffee accounting 10,34,35 and 21% of the total volume produced respectively (Workafes et al., 1999). The coffee growing areas of Ethiopia, characterized with high rainfall and suitable temperature and edaphic conditions encourage the growth of diverse weed flora ranging from abundant seed producing annuals to hard-to-control rhizomatous and stoloniferous perennial grasses and sedges. Experience has shown that weeds can be serious competitors. Perennial grasses, sedges and annual weeds with enormous amounts of seed production can easily smother coffee bushes and result in extremely low yields and small sized coffee beans which yield poor quality processed coffee. The worm, wet and humid condition prevailing in the coffee growing areas not only result in diverse weed flora also encourage the continuous growth of weeds necessitating weed control throughout the growing period. As a result, weed control is one of the major cultural operations which entails high cost Bayissa et al., 1988.

The majority of coffee producers (90%) are subsistence farmers heavily dependent on manual slashing and digging of the perennial weeds who cannot afford the purchase of chemical inputs. As a result, the traditional practices of slashing and digging encourage the multiplication and spread of the perennial weeds in coffee. However, the use of herbicides for weed control in coffee is growing steadily in the state farms. Good weed management and effective weed control require as much better understanding of weed response to changes in cultural methods and the application of herbicides. Hence, Integrated Weed Management (IWM) is the most promising alternative strategy, because it emphasizes the proper utilization of cultural, mechanical and chemical methods for sustainable coffee production. This paper attempts to discuss the biological spectrum of the weed flora associated with the Ethiopian coffee and the control practices in coffee production.

WEEDS IN COFFEE

Weeds in coffee are diverse (Table 1). The high rainfall, warm, and wet humid conditions prevailing in the coffee growing areas coupled with the weed control methods have encouraged a continuous and exuberant growth of highly competitive and difficult to control weed flora. Although systematic survey and hierarchical classification is lacking, based on visual scoring and subjective type of ranking different researchers have attempted to identify and categorize the major weed species in coffee (Paulos, 1985, Bayissa et al., 1988; Lakew, 1987).

According to survey made so far, a total of 52 species from 8 families were identified. As weeds are a moving target in continuous evolution be exposing only a small part of themselves, an actual flora (AF) represents only a limited percentage of the potential flora (PF) i.e. of the seed bank. The mere numbers and type of weed seeds in the soil, however, may not be correlated with the number and population of emerged weeds. Hence, species listed here as coffee weeds do represent a small portion of the potential coffee weeds of the future.

Weeds are nuisance to coffee production because of their competitiveness. As coffee is a slow growing crop, weed can establish well in a coffee field totally inhibiting its growth and productivity. They constitute various classes and families. On rather broader and practical considerations coffee weeds can be classified as C_3, C_4 and CAM plants based on their ecophysiological definitions which could make weed management practices easy and economical. C_4 weeds are well adapted to conditions of high irradiance (open sun) as opposed to C_3 weeds that properly flourish under low light intensities (excessive shading). Moreover, the C_4 perennial weeds are highly adapted to undisturbed conditions and are highly associated with coffee production than in annual crops were soil disturbance is carried out annually. On the other hand, CAM weeds could be prevalent under both conditions because of their complementary photosynthetic and respiratory cycles.

Most of the economically important coffee weeds are shade sensitive grassy and sedge species that are difficult to control manually because of their way of perpetuation and underground interconnected rhizome and tuber system which serve them as a storage organ and reproductive structure for their rapid multiplication. The fact that these weeds species could not tolerate low light intensities is however, an important feature in weed management strategy.

Table 1. List of noxious, important and potentially important weed species of coffee

Botanical name	Family	Growth nature	Ecophysiological definition	Economic importance
Digitaria abyssinica	Poaceae	Perennial	C_4	Noxious
Cynodon spp.	Poaceae	Perennial	C_4	Noxious
Cyperus spp.	Poaceae	Perennial	C_4	Noxious
Paspalum spp.	Poaceae	Annual	C_4	Noxious
Snowdenia spp.	Poaceae	Annual	-	Important
Phalaris paradoxa	Poaceae	Annual	-	Important
Elusine indica	Poaceae	Annual	C_4	Important
Bidens pilosa	Composaceae	Annual	C_3	Important
Ageratum conyzoides	Composacae	Annual	C_3	Important
Galinsoga parviflora	Composaceae	Annual	C_3	Important
Commelina spp.	Composaceae	Annual	C_3	Important
Plantago lanceolata	Plantignaceae	Annual	C_3	potentially important
Cynoglossum lanceolatum	Boraginaceae	Annual	C_3	potentially important
Guizotia scabra	Composaceae	Annual	C_3	Important
Sida spp.	Malvaceae	Perennial	-	Important
Oxalis spp.	Oxalidaceae	Annual	C_3	Important
Conyza spp.	Composaceae	Annual	C_3	Important
Hyperhenia spp.	Poaceae	Annual	-	potentially important
Phalaris spp.	Poaceae	Annual	-	potentially important
Tagetes minuta	Composaceae	Annual	C_3	potentially important
Tapinantus globiferous	Loranthacene ae	Annual	-	potentially important
Caylusea abyssinica	Resedaceae	Annual	-	potentially important
Solanum spp.	Solanaceae	Annual	-	potentially important
Corchorus spp.	Tiliaceae	Annual	-	Important
Nicandra physaloides	Solanaceae	Annual	-	potentially important
Datura stramonium	Solanaceae	Annual	-	potentially important
Digitaria scalarum	Poaceae	Perennial	C_4	Noxious

N.B: The economic classification as noxious, important and potentially important is based on the abundance and distribution of the species in coffee, the competitive ability of the species and how tolerant a species would be for different weed control methods

Table 2. 20 years summary of weather data of the coffee growing areas

Name	Rainfall	No. of rainy days	Temperature (°C)		Relative humidity (%)	AEZ
			Maxi.	Min.		
Jima	1593	163	27.0	11.4	65.1	SH_2-7
Gera	1878	181	25.2	10.4	70.8	H_2-7
Tepi	1731	164	30.0	15.4	-	H_1-7
Metu	1832	164	28.8	12.3	-	SH_1-7
Wonago	1600	205	25.0	8.7	-	H_2-7
Mugi	2513	176	-	-	-	SH_1-7
Bebeka	1744	154	30.0	15.0	-	PH_1-7
Goma	1800	172	29.5	14.4	-	SH_2-7
Gimbi	1700	-	25.8	13.9	-	-

Key: AEZ = Agro-Ecological zone; SH_1 -7 = Sub humid hot to worm low to mid high land mountains; SH_2 -7 = Sub humid tepid to cool mountains; H_1 -7 = Humid hot to worm low to mid highland mountains; H_2 -7 = Humid tepid to cool high land mountains; PH_1 –7 = Hot to warm per-humid mountains

CROP LOSS CAUSED BY WEEDS

Crop loss due to weed competition should be assessed in a given crop/weed association. The amount of crop loss will determine how serious weeds could be in a given crop. Crop loss in a given crop/weed interaction could vary depending on the type and density of weed species growing and the environmental factors available. The amount of crop loss could vary from location to location and from year to year under similar environmental conditions. Nonetheless, crop loss estimated at a specific location could give a general indication of the crop/weed interaction.

There are essentially two sides of economic loss due to weeds: On the one hand the damage caused by weeds to the crop and on the other the expenditure to control weeds. The coffee tree is highly susceptible to moisture and nutrient competition by weeds. The coffee feeder roots tend to lie close to the surface whereas competitive weeds can put down their deep roots to tap the underlying moisture. Because of the perennial weeds capacity to tap the underlying moisture, they continue to grow even during the dry period where the growth of coffee is temporarily checked. A crop loss assessment was conducted at Jima Research Center (JRC) in established coffee and in newly planted coffee to estimate the amount of crop loss due to weed competition. The result showed that yield loss amounted 65 percent when weeding was totally ignored (Table 3). On the other hand, in another experiment, Total N content of coffee seedlings was reduced by 54 percent when couch grass was allowed to compete full season (Table 4). These results clearly indicate that weeding is a serious matter in coffee.

Means followed by the same letter are not significantly different according to the Duncan's Multiple Range Test at 5% level.

WEED CONTROL

Slashing and Digging

Slashing and digging are the major methods of weed control employed by the majority of coffee growers (Paulos, 1985; Kassahun, 1994). Weed slashing is fast operation, useful for the control of broad leafed weeds but with little advantage on the control of perennial grasses and sedges. According to Kassahun 1994 the majority of coffee farmers used 2 slashing in

one crop season to control weeds which is hardly adequate to suppress weed growth and increase yield.

Research conducted at JRC have shown that slashing of perennial weeds beyond 4 weeks interval had no or little effect on yield suggesting that slashing interval is critical and should be performed with closer intervals in order to exhaust the underground reserves (Table 4). On the other hand, in a yield loss assessment study, it was found that coffee which was given 10 slashing per season resulted in 14 per cent yield loss indicating that even if weeds are slashed more frequently, there is always considerable amount of yield loss associated (Tables 3 and 4).

Table 3. Coffee yield as affected by different weeding methods. Q ha^{-1} clean coffee

Weed control method	Q ha^{-1} clean coffee				% loss of clean weeding
	1993	1994	1995	Mean	
No weeding	2.00	1.67	4.40	2.69	65
3 slashing	3.54	2.43	7.98	4.65	40
5 slashing	3.75	3.15	9.50	5.46	30
10 slashing	4.47	4.20	11.28	6.65	14
1 slashing followed by one time glyphosate application at 4 Lt ha^{-1}	4.04	2.96	9.94	5.65	27
Clean weeding	5.38	5.1	12.74	7.74	-
LSD 0.05	1.03	1.04	2.00		
0.01	1.45	1.44	2.87		

Table 4. Effect of frequency of clipping of couch grass on the growth and total leaf nitrogen content of coffee seedlings

Clipping interval (weeks)	Clipping frequency (no)	Couchgrass dry weight (g)	Coffee dry weight (g)	Reduction In total dry weight (%)	Total N (%)	% loss of weed free
1	32	280[a]	177[b]	43	2.00[b]	30
2	16	287[a]	168[b]	44	1.98[b]	31
4	8	678[b]	118[c]	72	1.76[c]	39
6	6	908[c]	88[d]	72	1.48[d]	49
8	4	930[c]	83[d]	70	1.33[d]	54
10	3	925[c]	82[d]	70	1.32[d]	51
weed free	-	-	307[a]	-	2.88[a]	-
unclipped	-	927[c]	85[d]	71	1.33[d]	54

Generally, solely dependence on slashing and digging for weed control in coffee is inefficient, costly and also detrimental to the coffee tree (Paulos, 1985; Bayissa et al., 1988; Tadesse, 1994). Slashing using the bushman knife usually wounds the coffee tree predisposing it to a fungal disease called *Gibberella xylariodes* which ultimately kills the coffee tree. Control by digging (cultivation) is not practical in established coffee since the attempt to dig out the rhizomes and tuber chains is highly injurious to coffee feeder roots. Furthermore, digging fragments and disperses the rhizomes in the field. Hence, thorough cultivation is recommended only during land preparation. However, digging is a common practice in the southern and eastern coffee growing areas. This practice is laborious, time consuming with little effect on the control of perennial weeds. Nevertheless, slashing and digging with proper timing could be vitally useful in IWM approach.

Cover cropping

A cover crop could be defined as a plant that provides ground cover to discourage weed emergence and subsequent growth. Because weed removal is interference with nature, a first step in minimizing this interference is to identify weed control method that will not contribute to soil degradation or adversely affect environmental quality. Land and crop management practices such as total removal of weed flora that exposes the soil for erosion or in any way degrade it are thus inappropriate for sustainable agriculture.

At JRC, study on cover cropping primarily for weed suppression and soil enrichment has gained due attention and is in progress. So far, forage legumes which are adaptable to the coffee ecology have been screened. The research have taken a renewed interest in cover crops because of their potential role in reducing chemical inputs and improving soil quality. Cover crops are now recognized as an important component of sustainable coffee production.

Successful cover cropping requires the selection of a species or mix that will provide specific desired benefits and that will be compatible with the overall production system. Furthermore, especially with cash crops, cover crops should be rotated periodically to ovoid the build up of plant specific pests. Cover cropping, like any new practice, should be approached slowly and methodically (Chuck et al, 1994). It may be valuable to test several species to find the most appropriate cover crop. It is usually a good approach to begin on a small scale in order to learn from mistakes without incurring unnecessary expenses. With persistence and creativity, cover cropping can provide many benefits in perennial crops like coffee with little extra cost.

Investigation at JRC has shown that noug (*Guizotia abyssinica* L.) at the rate of 20 kg ha^{-1} was found to effectively suppress the perennial grasses in coffee compared with chickpea, lentil, linseed and soybean (Paulos, 1987). Furthermore, the forage legume *Crotolaria zanzibarica* has shown outstanding suppression of weed growth at Bebeka state farm. This is because crotolaria was found to be fast growing in the humid low land area of Bebeka.

Nevertheless, along with the selection of suitable cover crop(s), the level of fertilizer required for the coffee tree growing with the cover crop, the seed rate and the proper time of planting of the cover crop need close investigation. However, it is indispensable to note that the use of cover crops alone does not warrant the control of weed growth to a level where yield is not affected unless cover cropping is prudently unutilized in IWM program.

Mulching

The effect of mulching may be attributed to various physical aspects. Mulching is the covering of the soil with a layer of dry vegetative materials. Its benefits include conservation of soil moisture, control of soil erosion, improvement of soil structure, supply of mineral nutrients in case of decomposition, regulation of soil surface temperature and suppression of weeds (Opile, 1995).

The effect of some mulch materials on the control of couch grass was tested at JRC between 1978 and 1981. These mulch materials tested were corn cob, coffee husk, elephant grass and false banana leaf. The result showed that the mulch materials tested did not show satisfactory control of couch grass. However, some nutritional contribution from some of the mulch materials to the coffee tree has been observed (Paulos, 1985).

As a matter of fact, the use of mulch materials alone cannot solve the weed problem in coffee particularly in a field with high infestation of perennial weeds. However, mulching can be satisfactorily utilized in integrated program with other cultural and chemical control methods.

Chemical weed control

Herbicides will remain an essential part of coffee production, but their use need to be minimized to meet the demands of both the farmer and the general public. Much of the reduction can be achieved not only by reducing the number of applications to the crop but by using a reduced dose appropriate to the situation by combining with other cultural practices.

Integrating chemical and cultural control can reduce the level of herbicide use in three ways:

1) Reducing weed numbers to a level where herbicide application is not necessary or where herbicide dose can be reduced.

2) Delaying weed emergence and/or weed establishment and hence reducing the herbicide dose required.

3) Killing weeds that have already received a low rate of herbicide.

Table 5. List of recommended herbicides from JRC

Trade name	Common name	Recommended Rate (l./ha)	Mode of Action	Target Weeds
Round-up	Glyphosate	1-4	Systemic	All weeds
Dowpon	Dalapon	7-8	"	Grasses
Fusilade super	Fluazifop-butyl	2-4	"	Grasses
Gramoxone	Paraquat	1.5-2	Contact	Broad leafed
Kalache 360 SL	Glyphosate	1-4	Systemic	All weeds
Glyfos	Glyphosate	1-6	Systemic	All weeds
Clinic	Glyphosate	1-1.5	Systemic	Broad leafed
Basta	Glufosinate	1.5-2.0	Partial systemic	Broad leafed
Glysogan T	Glyph. + Terbu	1.0-1.5	Soil acting	Broad leafed
Touch down	Sulfosate	1-6	Systemic	All weeds

Integrated weed management

Integrated Weed Management uses all available knowledge to manage weeds so that they do not cause economic loss nor adversely affect the environment. IWM has the potential to increase coffee yields and quality and reduce time of input and operation costs among coffee farmers. It also prevents the incidence of herbicide resistance by weeds, removes the difficult weeds and reduces environmental pollution and possible reduction of drudgery.

The implementation of IWM should involve strategies whose components have been carefully selected, field tested in selected ecologies and adapted to the needs of the farmers who will use them. Indicators to the success of IWM include soil quality, crop productivity and water quality; all of these are related to the rationale of IWM, hence IWM can be linked to agro-ecosystem health. Hence, cover cropping, mulching, slashing and digging, shading, land preparation methods and herbicides can be prudently integrated depending on the environmental situation where the coffee is growing to obtain the maximum benefits of IWM program.

Table 6. Effect of integrating different weed control methods on coffee yield at Jima (Melko)

Treatment	Clean coffee yield Qha^{-1}			Mean	% yield loss of clean weeding
	1995	1996	1997		
1	4.92	4.10	5.82	5.28	59.2
2	7.50	6.28	12.33	8.70	31.0
3	6.00	4.29	7.08	5.79	54.0
4	7.95	8.12	12.20	9.42	25.4
5	5.67	5.92	7.43	6.34	49.0
6	8.85	6.03	11.86	8.91	29.5
7	8.44	8.31	11.83	9.52	24.6
8	13.72	8.56	15.61	12.63	-
9	2.89	3.11	4.48	3.49	72.4
LSD 0.05	2.5	2.0	5.21		
LSD 0.01	4.7	4.2	7.18		

Key to treatments: 1 = 2-3 slashing; 2 = 1 time Roundup applied at 4 $Ltha^{-1}$; 3 = Noug (Guizitia abyssinica L.) cover crop applied at 20 kg ha^{-1}; 4 = 1 time slashing followed with Roundup applied at 1.5 / Lha^{-1}; 5 = 1 time slashing followed with noug cover crop at 20kg ha^{-1}; 6 = 1 time Roundup applied at 1.5 /Lha^{-1} followed with noug cover at 20kgha^{-1}; 7 = 1 time slashing followed with Roundup applied at 1.5 /Lha^{-1} followed with noug cover at 20 kg ha^{-1}; 8 = Clean weeding; 9 = 1time slashing (farmers practice)

Table 7. The relative merit of different weed control methods with regard to economic,agronomic and environmental parameters

	Slashing and Digging	Mulching	Cover cropping	Chemical	Integrated
Cost	+	+++	++++	++	++++
Time	+	++	+++	++++	++++
Yield benefit	+	++	+++	+++	++++
Crop safety	+	+++	+++	+++	++++
Soil moisture	+	+++	+++	+	++++
Soil erosion	+	++++	++++	+	++++
Soil nutrient benefit	++	++++	++++	+	++++
Weed flora change to undesirable types	+	++	+++	+	++++
Overall sustainability	+	++	++	+	++++

Key: + = Low merit; +++ = Medium merit; ++ = Fair merit; ++++ = High merit

REFERENCES

Bayissa Mormene, Paulos Dubale and Damenu Tulu. 1988. Approaches to weed control practice in large scale coffee plantation in Ethiopia. Paper presented to the Ethiopian Weed Science Committee. April 1988, Addis Ababa.

Chuck I., Mark V.H., Robert L.B. and Miller P.R.. 1994. Selecting the right cover crop gives multiple benefits. California agriculture, **48**: 5 pp43-48.

Kassahun Syoum. 1994. Farmers weed management in coffee based forming system of the Jima area. Paper presented on the Second Annual Conference of Ethiopian Weed Science Society, 15-16 Dec. 1994.

Lakew Belaineh.1987. Management practices and productivity of the cultivars that are resistant to Coffee Berry Disease (CBD) within the Coffee state farms. In Paulos Dubale(ed).). Proceedings of the first Ethiopian Symposium on coffee, Addis Ababa, Ethiopia, 20-23 August 1986. (In press).

Opile W.R. 1995. African coffee : an overview. ASIC, Kyoto, 9-14 avril, Japan

Paulo Dabale. 1985. Review of weed control research in Ethiopia. In Tesedeke Abate (ed.). Proceedings of Review of crop protection Research in Ethiopia. Addis Ababa, Ethiopia. 4-7 February 1985. IAR.

Tadesse Eshetu. 1994. 1994. Effect of clipping frequency of Couch grass (Digitaria abyssinica) on the growth of young coffee. Paper presented on the Second Annual Weed Science Conference, 15-16 Dec. 1994. Addis Ababa, Ethiopia.

Workafes Woldesadik and Kassu Kebede.1999. Coffee Production Systems in Ethiopia. Proceedings of the Workshoe on Control of Coffee Berry Disease (CBD), in Ethiopia, Addis Ababa (Ghion Hotel), 13-15 August 1999.

Integrated Sustainable Production Systems for Coffee in India

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SUMMARY

Coffee in India is grown under well-established mixed shade canopy comprising of evergreen, leguminous trees. Major coffee growing regions are situated in the ecologically sensitive forest hill tracts of the Western Ghats. In these areas, coffee is often cultivated with inter-crops like pepper, cardamom, orange etc. India is a major contributor of both arabica and robusta coffees, because of highly suitable growing conditions. Thus, the economy of plantation districts in south India is intricately linked to coffee.

The economy of coffee plantations has suffered a setback in the recent past owing to unattractive prices for coffee and associated crops and escalation in the cost of cultivation. The situation at present is quite alarming with coffee prices plummeting to all time low with no signs of immediate recovery due to global surplus. If this situation persists for few more years, there is every possibility of growers abandoning the plantations and resorting to large-scale timber extraction. This could be disastrous for the fragile ecosystem of the coffee growing regions. Thus, there is an immediate need to resurrect and sustain the economy of coffee plantations on one hand and to preserve the ecological balance of the Western Ghats on the other.

Coffee areas in India are suitable for diverse agricultural activities such as production of spices, horticulture crops, mulberry for silk production and other animal husbandry practices like bee keeping, dairying, fisheries etc. Integrating these diverse activities would go a long way in sustaining the economy of small coffee growers besides preserving the fragile ecosystem in the coffee growing regions. In this paper, an approach for Integrated Sustainable Production Systems in Coffee is being discussed with few case studies on the successful diversification attempts in the coffee plantations in India.

INTRODUCTION

Coffee cultivation forms the backbone of economy of many developing countries, where it provides employment opportunities to more than 20 million people in cultivation, processing and trade, while it's consumption is mostly concentrated in the developed countries. The global coffee prices exhibit 'Boom and Bust Cycle' associated with short supplies and surplus production scenarios. The violent fluctuations in supply dependent prices of coffee cause enormous setback to the economy of coffee producing countries.

In India, coffee cultivation occupies only 0.24% of cropped area, with a 0.10% share in GDP. It is cultivated in about 340,000 hectares with an annual production of 292,000 MT out of which arabica production forms 40% and the remaining 60% robusta. But the coffee industry occupies an important position in terms of foreign exchange earnings, employment opportunities in backward hilly areas and preservation of biodiversity in the ecologically sensitive forest hills. Coffee exports from the country occupy seventh position among total

agri-exports with annual foreign exchange earnings to the tune of Rs. 20 billions. The industry provides employment to nearly half a million people in cultivation, processing and trade. More significantly, coffee is cultivated in the ecologically sensitive Western and Eastern Ghats under the evergreen forest shade tree canopy, thus helping in preserving the ecosystem.

Like in many producing countries, coffee cultivation in India is predominantly a small grower enterprise with nearly 94% of 150,000 holdings coming under less than 4 hectare category. Coffee yields in the small holding sector are quite low because of lack of awareness about improved cultivation methods and due to traditional farming approaches.

In recent years, the economy of coffee plantations has suffered a setback owing to unattractive prices for coffee and escalation of cost of cultivation. With the emergence of new players with very high out put levels, competition from other beverages and almost stagnant consumption in major consuming countries, the price situation is not likely to improve in the near future. If the present low price situation persists for few more years, there is every possibility of growers indulging in indiscriminate timber extraction and abandoning of their plantations resulting in large-scale unemployment in backward hilly areas. This could be disastrous not only for the socio-economic condition of coffee growers but also for the fragile ecosystem in the coffee growing regions.

Besides, the awareness about the environment concerns and food safety is rapidly increasing worldwide. The importing countries are insisting that the produce must be free from physical and chemical contaminants, which cause health hazards. The Sanitary and Phytosanitary (SPS) measures contemplated under the WTO agreement are a step towards this direction. In another development, consumers in some countries are favouring eco-friendly food products. Thus, the growers need to respond to the changing scenario by resorting to alternate production systems to ward off market risks, to ensure sustainable returns from holdings and to produce coffees acceptable to the global consumers.

INTEGRATED SUSTAINABLE PRODUCTION SYSTEMS

Sustainable production is much debated topic these days. Whatever may be the definition, the integrated sustainable production system aims at optimum utilization of all available resources in the farming unit towards protecting the economy of farmer from market vagaries and to preserve the surrounding environment. Based on scientific information generated over the years on various aspects of coffee cultivation at the Central Coffee Research Institute, the major components that could be integrated towards the sustainable coffee production are discussed in this paper.

Soil management

As coffee is grown on the slopes of hill ranges with heavy rainfall, soil erosion is a serious problem especially in young clearings and exposed conditions. At the same time, soil moisture is a limiting factor for the growth of coffee during dry period. Several soil conservation measures like contour planting, terracing and cradle pits/renovation trenches have proved beneficial under Indian conditions. Similarly, cultural measures like scuffling, mulching and cradle pits were found to help in conservation of soil moisture (Raghuramulu et al., 1996).

Shade management

Right from the beginning coffee is being cultivated under a mixed shade canopy in India. The recommended practice is to go in for two-tier shade comprising of top canopy by permanent shade trees and a lower canopy by temporary shade plants like *Erythrina lithosperma* (Dadap). The trees belonging to great fig tree family and *Albizzia* sps. are most commonly preferred shade trees in India. Apart from these, the *Artocarpus integrifolia* (jack tree), *Cedrella toona* (gandhagarige), *Pterocarpus marsupium* (Honne), *Syzygium jambolana* (Nerala), *Terminalia bellarica* (tare tree), *Dalbergia latifolia* (Beete) and *Grevellia robusta* (Silver Oak) add value to the coffee plantations in terms of timber and fruit etc.

The benefits of shade are too many in coffee plantations. Shade trees provide the required micro-climate for growth of coffee and prevents crop exhaustion, recycle nutrients from sub-soil and improves fertility, check soil erosion, increase the infiltration rate, provides habitat for wide range of flora and fauna and fuel wood to the native people.

It is estimated that, in coffee plantations covered with a mixed shade canopy, shade trees contribute a leaf litter of nearly 10 MT per hectare per annum, which is equivalent to a nutrient recycling of 100 kg nitrogen, 35 kg phosphorus and 45 kg potassium and many micronutrients (Krishnamurthy Rao, 1989). In another study, coffee estate with well-maintained shade is reported to harbour nearly 70 different types of birds indicating eco-friendliness of coffee cultivation in shade (Raghuramulu, 2001). Apart from the above, shade plays a greater role in management of many pests and diseases of coffee.

Integrated Nutrition Management

Coffee soils are well drained and are usually subjected to leaching of nitrogen and potash. While because of their acidic nature, most of the applied phosphorus is rendered immobile by fixation. Integrated nutrition approach involving soil enrichment, recycling of organic matter and use of bio-fertilizers would enhance the use efficiency of nutrients, thus minimizing the dosage of fertilizers.

Soil enrichment

Successful crop production systems require a fertile soil for supporting normal growth of crops. The fertility of the soil can be improved by cultivation of green manure crops like *Crotolaria anagyrioides, Tephrosia candida,* cow pea, horse gram etc. in the early years of coffee plantation. The green manure crops should be sown during *Kharif* season (May-June) and ploughed back to the soil by the end of monsoon (September). The green manure crops contribute around 4-5 MT of organic matter per ha. apart from suppressing the weed growth in the early years (Raghuramulu and Naidu, 1995).

Recycling of organic matter

Apart from shade trees leaf litter, the coffee processing by-products are major source of organic matter in coffee estates. It is reported that, every tonne of clean coffee produced on the estate would result in one tonne of processing by-products like pulp/ cherry husk, which contain 14-15 kg nitrogen, 3.2-3.7 kg phosphorus, 29-37 kg potassium (Jayarama et al. 1996).

Use of biofertilizers

In coffee, use of *Biophos* (a biofertilizer containing phosphorus solubilising microorganisms) has been found to increase the yield by 39% with a saving of 30% of phosphorus dosage (Anonymous, 2000).

Integrated Plant Protection

In sustainable production, emphasis is on maintaining the health and vigour of the plants so as make them more tolerant to the pests and diseases. By adopting an integrated approach involving shade management, integrated nutrition, proper bush management and utilization of timely and appropriate protection measures, the crop losses due to pests and diseases could be minimized effectively. Some of the approaches for management of major pests and diseases of coffee are as follows:

Coffee leaf rust

Leaf rust is a major disease affecting arabica coffee in almost all the coffee growing countries. This disease takes an upper hand when the plant suffers exhaustion due to heavy bearing. In India, providing optimum shade and regular light pruning were found to help in preventing exhaustion of coffee bushes and thereby minimizing the incidence of leaf rust disease. Besides these, use of resistant/tolerant varieties and prophylactic sprays with 0.5% Bordeaux mixture were found to be adequate in minimizing the damage by leaf rust disease.

Black rot

This disease is endemic to high humidity areas and could be effectively checked by providing proper ventilation through pruning and sprays with 1% Bordeaux mixture.

Root diseases

The root diseases could be checked by application of liberal doses of organic composts and bio-suppression agents like *Trichoderma*.

White stem borer

This dreaded pest of arabica coffee is endemic to India and some Asian countries only. Incidence of this pest could be minimized by an integrated approach like providing optimum shade, regular tracing and uprooting of affected bushes, removal of loose bark on the stem surface and spraying the stem surface with lime solution.

Coffee berry borer

A common pest in almost all the countries, berry borer could be tackled by an integrated approach involving, phyto-sanitation, clean and timely harvesting, removal of off season berries, and use of timely sprays of entomo-pathogenic fungi *Beauveria bassiana* as well as need based application of chemicals.

Similarly, integrated management tools are available for the sucking pests like mealybugs and green scale etc. The problem of nematodes could be effectively tackled by use of resistant rootstocks.

Mixed Cropping

Mixed cropping in coffee plantations is in vogue right from the earliest days of coffee cultivation in India. However, it was only much later that Coffee Board generated systematic data on the compatible crops in coffee and economics of mixed cropping in coffee.

In a study on intercropping in young robusta coffee fields, it was observed that ginger and elephant foot yam can be profitably grown as intercrops during initial two years without any adverse effect on the growth of coffee. The net returns per rupee invested was 1.9-2.3 in case of ginger and 1.4 to 3.2 in case of elephant foot yam (Table 1) (Ramakrishnan Nair, 1976).

Table 1. Economics of intercropping in young coffee

Crop	Cost of cultivation/ac.(Rs.)	Net returns/ acre (Rs.)	C:B ratio
Ginger	4183	8768	1:2.1
Turmeric	3910	10,205	1:2.6
Yam	2911	6689	1:2.3

A long-term study was carriedout by Coffee Board under the ICO sponsored project “Agriculture Development of Small Coffee Farmers” during 1973-1987, with an objective of increasing the overall productivity of the unit by increased production of coffee and to convert farms into economically viable units by introducing other crops to supplement the overall income. In this study coffee was intercropped with banana, pepper and orange in different planting designs. The cost:benefit analysis over a period of 14 years has brought out very interesting findings. In both arabica and robusta, mixed cropping with pepper + orange + banana, would fetch almost equal returns as from that of their pure blocks. However, highest benefit was obtained when pepper is grown as an intercrop in arabica as well as robusta (Table 2) (Bheemaiah and Mehboob Shariff, 1989).

Table 2. Economics of mixed cropping in coffee

Crop combination	C:B ratio
Arabica monocrop	1:2.09
Arabica + Banana +Orange + Pepper	1:2.20
Arabica + Pepper	1:3.10
Robusta monocrop	1:1.08
Robusta + Banana +Orange + Pepper	1:1.32
Robusta + Pepper	1:2.29

In recent years, few enterprising small growers have successfully introduced vanilla in coffee estates. The humus rich soils, cool climates, partially shaded conditions in coffee are ideally suited for vanilla cultivation. In these estates vanilla vines are trained on to trellis all along the roads and paths. Each grownup vine produces 2-3 kg of fresh pods, which fetch an income of Rs. 600 to 800 per year (Personal communication). Vanilla cultivation requires regular manual labour to perform assisted pollination of flowers, thus restricting it’s cultivation to small family holdings.

Thus systematic mixed cropping in coffee estates is a good tool to supplement the income from unit holding.

Diversification

Diversification of farm activities is not a common practice in coffee plantations in India except in few smallholdings, which maintain few local breed cattle. Farm diversification encompasses allied activities like animal husbandry, sericulture, bee keeping etc. in coffee plantations apart from crop diversification. The main objective here is optimum utilization of resources within a farm so as to provide gainful employment to the families involved in coffee cultivation. This concept may not be suitable for large coffee holdings, which operate on hired labour but ideally suits for smallholdings where family labour is engaged in farming activities.

The concept of farm diversification in coffee is not studied in detail. However, few enterprising coffee growers have tried the new areas of diversification and achieved good success. Some of the promising activities in farm diversification include dairy farming, sericulture and bee keeping. The scope of introducing each one of these components in coffee plantations is discussed here under.

Dairy farming

Small and marginal coffee farmers do maintain few local cows, which are low yielding and non-remunerative. As an alternative, high yielding cross-bred cows can replace local cows. The cost of maintaining two cross-bred cows would come to Rs. 15,000 including the cost of cows, construction of shed, feed and other maintenance expenditure. On an average 24-30 litres of milk can be obtained from two cross-bred cows daily for about 280 days in a year (Korikanthimath, 1999). If the selling price of milk is taken as Rs. 7 per litre, the net returns from two milch cows would be Rs. 32,000 to 43,800 (Table 3), which is much more attractive.

Table 3. Economics of maintaining 2 crossbred milch cows

Cost of 2 crossbred milch cows	Rs.10,000
Cost of shed, feed etc.	Rs.5,000
Milk yield per cow	12-15 LPD
No. of milking days /year	280
Net income/ year at a selling rate of Rs.7/ litre	Rs.32,000 - 43,800

The fodder for cows can be grown on areas unsuitable for coffee like steep slopes and marginal areas with in an estate. The manure obtained from cow sheds forms a good source of organic manure on the estate. However, the major problem associated with dairy farming is marketing of milk, because of inadequate transport and chilling facilities in the remote plantation areas.

Sericulture

The climatic conditions in coffee tracts are ideally suited for mulberry cultivation and silkworm rearing, except during the monsoon months. Sericulture was introduced on an experimental scale in coffee areas as early as 1980 s, but it could not make any appreciable impact due to technology gap and support system. However, recent technologies make sericulture more economical and less labour dependent. The coffee areas are found suitable for production of silkworm seed due to certain inherent advantages like enforced crop holiday during monsoon months.

According to Dr. Dandini (2001), mulberry cultivation in coffee plantation areas can be practiced in the form of block plantations of low bush type with irrigation facilities and dwarf trees types as a shade to coffee. Few enterprising coffee growers have successfully adopted sericulture in their plantations in Coorg district of Karnataka with very attractive income upto Rs. 60,000 to 70,000 per acre, which is much higher than returns from coffee (Table 4).

Table 4. Economics of Sericulture in one acre mulberry plantation

Leaf yield from 1 acre of mulberry	8000-10000 kg/yr
No.of rearing crops/ year	3-4
Total cocoon yield/ year	300-400 kg
Cost of production/ year	Rs.36,000-48,200
Net income	Rs.54,000-72,000

Bee keeping

The diverse flora in the evergreen forests of coffee growing regions offers ideal condition for honey bees. Bees assist in pollination of coffee especially the cross-pollinated robusta coffee there by help in obtaining better fruit setting. Bee keeping can form an important subsidiary activity in coffee plantations especially in small holdings. Honey extraction and processing is quite simple and marketing of honey is not a constraint. It has been reported that a net profit of Rs.20,125 could be obtained from 4th year onwards by maintaining 25 bee hives (Korikanthimath, 1999).

CONCLUSION

Integrated production systems is a sustainable alternative to mono cropping of coffee. The sustainable approaches in coffee cultivation, mixed cropping and diversification would help to maximize the returns from unit land holding and at the same time increases the bio-diversity in coffee regions. However, all the components discussed above may not fit universally into the coffee system, because of differences in climatic conditions, holding sizes, availability of finances and manpower. Thus there is a need to study the integrated production concept in different regions so as to identify most suitable integrated package for each given region. Adaptive research through farmers participation will be a better approach for identifying more appropriate production systems in diverse conditions.

REFERENCES

Anonymous, 2000. Coffee Guide. Central Coffee Research Institute, Coffee Board Research Department, India, 2000.

Bheemaiah M.M. and Mehboob Shariff, 1989. Multiple cropping in coffee. Indian Coffee. **53** (12): 9-13.

Dandini S.B., 2001. Sericulture in coffee estates. A paper presented in the Workshop on "Integrated Sustainable farming Systems Approach in Kodagu District" held on 4th February 2001 at Madikeri, India.

Jayarama Krishnappa Naik C.S., Ananda Alwar R.P. and Naidu R., 1996. Biodegradable organic matter as potential source of coffee nutrition in coffee plantations in India. Indian Coffee. **60** (3): 3-8.

Korikanthimath, 1999. Alternate farming systems in high ranges of western ghats with reference to Coorg district in Karnataka – Part II. Indian Coffee. **63** (6): 7-11.

Krishnamurthy Rao, 1989. Nutrition management of plantation crops. J. of Plant. Crops. **16** (suppl): 403-411.

Raghuramulu Y. and Naidu R., 1995. Prospects of organic coffee production in India. Indian Coffee. **59** (12): 3-5.

Raghuramulu Y., Muralidhara H.R. and Manjunatha A.N., 1996. Soil and water conservation measures in coffee. In “Proc. of the National Seminar on Drought Management in Plantation Crops”, Pages II-43-48, 1996, Kottayam, India.

Raghuramulu Y., 2001. Benefits of growing coffee under shade in India. Indian Coffee. **65** (4): 11-12.

Ramakrishnan Nair T.V., 1976. Intercropping in young robusta coffee. Indian Coffee. **40** (2&3): 70-74.

Effets de quatre légumineuses forestières sur la production de *Coffea canephora*, var. Robusta au Togo

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SUMMARY

With the aim of identifying efficient forest leguminous plants capable of improving soil fertility and protecting the environment in coffee growing in Togo, four species were tested in 1995 at the CRA-F station at Tové. They were *Erythrophleum guineensis*, *Albizzia adianthifolia*, *Albizzia lebbeck* and *Samanea saman.* They were compared to an absolute control(without fertilizer) and to a reference control (usual fertilizer dosage). After 2 years of haverst 1998 and 1999 the experiment were suffered of the rigours of the drought of 1999-2000 and the plants had to be pruned. This situation permitted:

- a measurement of the aptitude of the species to protect the coffee plant in the event of a prolonged drought (mortality readings)
- an evaluation of the capacity of Leguminous plants to improve on the fertility of the soils (analysis of soil samples)
- a measurement of productivity (yields)

The results show clearly *Albizzia lebbeck* as the best Leguminous plant to

- protect coffee plants: 4,16% mortality rate
- support productivity: 1261 kg/ha of marketable coffee (compared to control with usual fertilizer dosage: 907 kg/ha)

RÉSUMÉ

Dans le but d'identifier des légumineuses forestières performantes amélioratrices de la fertilité des sols et protectrices de l'environnement en caféiculture togolaise, quatre espèces ont été mises en essai comparatif en 1995 à la station du CRA-F à Tové. Il s'agit de *Erythrophleum guineensis*, *Albizzia adianthifolia*, *Albizzia lebbeck* et *Samanea saman.* Elles sont comparées à
un témoin absolu (sans engrais) et à un témoin de référence (engrais à la dose vulgarisé). Après deux années s de récoltes 1998 et 1999, l'essai a subi la rigueur de la sécheresse 1999-2000 et a dû être recepé. Cette situation a permis de:

- mesurer l'aptitude de ces espèces à protéger le caféier en cas de sécheresse prolongée (relevés de mortalité),
- évaluer la capacité des légumineuses à améliorer la fertilité des sols (analyses des échantillons de sol).
- mesurer la production (rendements).

Les résultats dégagent *Albizzia lebbeck* comme meilleure légumineuse à

- protéger les caféiers: 4.16% de taux de mortalité

- soutenir la production: 1261 kg/ha de café marchand (par rapport au témoin engrais dose vulgarisée: 907 kg/ha)

INTRODUCTION

Le café se classe au 2ème rang des produits agricoles exportés au Togo. Sa culture occupe quarante mille familles et connaît trois périodes sur le plan historique.

La première période va de 1930 à 1975 et est caractérisée par un système extensif. La deuxième période (1975-1992) est celle de la promotion du système intensif. Ces deux périodes ont connu une stabilité relative (prix, production..) dans le temps.

La troisième période (1992 à nos jours) est constituée de bouleversements économique, social et climatique. Les bouleversements économique et social sont respectivement relatifs d'une part aux fluctuations des prix d'achat aux producteurs, à l'absence du crédit agricole, à la suppression des subventions et à l'augmentation des prix des intrants et d'autre part au relâchement de l'encadrement technique des producteurs et à l'introduction de nouvelles approches de la vulgarisation agricole. Tandis que les bouleversements climatiques concernent la diminution de la quantité et de la mauvaise répartition des pluies et l'allongement de la saison sèche. Face à cet ensemble de changements, le producteur n'a pas pu s'adapter. En conséquence on note un découragement des producteurs exprimé par: une diminution du nombre de désherbages, d'égourmandages, une insuffisance ou absence d'épandage d'engrais, un abandon des parcelles voire leur disparition surtout pour les caféières en plein soleil.

C'est pour identifier les meilleures légumineuses forestières aptes à protéger ces caféières contre les aléas climatiques devenus fréquents et sévères, et améliorer la fertilité des sols en caféiculture togolaise que quatre légumineuses forestières ont été associées aux caféiers dans un essai comparatif en 1995 à la station centrale du Centre de Recherche Agronomique de la zone Forestière à Tové.

En effet, en agriculture tropicale, des espèces végétales associées aux cultures permettent d'obtenir de bons rendements comparables à ceux des engrais chimiques ou même mieux (Chikasa, 1994). L'intégration dans les systèmes agricoles de légumineuses forestières permet de satisfaire les besoins des sols en plusieurs éléments tels que l'azote (Giffard, 1964; Aranguren et al., 1982; Wijewardene, 1984; Kang et al., 1985) le phosphore (Pierter et al., 1982; Giffard, 1964; Kang, 1989) le potassium et les autres bases échangeables (Giffard, 1964; Kretzchmar et al., 1991).

Au Togo, en milieu paysan, plusieurs légumineuses forestières existent naturellement dans les caféières et ont un effet bénéfique sur la production du café. Deux d'entre elles *Albizzia adianthifolia* et *Albizzia zygia* induisent des rendements moyens de1012 kg et 472 kg de café marchand à l'hectare (Koudjéga et Djiékpor, 1997).

La présente communication expose les premiers résultats de cet essai qui vise à élargir la connaissance des légumineuses en vue de leur association aux caféiers de manière à obtenir une production optimale et durable.

MATÉRIEL ET MÉTHODES

Matériel

Les Légumineuses forestières, étudiées sont les espèces suivantes: *Erythrophleum guineensis, Albizzia adianthifolia, Samanea saman, Albizzia lebbeck* .

Coffea canephora var Robusta est un mélange clonal (clones 149, 181, 182, 197, 375, 461).

- Echantillons de sols (6 échantillons)
- Données pluviométriques (1996-2000) de la station centrale du CRA-F à Tové.
- Matériel de laboratoire (pour analyse de sol)

Méthodes

Dispositif expérimental

Les traitements ont été disposés en randomisation totale. La surface de l'essai a été de 0.63 ha.

La parcelle élémentaire a couvert 262.5 m^2; elle a porté 48 caféiers dont 24 utiles et un pied de légumineuse.

L'essai a comparé 6 traitements répétés 4 fois. Ce sont:

T1 = *Erythrophleum guineensis*,
T2 = *Albizzia adianthifolia*,
T3 = *Samanea saman*,
T4 = *Albizzia lebbeck,*
T5 = NPK 20-10-10 à 400 kg/ha (témoin de référence)
T6 = Témoin (absolu).

Techniques culturales

Les caféiers et les légumineuses forestières sont plantés au même moment. Pour les caféiers la densité de plantation est de 1333 pieds/ha avec des écartements de 3,00 x 2.50 m. Tous les caféiers ont reçu de l'engrais les deux premières années. Les légumineuses sont mises à une densité de 38 arbres/ha et n'ont pas reçu de l'engrais.

Analyse de sol

Des échantillons composites (1 par traitement) ont été prélevés à une profondeur de 20 cm.

Ces échantillons de sol sont analysés au laboratoire de l'Institut togolais de la recherche agronomique à Lomé.

Les procédés suivants sont utilisés pour ces analyses:

L'azote total est déterminé par la méthode de Kjeldahl. Le dosage du phosphore total est fait par la méthode colorimétrique. La teneur en carbone total et en matière organique est obtenue par colorimétrie directe. Le phosphore assimilable est dosé par la méthode Olsen et Jackson modifiée. Le pH est déterminé électroniquement sur un pH-mètre à lecture directe.

Les observations ont porté sur la production des caféiers, le nombre de plants morts, la superficie au houppier des légumineuses et la teneur en différents éléments des échantillons de sol. Une analyse de la variance avec l'utilisation du test de Newmann et Keuls au seuil de 0,05 a servi à détecter les différences.

RÉSULTATS

L'analyse de la variance ne montre pas de différence significative entre les traitements pour ce qui concerne les observations relatives à la production 1998, la mortalité 1999-2000 et la surface au houppier des légumineuses. En revanche *Albizzia lebbeck* semble favoriser la production pour l'année 1999.

Tableau 1. Production 98 et 99, mortalité 1999-2000, superficie au houppier des légumineuses

Traitements	Erythrophleum guineensis	Albizzia adianthifolia	Samanea saman	Albizzia lebbeck	NPK	Témoin
Produtcion 98 (kg/ha)	496	199	270	407	346	361
Production 99 (kg/ha)	839 ab	612 b	847 ab	1261 a	907 ab	932 ab
Mortalité 99-2000 (%)	14.58	34.37	32.29	4.07	25	28
Superficie au houppier (m^2)	43.16	85.60	125.69	138.97		

DISCUSSION

L'analyse de la variance à un facteur n'indique pas de différence significative entre les traitements en 1ère année de production. Le traitement *Erythrophleum guineensis* a la moyenne de production la plus importante (363 kg/ha de café marchand; voir Tableau 1); ceci exprime la précocité de son effet. La 2ème année de production révèle une différence. On note 3 groupes a, ab et b. (Tableau 1). Le rendement moyen de la parcelle est 899,89 kg/ha de café marchand; les rendements exprimés par les témoins "absolu" et "engrais" sont similaires. En conditions favorables de sol et de pluies l'engrais n'a que peu d'effet. *Albizzia lebbeck* induit un rendement de 1261 kg/ha de café marchand. Le traitement NPK a un rendement (907 kg/ha de café marchand) légèrement supérieur aux rendements des 3 autres légumineuses. Ce qui confirme toujours l'intérêt de l'engrais dans certaines conditions.

Par ailleurs la pluviométrie moyenne, ces 4 dernières années (1966-1999) à la station de Tové est de 1537,5 mm répartie en 96 jours et la période sèche ne dépasse pas 2 mois. Les caféiers ont donc vécu dans des conditions normales. La saison sèche 1999-2000 est marquée par une période de 90 jours avec une seule journée de pluie d'une hauteur de 4 mm. De plus l'harmattan a été très rigoureux. Ces conditions défavorables ont entraîné le dessèchement et la mort de nombreux caféiers (Tableau 1) et c'est *Albizzia lebbeck* qui a fournit la meilleure protection aux caféiers et ceci grâce à sa bonne extension horizontale exprimée par la surface au houppier (Tableau 1).

Tableau 2. Teneur en différents éléments des échantillons de sol

Traitements	Erythrophleum guineensis	Albizzia adianthifolia	Samanea saman,	Albizzia lebbeck	NPK	Témoin
Matière organique %	2.917	2.299	2.00	3.482	2.410	2.910
Carbone %	1.692	1.334	1.163	2.024	1.328	1.688
Azote total %	0.112	0.112	0.095	0.168	0.112	0.146
C/N	15.10	11.91	12.21	12.04	11.85	11.59
Phosphore total ppm	231	254	206	263	276	356
Phosphore assimilable ppm	20	19	22	20	26	21
PA/PT %	8.65	7.48	10.67	7.60	9.42	5.89
Potassium meq/100g	0.270	0.301	0.204	0.286	0.332	0.469
Somme des Bases meq/100	1.334	1.365	1.050	1.491	1.008	1.683
CEC meq/100	8.33	8.33	8.33	11.10	2.77	5.55
Saturation	16.01	16.38	12.60	13.43	36.38	30.32
Ph eau	6.34	6.34	6.8	6.04	5.94	6.38

Le Tableau 2 montre la teneur du sol en éléments minéraux. Pour la matière organique, il vaut mieux raisonner sur le carbone (que l'on dose vraiment) plutôt que la matière organique que l'on exprime de façon approximative. Par rapport au carbone, *Albizzia lebbeck* se place en meilleure position. Les valeurs du rapport C/N de toutes les légumineuses sont supérieures à celles des témoins. Elles indiquent une meilleure activité de décomposition de la matière organique qui au contraire s'accumule au niveau du témoin.

Pour l'azote, les différences de production observées entre le témoin absolu et *Albizzia lebbeck* indiquent que les formes assimilables d'azote sont faibles au niveau du témoin malgré une importante teneur d'azote total. Il en est de même pour le phosphore où le rapport Phosphore assimilable/Phosphore total PA/PT le plus faible (Tableau 2).

Les valeurs de capacité d'échange cationique des légumineuses sont supérieures à celles des 2 témoins. La valeur de la C.E.C est une précieuse indication quant à la fertilité potentielle des sols. Pour le pH le traitement NPK présente un début d'acidification des sols: valeur de Ph le plus faible (Tableau 2).

CONCLUSION

L'essai dégage ***Albizzia lebbeck*** comme la plus efficace des 4 légumineuses testées. Par son développement horizontal, son apport de matière organique, sa facilité à rendre le phosphore assimilable, cette légumineuse protège bien les caféiers contre la sécheresse et l'harmattan qui sont les deux facteurs climatiques défavorables à la culture du café au Togo. Pour le moment

elle induit le meilleur rendement. Les autres légumineuses présentent des caractéristiques intéressantes. Pour des raisons de biodiversité il faut un mélange de légumineuses forestières dans les caféières; c'est pourquoi les 3 autres n'ont pas encore perdu d'intérêt.

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RÉFÉRENCES

Aranguren J., Eslalante G., Herrera R.,1982- Nitrogen cycle of tropical perrennial crops under shade trees. In plant and soil 67, Nigeria pp 13-50.

Chikasa P., 1994; «l'arbre miracle»: mieux que les produits chimiques, L'AGRICULTEUR AFRICAIN, janvier 1994. P.42.

Giffard P.L., 1964 - Les possibilités de reboisement en *Acacia albida* au Sénégal. Bois et forêts des tropiques. In Cahier: conservation n° 27, Rome pp. 28-44.

Kang B.T., Gimme H. et Wilson G.F., 1985. Alley cropping sequentially cropped maize and cowpea with Leuceana on sandy soil in south Nigeria , plant and soil , pp 267-277.

Kang B.T., 1989; Agriculture en couloir: gestion du sol et production. Guide de recherchede l'IITA, Ibadan , Nigeria 29 p.

Koudjéga T.et Djiékpor E. K., 1997; Influence de *Albizzia adinthifolia* sur la production de Coffea canaphora var Robusta au Togo. 17ème Colloque ASIC. Naïrobi

Kretzchmar Manu A. et Geiger Sc , 1991- The interactive effet of fertilizer and millet residues on P dynamique of sandy Nigerien soil. Agronomy abstracts. American society of agronomy 59 p.

Pieter B., Pieterl van D. et Anneke van der Veer, 1982 E, grais vert et autres formes d'améliorations dans les pays tropicaux, revue de la CTA, Wageningen, Pays - Bas 6 p.

Wijewardene, 1984- Conservation farming. The Sri Lanka Ministry of Agriculture, Colombo 208 p.

Réponse aux carences minérales de clones sélectionnés *Coffea canephora* Pierre

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SUMMARY

A trial comparing twelve high yielding *C. canephora* clones was established in Macenta (Guinea) on an acidic, unsaturated soil, with calcium/potash misbalance and high aluminium content. Another trial at Lola on a fertile sol is used as control. Assessments on mineral deficiencies symptoms, growth and yield were made and analysed. Leaf contents were analysed.

Highly significant differences are found for these criteria in the first trial. Some clones exhibit normal growth and yield without deficiency symptoms, whereas others show conspicuous deficiency symptoms and abnormal development. These are correlated with the proportion of K and Ca in the sum of cations as measured in the leaves. The role of Al in the absorption of P and of exchangeable bases is discussed.

Based on this variability between the clones, it seems possible to select clones for their ability to grow and yield in unsaturated soils with a high exchangeable Al content. Propositions are made for further investigation on this tolerance.

Key words: *Coffea canephora*, Coffee breeding, Guinea, mineral deficiency, soil aluminium, calcium, magnesium, potassium.

INTRODUCTION

Dans la sélection du caféier *Coffea canephora* Pierre mise en oeuvre depuis le début du siècle, l'adaptation du matériel végétal à des conditions de culture peu favorables n'était qu'un critère secondaire, l'installation de faisant généralement sur défriche de forêt. Cependant, depuis quelques décennies, la diminution des surfaces entraîne une rotation de plus en plus rapide des jachères; les propriétés physiques et chimiques des sols se dégradent. Les petits planteurs utilisent peu d'engrais. Les nouvelles caféières sont souvent plantées après jachère ou cultures vivrières, ou par replantation de vieilles caféières. La recherche de matériel végétal adapté à ces conditions de culture présente donc un intérêt certain.

De nombreux travaux ont été menés sur l'adaptation des plantes annuelles à des conditions d'alimentation minérale déficitaire ou limitée (*low input system*). On a souvent mis en évidence une variation intraspécifique dans l'efficacité de l'absorption et de l'utilisation des nutriments et dans la résistance à des niveaux élevés d'éléments toxiques (Clark, 1982; Dambroth et Bassam, 1990). Mais peu de travaux sur *C. canephora* sont à signaler sur ce sujet. On a observé des différences dans les teneurs et les équilibres en éléments minéraux dans les feuilles, en relation avec la productivité des clones, dans des sols déséquilibrés en bases échangeables (Colonna, 1962). Les différences de réponse de plusieurs clones à l'apport

d'engrais ont été étudiées (Snoeck, 1982). Plus récemment l'interaction génotype – environnement dans les conditions de Côte d'Ivoire a été étudiée (Moreau, 1983).

La présente étude a été menée en Guinée forestière. Elle présente les résultats d'un essai de clones de *C. canephora* potentiellement productifs, installé sur une jachère dégradée. On a tenté de mettre en évidence les différences d'adaptation des clones, de proposer quelques éléments d'explication ainsi que des recommandations pratiques et des voies de recherche pour le futur.

MATÉRIEL ET MÉTHODES

Matériel végétal

Ces clones ont été sélectionnés en Côte d'Ivoire à partir de 1962: sept – 107^{H}, 126^{H}, 182^{C}, 197^{G}, 461^{H}, 477^{C}, 503^{C} – sont vulgarisés en Côte d'Ivoire depuis 1975 et cinq – 512^{H}, 526^{H}, 529^{H}, 588^{H}, 594^{H} – ont été sélectionnés plus récemment. Les clones marqués ^{C}, ^{G} et ^{H}, appartiennent respectivement aux groupes "Congolais", "Guinéen" ou sont de type hybride (Berthaud, 1986). Leur potentiel de production, dans des sols bien pourvus en éléments minéraux, est de l'ordre de 2 tonnes de café marchand à l'hectare (Capot, 1977; Montagnon et al., 2000).

Situation des essais et dispositif expérimental

Les observations ont porté sur deux essais établis en mai 1990. L'essai principal est situé à Macenta sur un terrain précédemment exploité par des cultures répétées de plantes vivrières – riz, manioc – et colonisé par *Imperata cylindrica*. La pluviosité annuelle moyenne sur trente ans est de 2891 mm. La courbe de répartition est uni modale avec un maximum en août et une courte saison sèche en décembre et janvier. Les douze clones y sont testés.

Un deuxième essai est situé à Lola sur une jachère fertile à *Pennisetum purpureum* et sert de référence. La pluviosité est plus faible qu'à Macenta (1496 mm en 1992). Seuls six clones sont testés (107, 197, 126, 182, 461, 477).

Tableau 1. Fertilisation des essais (Urée, 17-17-17 et dolomie)

Macenta	**N**	**P**	**K**	**MgO**
1990	23 U (Urée)	+ 2 kg compost par pied		
1991	41 U	7 U	27 U	40 U
1992	Arrêt de la fertilisation			
Lola	**N**	**P**	**K**	**Mg**
1990	15 U (urée)			
1991	52 U (urée)	0	0	0
1992	Arrêt de la fertilisation			

Le dispositif est en blocs de Fisher à 8 répétitions d'une ligne de 6 pieds par clone. La densité est de 1667 pieds par hectare. Les caféiers sont conduits en croissance libre sur 3 tiges. Les deux essais sont installés en plein soleil. Les apports d'engrais ont été faits sur la base des analyses de sols, mais arrêtés en année 2 (Tableau 1).

Caractéristiques des sols (Tableau 2)

Dix prélèvements de terre par échantillon, pris entre 0 et 20 cm de profondeur, distribués sur l'ensemble de l'essai, ont été effectués en novembre 1992. Les analyses ont été réalisées au CIRAD (France). Les méthodes sont décrites sur www.cirad.fr/activities/labo_analyse

Tableau 2. Caractéristiques des sols des essais

			Macenta	Lola				Macenta	Lola
Argiles (%)			32,4	27,8	Mn ech ((meq/100 g)			0,01	0,08
Limons (%)			13,6	11	Al ech (meq/100 g)			1,72	0,14
Sables totaux (%)			54	61,2	H ech (meq/100 g)			0,11	0,04
C organique (%)			3,69	2	S (meq/100 g)			0,75	4,76
Mat organique (%)			6,35	3,44	K/S (%)	Normes	3-10	18,67	2,73
N total (‰)			1,96	1,78	Mg/K (%)		2-5	1,64	8,85
P total (ppm)			396	-	Ca/K (%)		3-14	2,28	26,31
P Olsen (ppm)			41,2	15,98	Mg/Ca (%)		0.2-0.8	0,78	0,34
K ech (meq/100 g)	Normes	0.4-0.6	0,14	3,12	CEC (meq/100 g)			3,12	5,05
Ca ech (meq/100 g)		6-7	0,32	23,48	S/CEC (%)			23,48	94,33
Mg ech (meq/100 g)		1-1.5	0,23	0,22	B soluble (ppm)			0,22	-
Na ech (meq/100 g)			0,05	0,06	Zn (DTPA) (ppm)			0,47	-
Normes : valeurs mesurées et normes calculées pour un taux d'argiles+limons de 46%					pHeau			4,96	5,55

Le sol de Macenta est ferrallitique désaturé (roche mère gneissique), argilo-sableux, et moyennement pourvu en matière organique. Sa teneur en phosphore assimilable est acceptable pour les caféiers (Snoeck et Snoeck, 1988). Les bases échangeables sont toutes très déficitaires. On note un déséquilibre en faveur du potassium, au détriment du calcium et du magnésium. Les teneurs en potassium et sodium sont probablement sous-estimées du fait de la méthode d'extraction. La teneur en aluminium échangeable est élevée (67% des cations échangeables). Malgré l'acidité du sol, la teneur en manganèse assimilable est très faible (0.01 meq/100 g). Les teneurs en bore soluble (0.22 ppm), et en zinc (0.47 ppm) sont faibles. Le sol de Lola est faiblement pourvu en matière organique, P et K, mais les teneurs en Ca et Mg sont optimales. Le taux d'Al échangeable est très faible. Les conditions sont donc proches de celles où ces clones ont été sélectionnés.

Echantillonnage et méthodes d'analyse minérale des feuilles

Les échantillons de feuilles et de sol ont été prélevés environ un mois avant la récolte: feuilles du troisième nœud sur les rameaux fructifères à mi-hauteur de l'arbre, dans l'interligne (Snoeck, 1984). Quatre prélèvements par clone ont été réalisés au hasard sur les 8 blocs, puis analysés séparément pour tous les éléments, sauf Mn et Al (échantillons regroupés). Les analyses ont été faites au Cirad (voir plus haut).

Observations des caféiers

Symptômes de carence

Lors du prélèvement foliaire (30 mois), un relevé détaillé des symptômes a été effectué. Les clones ont été classés en fonction des types de symptômes et de leur intensité (encadré).

Croissance et de la production

Une notation visuelle globale de chaque pied (0 = mort, 5 = croissance et développement très bons) et une mesure du diamètre au collet ont été effectuées. La production de cerises fraîches a été pesée par parcelle élémentaire durant trois ans.

Type 1: symptômes caractéristiques de la carence magnésienne: sur feuilles âgées, chlorose internervaire démarrant près de la nervure centrale avec brunissement.
Type 2: symptômes caractéristiques de la carence calcique: sur feuilles jeunes, chlorose marginale (avec parfois nécrose), la feuille prend une forme convexe (photo 1).
Type 3: symptômes complexes : raccourcissement des entre-nœuds souvent associé à la diminution de la surface, à la déformation et/ou à la chlorose des jeunes feuilles avec/sans chute des feuilles. Parfois port en entonnoir.

Niveau 0: pas de symptômes.
Niveau I: symptômes ponctuels légers; production et croissance normales.
Niveau II: symptômes modérés; croissance et production peu affectées.
Niveau III: troubles de croissance; la production est fortement affectée.
Niveau IV: croissance très fortement affectée entraînant un arrêt du développement, dans certains cas une impossibilité de fructification, et parfois la mort.

Analyses statistiques

Elles ont été faites avec les logiciels STATITCF (ITCF) et CSTAT (CIRAD) pour l'analyse de variance et les calculs de corrélation, et avec le logiciel OPEP (INRA) pour la détermination des corrélations environnementales et génétiques.

RÉSULTATS

Symptômes visuels de carence

A Macenta, des différences spectaculaires ont été observées entre les clones pour la croissance, l'expression de symptômes de carences minérales dès la première année et, plus tard, pour la production (photos 2 et 3). Dans l'essai de Lola, seul le clone 477 présentait quelques décolorations internervaires attribuables à une légère déficience magnésienne.

A Macenta, les premiers symptômes de carence sont apparus un an après plantation avec des différences très marquées selon les clones. Les premiers clones atteints ont été: 107, 182, 197, 503, 529. A 30 mois, 126 et 526 présentent peu ou pas de symptômes, alors que 197 et 107 présentent des symptômes de carences multiples. Les autres clones présentent des symptômes variables.

Tableau 3. Essai de Macenta. Type et intensité des symptômes de carence relevés à 30 mois

	Intensité croissante				
Clone	**Niveau 0**	**Niveau I**	**Niveau II**	**Niveau III**	**Niveau IV**
Pas de symptômes	126, 526				
Carence magnésienne		126, 526	477		
Carence calcique			529	182	
Carences multiples			477, 512, 529, 588	182, 503, 461, 594	107,197

Croissance et production (Tableau 4)

Pour la croissance et le développement, les clones 526, 477, 126, 529 et 512 sont significativement supérieurs aux autres clones. Pour la production, le classement est similaire et les écarts sont accentués; les clones 126, 526 et 588 se détachent nettement devant les autres clones. A Lola, les clones présentent des rendements équivalents, excepté les clones 477 et 182, du fait de mauvaises conditions de floraison (pluie insuffisante).

Composition minérale des feuilles (Tableau 5)

A Macenta, l'analyse montre un effet clonal hautement significatif pour la composition en K, Ca, Mg en valeur absolue et relative. La confrontation avec les niveaux critiques observés chez *C. canephora* et la proportion respective de ces cations met en évidence un déséquilibre en faveur de K, au détriment de Ca et surtout de Mg chez plusieurs clones, les plus affectés présentant des rapports K/S supérieur à 70% et Ca/S inférieur à 20%. Des symptômes de carence calco-magnésienne et des déséquilibres comparables entre éléments minéraux ont été observés sur des sols du même lieu (Loué, 1962). Enfin (obs. personnelle) cette relation se retrouve aussi au niveau des individus d'un même clone. Les teneurs en N, B et Zn sont normales à faibles, et les teneurs en P à la limite de la déficience malgré un sol bien pourvu en cet élément. Pour tous ces éléments, l'analyse de variance met en évidence des effets clonaux significatifs – sauf pour Zn, mais l'on n'observe pas de relation évidente entre ces teneurs et l'intensité des symptômes de carence. Les teneurs en Mn sont élevées malgré un sol faiblement pourvu. A Lola, les teneurs en P et Zn sont faibles, celles en N et B sont normales. Les niveaux et les équilibres K, Ca et Mg sont optimaux. Les teneurs en Mn sont élevées mais on cite des teneurs de 240 ppm sur *C. canephora* en Côte d'Ivoire sans symptômes de toxicité (Loué, 1960). Les teneurs en Al sont deux fois moins élevées qu'à Macenta.

Tableau 4. Diamètre au collet (en cm) à 2 ans et demi, rendement moyen (kg de café marchand/ha/an) et note de croissance et développement à 3 ans

Clone	Lola		Macenta					
	Rendement moyen sur 2 ans		Rendement moyen sur 2 ans		Diamètre au collet		Croissance et développement	
	Rendement	Groupe*	Rendement	Groupe*	cm	Groupe*	Note	Groupe*
107	1521	a	43	e	5.00	d e	1.48	e
126	2418	a	1808	a	6.23	b	4.01	a b
182	505	b	266	c d e	6.35	b	2.48	c d
197	1708	a	97	d e	4.61	e	1.40	e
461**	2045	a	363		6.12		1.64	
477	579	b	545	c d e	6.44	b	4.12	a b
503	-	-	303	c d e	5.78	b c	1.92	d e
512	-	-	666	c	5.91	b c	3.50	b
526	-	-	1458	a b	7.11	a	4.62	a
529	-	-	617	c d	6.05	b c	3.71	b
588	-	-	1159	b	6.04	b c	2.91	c
594	-	-	539	c d e	5.34	c d	2.28	c d
Moyenne	1462		682		5.90		2.95	
ETR	705,2		371		0.54		0.57	

Les valeurs suivies d'une même lettre ne sont pas significativement différentes au seuil de 5% par le test de Newmann et Keuls. Le clone 461 (Macenta) a été exclu de l'analyse de variance en raison d'un effectif insuffisant*

Corrélations entre la composition foliaire et les performances des clones de l'essai de Macenta (Tableau 6)

On observe une liaison forte entre les paramètres de croissance et de production et les teneurs en K et Ca. La carence magnésienne très importante ne semble pas avoir d'influence sur la production et de la croissance.

La composante génétique est prépondérante dans la corrélation entre la composition minérale en K, Ca et Mg et la note de croissance et de développement (Tableau 7). Elle est équivalente à la composante environnementale dans le cas de la production.

DISCUSSION ET CONCLUSIONS

Le comportement des clones de l'essai de Macenta s'explique par un environnement défavorable, mais aussi par la réaction de ces clones dans cet environnement . A l'origine des troubles observés, on peut citer la pauvreté en bases échangeables, leur déséquilibre en faveur de K, et la teneur élevée en Al échangeable du sol.

Une étude approfondie de la nutrition minérale des clones de l'essai de Macenta ne peut être envisagée ici compte tenu du caractère ponctuel des données recueillies et de l'absence d'un protocole orienté, dès la conception de l'essai, vers une problématique physiologique.

On a montré sur *C. canephora* que, sur des milieux nutritifs différents, la somme des éléments cationiques K+Ca+Mg était relativement stable mais que la valeur du rapport K/(Ca+Mg) était corrélée positivement avec la valeur du même rapport dans le milieu nutritif. La toxicité de Al serait surtout indirecte (Boyer, 1976). Il bloquerait l'assimilation de P au niveau des

racines et de Ca au niveau des membranes cellulaires, entraînant des signes divers de malnutrition. Il favoriserait l'absorption de Mn. Dans cet essai, l'action néfaste de Al parait donc confirmée par l'accumulation dans le végétal de K et de Al et par la faible teneur en Ca, Mg et P bien que P soit en quantité suffisante, et assimilable, dans le sol. La teneur élevée en Mn, malgré un sol très déficitaire, pourrait être expliquée par l'effet synergique de l'aluminium. En l'absence de données plus précises au cours du temps, on peut donc supposer que cette perturbation sévère des processus de croissance, pour les clones les plus sensibles, est due à des déficiences multiples en Ca, Mg, P – et peut-être en oligo-éléments – résultant de la pauvreté et du déséquilibre du sol, et de la teneur élevée en Al échangeable.

On constate une grande variabilité de comportement de ces clones dans des conditions d'alimentation déficitaire et/ou toxique. Dans des conditions d'alimentation normale (Côte d'Ivoire, Lola) ces clones sont équivalents.

Les clones qui arrivent à maintenir un équilibre interne entre les ions K, Ca – et dans une moindre mesure Mg – compatible avec le bon fonctionnement de la plante sont peu ou pas affectés dans leur production et leur croissance. Des carences sévères apparaissent, ici, pour un rapport K/S supérieur à 70%.

Enfin, les seuils de déficience et de toxicité peuvent fluctuer en fonction du génotype mais aussi être influencés par les teneurs en d'autres éléments ainsi que par les conditions environnementales (Bouharmont, communication personnelle).

Si l'analyse du sol permet de prédire le comportement moyen des caféiers, l'observation visuelle des symptômes de carence et l'analyse des feuilles peuvent être utiles, en particulier, teneurs et équilibres en K et Ca, qui semblent bien corrélées avec la croissance et la production.

Des études complémentaires seraient donc indispensables pour déterminer les composantes et le déterminisme de cette "tolérance". Sans exclure un effet dû au hasard, c'est dans les hybrides intergroupes qu'on trouve les génotypes les moins sensibles aux variations du milieu. Cette tolérance pourrait constituer un critère de sélection intéressant par les possibilités de développement offertes sur de vastes superficies en particulier en Amérique du Sud où le seul recours actuellement est l'application d'amendement calcique (Pavan et al., 1982). Les hybrides de clones en cours de confirmation en Côte d'Ivoire (Montagnon, 2000) offrent une plus large base de sélection.

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Tableau 5. Teneurs moyennes en éléments minéraux dans la troisième paire de feuilles selon les clones. Classement selon de la teneur relative en potassium croissante. Relation avec l'intensité des symptômes observés

Clone	N %	P %	K %	Ca %	Mg %	B ppm	Zn ppm	Mn ppm	Al ppm	S	K/S %	Ca/S %	Mg/S %	Intensité des symptômes
107	2,82	0,13	2,45	0,65	0,12	35	8,3	100,8	564,4	3,22	76	0,2	4,6	IV
107	2,5	0,12	1,49	1,75	0,42	72,45	6,93	187,2	-	3,66	41	0,5	0,1	-
126	2,63	0,11	1,8	1,17	0,2	38,9	11,3	136,8	502,6	3,17	57	27,7	8,4	0-I
126	2,36	0,12	1,18	2,47	0,44	94,05	7,13	272,9	246,1	4,09	29	-	-	-
182	2,32	0,11	2,3	0,58	0,18	19,8	13,2	67,2	463,8	3,06	75	12	7,4	III
182	1,88	0,09	1,93	1,61	0,31	62,42	8,4	237,7	378,1	3,85	50	-	-	-
197	2,53	0,1	2,4	0,97	0,1	40,7	10	157,8	587,7	3,47	69	18,7	3,9	IV
197	2,25	0,1	1,35	2,25	0,35	90,17	8,48	312,8	186,3	3,95	34	-	-	-
461	2,48	0,11	2,1	0,75	0,21	30,8	7,9	49	420	3,06	69	16,1	8,7	III
461	2,35	0,13	1,58	1,87	0,33	94,9	7,58	135,2	-	3,78	42	-	-	-
477	1,9	0,1	2,03	0,92	0,26	19,9	9,4	65,9	395	3,21	63	18,9	10,4	II
477	1,89	0,1	2,03	1,68	0,22	63,58	12,98	154,7	294,7	3,93	52	-	-	-
503	2,97	0,16	2,11	0,81	0,24	24	9,4	59,1	391,9	3,16	67	16,7	9,7	III
512	2,68	0,13	2,06	1,2	0,15	32,8	10,3	90,4	589	3,41	60	24,1	5,8	II
526	1,79	0,1	1,63	0,92	0,2	33,3	8,9	105,6	331,7	2,75	59	22,7	9,7	0-I
529	2,23	0,1	1,69	0,95	0,2	26,3	8,3	76,8	379,7	2,84	60	22,7	9,3	II
588	2,58	0,11	1,94	1,13	0,27	28,3	8,3	66,2	503,9	3,34	58	22,9	10,5	II
594	2,17	0,11	2,1	0,72	0,18	24,5	10,9	64,3	331,1	3	70	15,6	7,5	III
Niveaux critiques sur C. canephora[15]														
moyen-élevé	3,3	0,15	2,5	1,8	1,1	150	30	80						
bas-moyen	2,8	0,12	1,5	1,2	0,3	40	10	60						
déficient-bas	1,8	0,11	0,8	0,8	0,2	25	7	30						

Tableau 6. Macenta: Corrélation entre la composition minérale des feuilles, la production moyenne des deux premières années et la note de croissance et développement à 3 ans 1/2

	N	P	K	Ca	Mg	B	Zn
Production sur 2 ans	- 0.198	-0.259	**- 0.739**	**0.685**	*0.350*	*0.336*	-0.085
	NS	NS	**HS**	**HS**	*S*	*S*	NS
Croissance et Développement	**- 0.401**	-0.281	**- 0.690**	**0.599**	**0.454**	-0.003	-0.084
	HS	NS	**HS**	**HS**	**HS**	NS	NS

HS= significatif au seuil de 1%, S=significatif au seuil de 5%, NS= non significatif

Tableau 7. Corrélations génétique (G) et environnementale (E) entre K, Ca, Mg et leur teneurs relatives dans les feuilles, la production moyenne des deux premières années et la note de croissance et développement à 3 ans et demi

		K	Ca	Mg	K/S	Ca/S	Mg/S
Production sur deux ans	G	- 0.822	0.672	0.303	- 0.839	0.814	0.423
	E	- 0.642	0.748	0.425	- 0.740	0.811	0.499
Croissance et développement	G	- 0.916	0.662	0.563	- 0.928	0.810	0.667
	E	- 0.362	0.412	0.243	- 0.403	0.449	0.258

RÉFÉRENCES

Berthaud J. (1986) Les ressources génétiques pour l'amélioration des caféiers africains diploïdes. Evaluation de la richesse génétique des populations sylvestres et de ses mécanismes organisateurs. Conséquences pour l'application. Collection Travaux et Documents n° 188, ORSTOM (Paris), 379 p.

Boyer J. (1976) L'aluminium échangeable: incidences agronomiques, évaluation et correction de sa toxicité. Cahiers ORSTOM Série Pédologie, **14** (4): 259-269.

Capot J. (1977) L'amélioration du caféier Robusta en Côte d'Ivoire. Café Cacao Thé, **21** (4): 233-244.

Clark R.B. (1982) Plant response to mineral element toxicity and deficiency. In: Breeding Plants for Less Favorable Environments, Christiansen M.N., Lewis C. F. Eds., John Wiley & Sons (New-York), p. 71-142

Colonna J.-P. (1962) Différences dans la nutrition minérale et dans la production de clones adultes de *Coffea canephora* var. robusta. C. R. Acad. Agric. France, **48**: 615-628

Dambroth M., El Bassam N. (1990) Genotypic variation in plant productivity and consequences for breeding of « low-input cultivars ». In: Genetic Aspects of Plant Mineral Nutrition, El Bassam N., Dambroth M., Loughman B.C.Eds, Kluwer Academic Publishers, Dordrecht, The Netherlands, p.1-7.

Loué A. (1960) Nouvelles observations sur les oligo éléments dans la nutrition du caféier *C. canephora* P. Café Cacao Thé. **4** (3): 133-149.

Loué A. (1962) Etudes des carences et déficiences en potassium, calcium et magnésium chez le caféier robusta. IFCC Bulletin (Paris), **4**, 48 p.

Montagnon C., Cilas C., Leroy T., Yapo A., Charmetant P., 2000. Genotype-location interactions for *Coffea canephora* yield in the Ivory Coast. Agronomie **20**, 101-109, INRA, EDP Sciences

Montagnon C., 2000. Optimisation des gains génétiques dans le schéma de sélection récurrente réciproque de *C. canephora.*Thèse de doctorat, ENSA Montpellier France.

Moreau R. (1983) Evolution des sols sous différents modes de mise en culture en Côte d'Ivoire forestière et pré forestière. Cah. ORSTOM, sér. Pédol., **20** (4): 311-325.

Pavan M.A., Bingham F.T., Pratt P.F. (1982) Toxicity of aluminium to coffee in Ultisols and Oxisols amended with $CaCO_3$, $MgCO_3$, and $CaSO_4$,H_2O. Soil Sci. Soc. Am., **46**: 1201-1207.

Snoeck D., Snoeck J. (1988) Programme informatisé pour la détermination des besoins en engrais minéraux pour les caféiers arabica et robusta à partir des analyses de sol, Café Cacao Thé, **32** (3): 201-218.

Snoeck J. (1982) Méthodologie des recherches sur la fertilisation minérale du caféier robusta en Côte d'Ivoire. I. Influence du matériel végétal . In: 10e Colloque Scientifique International sur le Café, Bahia, ASIC (Paris), p. 467-476.

Snoeck J. (1984) Chap.14: Caféier. In: L'analyse végétale dans le contrôle de l'alimentation des plantes tempérées et tropicales. Martin-Prevel P., Gagnard J., Gautier P., Technique et Documentation, Lavoisier Ed. (Paris), 473-486.

Vers une identification des cafés-terroir au Honduras: Caractérisation physique, phytotechnique et biologique des caféières honduriennes

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SUMMARY

In the case of coffee, the intervention and location factors which must enter in the definition of the “terroirs” are not well-known. This study aimed to identify some of these factors and to use them in a characterisation of Honduran “terroirs”. For this purpose, a survey was conducted in six areas of Honduras with a total of 52 plots sampled. This survey allowed to study the environment, the crop management, the characteristics of the yield, and the quality of the beverage through an holistic approach. The data were analysed by a series of multivariate analysis. Our results show that the quality of the beverage is especially connected to the altitude of the plots, the rainfall, the soil acidity, the percentage of shade, the yield of the trees, and the bean-size. The most appreciated, aromatic and balanced beverages, are associated with the plantations located at high altitudes (1115 m on average), with a medium annual rainfall (1726 mm), a slightly acid and rich soil, a medium percentage of shade (48%), a high yield (464 fruit-bearing nodes per coffee-tree) and big beans (screen 17). These results allow to identify zones of quality coffee production in Honduras in the areas of El Paraíso, Comayagua, Olancho, and Marcala, but let also foresee the existence of good and bad years in relation with the rainfall, the yield and bean-size of the year into consideration.

INTRODUCTION

La hausse de la production mondiale de café et la consommation stagnante contribuent à maintenir les prix à des niveaux excessivement faibles. Dans ce marché saturé et difficile, l’avenir du café centraméricain réside dans son aptitude à se démarquer des autres cafés du monde par la production d’un café dont la qualité et la typicité seront reconnus. L’identification de cafés-terroir constitue donc un axe de recherche d’importance majeure pour la caféiculture centraméricaine.

La notion de terroir a évolué au cours de ces dernières années. On empruntera à J. Salette et al. (1998) les définitions qui suivent. La définition classique du terroir est basée sur une description du milieu physique. «Un terroir est un agro-écosystème caractérisé, doté d’une capacité à donner des produits particuliers auxquels il confère une originalité, un caractère propre.» Dans le cas du café, c’est cette définition qui est certainement la plus utilisée. Ce sont les cafés «d’origine» que l’on trouve dans les grandes surfaces. Le milieu physique n’est cependant pas le seul élément à prendre en compte dans la définition du terroir. L’intervention de l’homme en est un élément clé, car elle peut conduire à une valorisation plus ou moins réussie des potentialités du milieu physique et donc à des produits de qualités variées pour un même agro-écosystème. C’est la raison pour laquelle on considère aujourd’hui qu’un terroir est plus qu’une simple région de production. «Un terroir est un système d’interactions complexes entre un ensemble d’actions et de techniques conduites par des hommes, une

production agricole et un milieu physique à valoriser par un produit auquel il confère une originalité particulière.»

Dans le cas du café, les facteurs qui ont une influence sur la qualité sont mal connus. Les facteurs les plus souvent cités sont l'altitude, la qualité de la récolte et les traitements post-récolte (Barel et Jacquet, 1994). Parmi les facteurs d'intervention avant-récolte, les variétés cultivées (Carvalho et al., 1990; Guyot et al., 1996; Moschetto et al., 1996) et l'ombrage (Guyot et al., 1996; Fernández et Muschler, 1999) sont les principaux facteurs dont les effets sur la qualité sont avérés.

L'objet de cette étude est d'identifier les facteurs avant-récolte qui, parmi les facteurs de situation, d'intervention et de production, doivent être considérés dans la définition des terroirs. Cette étude s'appuie sur une caractérisation globale des plantations, tant du point de vue physique, que phytotechnique et biologique.

MATÉRIELS ET MÉTHODES

L'approche méthodologique choisie pour aborder cette étude multifactorielle est l'enquête. Celle-ci a été menée en 1997 sur un échantillon de 52 parcelles.

Localisation des parcelles et unités enquêtées

Les 52 parcelles échantillonnées proviennent des régions du lac de Yojoa, de Santa Bárbara, de Comayagua, de El Paraíso, de Olancho et de Marcala. Les coordonnées géographiques des parcelles ont été déterminées à l'aide d'un GPS (Global Positionning System) (Figure 1). L'unité enquêtée est une parcelle compacte d'environ 170 caféiers parmi lesquels cinq plants ont été identifiés pour mener à bien des observations particulières qui seront précisées ci-dessous.

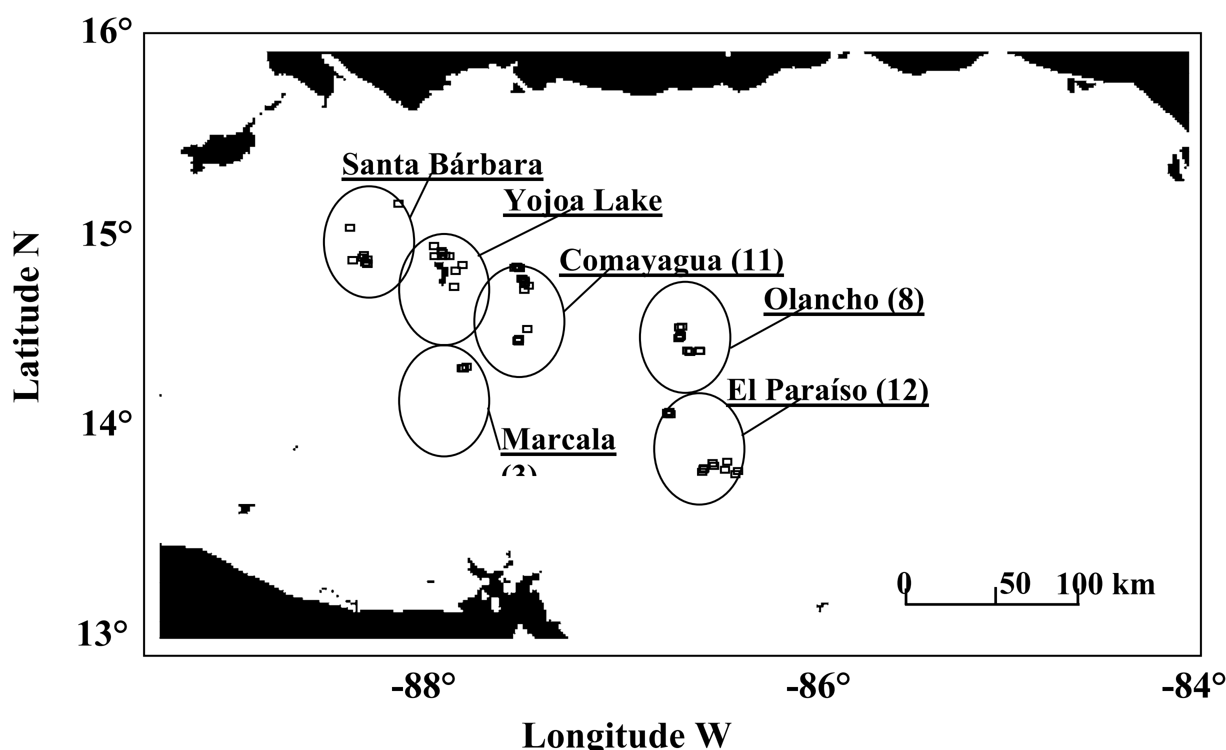

Figure 1. Plots localisation (squares) and their number per region (between parenthesis)

Climat

Le climat a été caractérisé par la pluviométrie annuelle, l'altitude et la latitude. Vingt-cinq pluviomètres ont été installés sur les parcelles en étude. Les relevés de certains pluviomètres ont permis de caractériser plusieurs parcelles à la fois en raison de leur proximité géographique.

Sol

Pour chaque parcelle, un échantillon composite de sol (10 sous-échantillons), prélevé peu avant le début de la saison des pluies, dans l'intervalle entre les lignes et à proximité des plants marqués, a été analysé. Le sol a fait l'objet d'une analyse de sa texture (pourcentages de sables et argiles) et de ses caractéristiques chimiques (pH, pourcentage de matière organique, teneurs en calcium, magnésium et aluminium)

Itinéraire technique

La variété plantée, le nombre de fertilisations et le nombre de pulvérisations fongicides et insecticides ont été documentés auprès du producteur. L'enquête ne concerne que des parcelles plantées de variétés de port nain, Catuaï ou type Caturra (Caturra, Pacas, Villasarchi). Le pourcentage d'ombrage a été évalué à l'aide d'un densiomètre sphérique (Lemmon, 1957). Cet appareil est constitué d'un miroir concave et quadrillé, qui orienté vers le haut, réfléchit le feuillage des arbres d'ombrage. Le quadrillage permet d'estimer la part de surface occupée par leur frondaison. Cette donnée a été déterminée au pied des caféiers marqués à deux reprises dans l'année: en début de la saison des pluies et pendant la récolte. Pour les analyses, la moyenne de ces deux valeurs a été utilisée.

Caractéristiques de production

Le nombre de nœuds fructifères a été compté sur les caféiers marqués. Sur chacun de ces caféiers, trois branches ont été identifiées. Le comptage du nombre de fruits et de feuilles jeunes sur ces branches a permis d'estimer la charge fruitière (nombre de fruits rapporté à la masse foliaire). La taille des plants a été mesurée.

Echantillonnage du café et traitement post-récolte

Sur chaque parcelle en étude, 45 kg de cerises fraîches, sans défaut, ont été prélevés au moment du pic de récolte. Les récoltes ont été réalisées sur les caféiers marqués, puis sur des caféiers proches des caféiers marqués, jusqu'à obtenir les 45 kg de café cerise. La manipulation des échantillons a été standardisée. Le dépulpage a eu lieu sur place, immédiatement après la cueillette. La fermentation a été réalisée dans des sacs de polypropylène. L'échantillon a été lavé dès que la durée de fermentation a été jugée suffisante (entre 24 et 48 heures suivant les cas). Le café a été mis à sécher sur des sacs de polypropylène propres, en patio, en évitant des températures excessives.

Granulométrie

Pour chaque échantillon de café, un sous-échantillon de 500 grains a été calibré à l'aide de neuf tamis d'ouvertures croissantes : de l'ouverture 12 à l'ouverture 20 (en $64^{ème}$ de pouce). Ce calibrage a permis de calculer la granulométrie moyenne de chaque échantillon.

Torréfaction

La torréfaction, de même durée pour tous les échantillons, a été réalisée dans les laboratoires du CIRAD. Un indicateur de la qualité de la torréfaction a été mesuré : la luminance. Celle-ci mesure l'intensité de la réflexion de la lumière, fonction de la couleur du grain torréfié. Un grain sous-torréfié de couleur claire aura une forte luminance alors qu'un grain sur-torréfié de couleur sombre aura une faible luminance. Six échantillons sous-torréfiés (luminance supérieure à 28 nits) ou sur-torréfiés (luminance inférieure à 24 nits) ont été exclus des analyses statistiques.

Tests organoleptiques

Les tests organoleptiques ont été réalisés dans le laboratoire d'analyses sensorielles du CIRAD. La qualité à la tasse des échantillons de café a été évaluée par six juges à travers sept critères sensoriels: l'arôme, le corps, l'acidité, l'amertume, l'astringence, le goût dit "vert" et la préférence. Ce dernier critère est une note de jugement d'ensemble au regard des critères précédents. La notation est basée sur une échelle de 0 à 5, où une note de 0 (respectivement 5) correspond à une absence (présence) totale du critère dans le café. Le goût "vert" est considéré comme un défaut. Dans les analyses, on s'intéressera uniquement à la note moyenne des différents critères attribuée par l'ensemble des juges aux différents échantillons.

Analyses statistiques

L'analyse statistique se décompose en deux étapes principales. La première étape consiste à créer des typologies de climats, de sols, d'itinéraires techniques, de caractéristiques de production (granulométrie incluse) et de qualités de cafés. Pour ce faire nous avons procédé en trois phases. (1) Les variables mesurées sont de nature hétérogène, quantitative pour certaines et qualitative pour d'autres. Les variables quantitatives ont été transformées en variables qualitatives pour permettre une analyse conjointe de l'information. (2) Puis, nous avons procédé à des analyses de correspondances multiples (ACM) pour chaque catégorie de variables. Cette analyse a abouti à la création d'axes indépendants entre eux sur lesquels les individus sont représentés. (3) Les coordonnées des individus sur les 3 premiers axes des ACM, ceux qui regroupent l'information la plus consistante, ont été utilisées dans une analyse de classification ascendante hiérarchisée (critère d'agrégation : moment d'ordre 2) qui a conduit à la création des typologies. Dans une seconde étape nous avons confronté ces typologies dans une analyse factorielle des correspondances simples à partir d'un tableau de contingences où les types de qualités sont en colonne et les autres typologies en ligne.

RÉSULTATS ET DISCUSSION

Typologies

Les Tableaux 1, 2, 3, 4 et 5 donnent respectivement les différents types de climats, de sols, de caractéristiques de production, d'itinéraires techniques, de qualités de café et la contribution des variables initiales dans ces typologies. La contribution des variables a été testée par une analyse de variance pour les variables quantitatives et un test du ι^2 pour les variables qualitatives. Dans ce dernier cas, la validité du test dépend de la taille des effectifs attendus. Gibbons (1976) considère qu'au plus 20% des effectifs théoriques peuvent être inférieurs à cinq. Dans les cas où les tests sont possibles, on vérifie que les variables initiales contribuent toutes significativement à la construction des typologies. Dans les autres cas, les valeurs du ι^2 élevées témoignent d'une forte liaison entre les variables et les typologies.

Table 1. Description of the different climates obtained by cluster analysis (average data or percentage of plots)

Climates	Latitude (°N)	Altitude (m)	Annual rainfall (mm)	Region [NC]	n
C1	14.9	849	3068	56% from Yojoa Lake 44% from Santa Bárbara	16
C2	14.5	1115	1726	25% from El Paraíso 25% from Comayagua 25% from Olancho 25% from Marcala	12
C3	13.8	867	1333	100% from El Paraíso	9
C4	14.6	954	1735	56% from Comayagua 44% from Olancho	9
All	14.5	942	2118		46
F	103.6**	10.4**	31.3**		
ι^2 (degrees of freedom = 15)				85.8[NT]	

***significant p<0.01; n: number of plots; NC: not considered in the cluster; NT: non tested (more than 20% of the expected values are less than 5)*

Table 2. Description of the different soils obtained by cluster analysis (average data)

Soils	Sand (% d.w.)	Clay (% d.w)	pH	Organic Matter (% d.w.)	(cmol $^{(+)}$/kg)			n
					Mg	Ca	Al	
S1	50.8	22.7	4.7	4.6	1.1	3.2	2.4	10
S2	54.9	18.3	5.0	3.9	2.6	9.5	0.3	15
S3	61.3	12.1	5.5	5.6	3.3	9.3	0.0	11
S4	35.6	31.4	5.5	2.6	4.8	15.3	0.1	10
All	51.3	20.6	5.2	4.2	2.9	9.4	1.3	46
F	11.3**	17.5**	11.2**	4.4*	8.3**	13.8**	32.8**	

**significant p<0.05; **significant p<0.0 ; n: number of plots*

Table 3. Description of the different production groups obtained by cluster analysis (average data)

Production groups	Height of the trees (m)	Producing nodes per tree	Fruits per leaf	Grain width (mm)	n
P1	2.38	384	4.8	6.5	13
P2	2.85	566	6.1	6.5	12
P3	2.07	464	6.2	6.8	9
P4	2.24	223	3.3	6.4	12
All	2.40	405	5.0	6.5	46
F	11.0**	11.9**	2.9*	5.2**	

**significant p<0.05; **significant p<0.01; n: number of plots*

Table 4. Description of the different management patterns obtained by cluster analysis (average data or percentage of plots)

Management Patterns	Shade (%)	Variety	Fertilisation	Pest management	n
M1	48	50% with Caturra type and 50% with Catuaï	75% once a year as a maximum and 25% twice a year at least	100% with no pest management practices	12
M2	45	56% with Catuaï and 44% with Caturra type	100% twice a year at least	100 % with at least one annual pesticide spray	9
M3	61	100% with Catuaï	56% once a year as a maximum and 44% twice a year at least	89% with no pest management practices and 11 % with at least one annual pesticide spray	9
M4	38	75% with Caturra type and 25% with Catuaï	50% once a year as a maximum and 50% twice a year at least	94% with no pest management practices and 6% with at least one annual pesticide spray	16
All	47	52% with Catuaï and 48% with Caturra type	52% twice a year at least and 48% once a year as a maximum	76% with no pest management practices and 24% with at least one annual pesticide spray	46
F	9.3**				
ι^2 (degrees of freedom = 3)		13.1^{NT}	12.1^{NT}	36.0^{NT}	

***significant p<0.01; n: number of plots; NT: non tested (more than 20% of the expected values are less than 5)*

Table 5. Description of the different quality groups obtained by cluster analysis (average data)

Quality groups	Aroma (0 to 5)	Body (0 to 5)	Acidity (0 to 5)	Bitterness (0 to 5)	Astringency (0 to 5)	Grassy taste (0 to 5)	Preference^{NC} (0 to 5)	n
Q1	2.7	3.0	1.8	3.3	2.7	1.9	2.4	7
Q2	3.1	2.5	2.7	2.5	2.3	0.7	3.2	22
Q3	3.3	2.9	2.4	3.0	2.2	0.4	3.3	9
Q4	3.4	2.6	2.7	2.7	2.1	0.1	3.4	8
All	3.1	2.7	2.5	2.8	2.3	0.7	3.2	46
F	3.0*	8.1**	7.7**	18.0**	7.4**	19.7**	5.2**	

**significant p<0.05; **significant p<0.01; n: number of plots; NC: not considered in the cluster*

Analyse factorielle des correspondances simples

La Figure 2, est la représentation graphique des deux premiers axes de l'analyse factorielle des correspondances simples. Elle regroupe 84% de l'information. Seuls les groupes dont la représentation est bonne (supérieure à 50% sur les deux axes) sont portés sur la figure. Les différents groupes de climats, de sols, d'itinéraires techniques, de caractéristiques de productions sont d'autant plus liés aux divers types de cafés qu'ils sont proches sur la représentation graphique. C'est ainsi qu'on peut dire que Q1 est plutôt associé à C4, S3, M4, P2, que Q2 est associé à C1, C3, S4, M2, P1 et Q4 à C2, S2, M1, P3. La Figure 3 est la représentation graphique des axes 1 et 3 de l'analyse factorielle des correspondances simples. Elle regroupe 75% de l'information. Comme dans le cas précédent, seuls les groupes dont la représentation est supérieure à 50 % sur les deux axes sont portés sur la figure. On observe que Q1 est plutôt associé à C1, S1, P2, que Q3 est associé à C3, S3, M2, P1, P4 et Q4 à C2, S2, M1, P3.

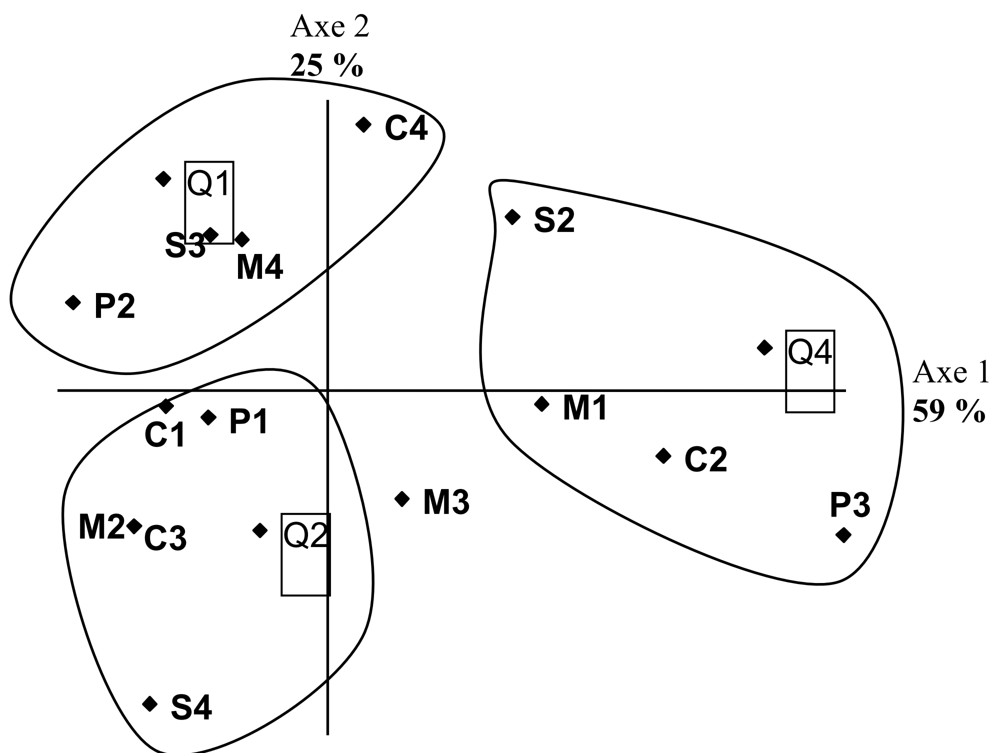

Figure 2. Factorial Correspondence Analysis (axes 1 and 2). For the signification of the symbols see Tables, 1, 2, 3, 4 and 5

Il est donc non seulement possible de trouver des liens entre la qualité et des caractéristiques plutôt régionales comme le climat et le sol mais aussi entre la qualité et des caractéristiques spécifiques de chaque parcelle comme l'itinéraire technique et les caractéristiques de production. Cela confirme d'une part l'importance des facteurs d'intervention avant-récolte et de production sur la qualité des cafés et d'autre part la nécessité de les considérer dans la définition des terroirs.

Les relations observées sur les Figures 2 et 3 peuvent être détaillées pour deux types de cafés, Q1 et Q4. Q1 regroupe les cafés les moins prisés, avec une note de préférence de 2,4 et Q4 correspond, au contraire, aux boissons les plus appréciées avec une note de 3,4 (Tableau 5). Q1 est constitué de boissons peu aromatiques et déséquilibrées. Les notes d'amertume, de corps et d'astringence sont en effet très supérieures à la note d'acidité. Le goût vert est très

prononcé (Tableau 5). A l'inverse, Q4 est constitué de boissons plus aromatiques et équilibrées. La note d'acidité est équivalente aux notes de corps et d'amertume. Les boissons de Q4 ne présentent pas de goût vert (Tableau 5).

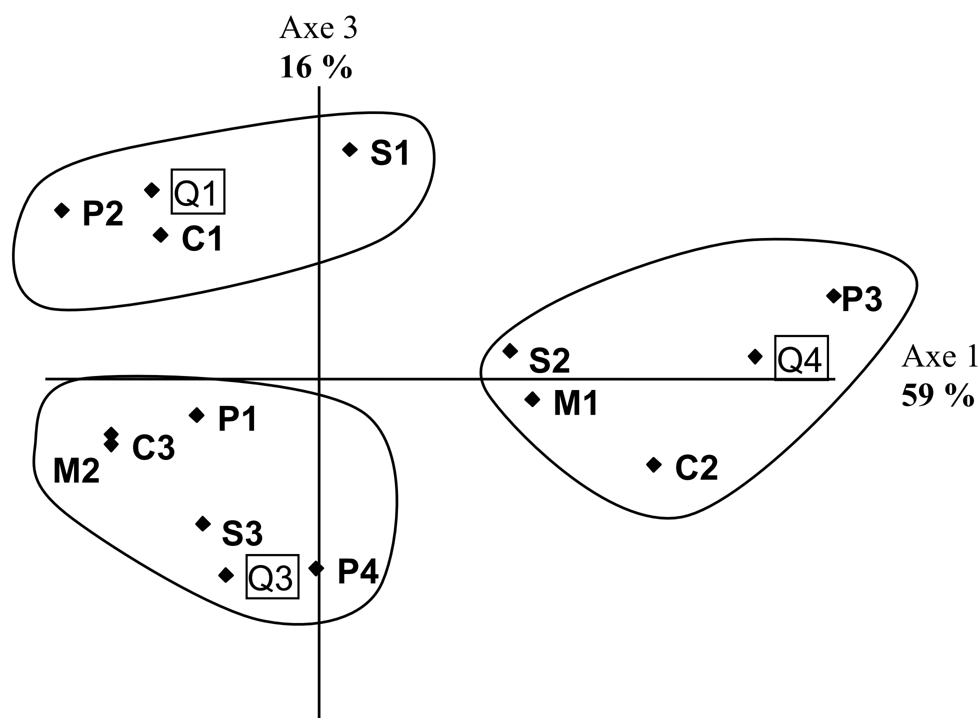

Figure 3. Factorial Correspondence Analysis (axes 1 and 3). For the signification of the symbols see Tables, 1, 2, 3, 4 and 5

Nos analyses ont permis d'associer à chacun des deux types de cafés au moins un climat. Pour Q1, il s'agit de C1 et C4 et pour Q4, il s'agit de C2. C2 correspond à des plantations d'altitude (1115 m) soumises à une pluviométrie moyenne (1726 mm sur l'année) (Tableau 1). Une diminution de l'altitude (C1,C4, Tableau 1) associée éventuellement à une augmentation de la pluviométrie (C1, Tableau 1) conduisent à une forte diminution de la qualité. Cette dernière relation, qui à notre connaissance est nouvelle, permet d'envisager l'existence de millésimes en relation avec la pluviométrie de l'année de production.

En ce qui concerne les sols, Q4 est bien lié à S2 et Q1 à S1 et S3. S2 regroupe des sols idéaux pour le caféier. Il s'agit de sols à texture adéquate, légèrement acides (pH de 5,0) et riches en bases (Tableau 2). Dès que les caractéristiques du sol s'éloignent de celles du sol idéal, la qualité du café diminue. On observe ainsi qu'une acidité trop prononcée, de faibles contenus en Mg et Ca, et des excès en Al (S1, Tableau 2) sont particulièrement nuisibles à la qualité du café. Ces résultats constituent une des premières illustrations des relations entre le sol et la qualité du café.

Pour ce qui est des caractéristiques de production, Q4 est associé à P3 alors que Q1 est lié à P2. Les parcelles de P2 ont des productions excessives de 566 nœuds fructifères par plant en moyenne. Les grains de 6,5 mm de largeur sont retenus par le crible 16 (Tableau 3). Une diminution de la production à 464 nœuds fructifères par plant, production qui reste encore élevée, associée à une augmentation de la granulométrie à 6,8 mm de largeur (crible 17), sont suffisantes pour améliorer la qualité du café (P3, Tableau 3). La relation entre excès de production et qualité du café que nous venons de mettre en évidence est nouvelle. Encore une

fois, on peut penser à l'existence de millésimes, mais cette fois-ci en rapport avec la production de l'année.

Pour l'itinéraire technique, Q4 est bien lié à M1 alors que Q1 est associé à M4. M1 correspond à des parcelles avec un ombrage moyen (48%), plantées de variétés type Caturra ou Catuaï et en général modérément fertilisées (Tableau 4). En revanche, M4 est constitué des parcelles qui ont le pourcentage d'ombrage le plus faible (38%). Il s'agit là d'une des rares démonstrations de l'effet bénéfique d'un ombrage modéré sur la qualité. Ces résultats confirment ceux de Guyot et al. (1996) et Fernández et Muschler (1999). Par ailleurs, les parcelles regroupées dans M4 sont plantées principalement de Caturra et, pour la moitié d'entre elles, sont abondamment fertilisées (Tableau 4).

CONCLUSION

Pour conclure, notre étude à permis de montrer que la qualité du café dépend aussi bien de caractéristiques d'ordre régional que d'attributs spécifiques des plantations. Parmi les caractéristiques importantes qu'il faudrait considérer dans la définition des terroirs, il y a l'altitude, la pluviométrie, l'acidité du sol, l'ombrage, la fertilisation, la variété plantée, la production et la granulométrie. Ces résultats permettent d'identifier des zones potentielles de production de café de qualité au Honduras, dans les régions de Comayagua, Olancho, Marcala et El Paraíso. Ils constituent aussi une mise en garde quant aux effets de la caféiculture intensifiée sur la qualité. Réduction de l'ombrage, fertilisation accrue, augmentation des productions d'une façon excessive, acidification des sols sont, en effet, des éléments distinctifs de la caféiculture intensifiée. Or, ces caractéristiques, d'après nos travaux, nuiraient à la qualité. Enfin, nos résultats montrent que la qualité peut varier dans le temps: on peut envisager qu'il existe des millésimes en relation notamment avec la pluviométrie, la production et la granulométrie de l'année considérée.

RÉFÉRENCES

Barel M., Jacquet M., 1994. La qualité du café: ses causes, sa détermination. Plantations, Recherche, Développement. **1** (1): 5-13.

Carvalho A., Alves Teixeira A., Fazuoli L.C., Guerreiro Filho O., 1990. Qualidade da bebida em espécies e populaçoes derivadas de híbridos interespecíficos de *Coffea.* Bragantia. **49** (2): 281-290.

Fernandez C.E., Muschler R.G., 1999. Aspectos de la sostenibilidad de los sistemas de cultivo de café en América Central. In: Desafíos de la caficultura en Centroamérica, B. Bertrand et B. Rapidel ed., Costa Rica, CIRAD, IICA, p. 69-96.

Gibbons J.D., 1976. Non parametric methods for quantitative analysis. New York, USA, Holt, Rinehart and Winston, 463 p.

Guyot B., Gueule D., Manez J.C., Perriot J..J., Giron J., Vilain L., 1996. Influence de l'altitude et de l'ombrage sur la qualité des cafés Arabica. Plantations, Recherche, Développement. **3** (4): 272-283.

Lemmon P.E., 1957. A new instrument for measuring forest overstory density. Journal of forestry. **55** (9): 667-668.

Moschetto D., Montagnon C., Guyot B., Perriot J.J., Leroy T., Eskes A.B., 1996. Studies on the effect of genotype on cup quality of *Coffea canephora*. Tropical Science. **36**: 18-31.

Salette J., Asselin C., Morlat R., 1998. Le lien du terroir au produit: analyse du système terroir-vigne-vin ; possibilité d'application à d'autres produits. Sciences des Aliments. **18**: 251-265.

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The Impact of ISO 14001 Certification on Cost, Quality and Management Behaviour

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The implication of the ISO 14001 certification on environmental management system goes beyond any ecological approach. It is a new way to produce coffee with positive consequences on quality and management behavior. Processes incorporated to assure ecological control had a dramatic effect on human attitude in the farm and beyond its frontiers. Eco-training programs changed agri-technicians' point of view around the region.

The ISO 14001 procedure increases cost. To compensate it, farming techniques and processing had to be enhanced as well marketing strategy to reward Eco-value.

The quality of beans improved as a consequence of some strict controls and the Eco-philosophy introduced by the Environmental Management System. Protecting the forests and its wild life, it was possible to recuperate the beauty of the savannas lost long time ago.

In summary, the ISO 14001 approach can be the best alternative to protect the ecosystem by controlling environmental impact without destroying productivity.

CHAPTER ONE

Environmental responsibility

Environmental impact reduction
Soil, water and botanic preservation
Biodiversity conservation
Native and fruit species reforestation
Rivers and waterfalls control

CHAPTER TWO

Social responsibility

Rural workers valorization
Labor laws attendance
Employees training sessions – more than 4.800 hours
Health care and workers protection
Children education

CHAPTER THREE

Community responsibility

Environmental classes at public schools
Environmental workshops on the farm
Quality on coffee workshops

Partners with good practices development
Ecological tours at farm's footpaths

CHAPTER FOUR

Environmental protection strategy

EAP – Environmental Adequacy Program – ESALQ USP
ISO 14001 certification
DPaschoal Foundation – more than 6 million books distributed including "Chico Silva - I Seed Coffee, I Harvest Magic"

CHAPTER FIVE

ISO 14001 and ESALQ-USP- AEP

Beginning of project 1996 - Environmental conscientiousness.
Residues management infrastructure and natural resources preservation.
Emissions control and Recycling
Certification in February, 2000.

CHAPTER SIX

Environmental and Social Responsibility Policy

"Daterra recognizes the potential impact that could be caused by coffee plantation and is engaged on actions focusing sustained production balancing economic development and environmental preservation. Continuous improvement on legislation attendance, education and employees environmental conscience, and interaction with communities focused on environmental preservation, is our lifework."

The Effect of Established Shade Trees on the Yield of Arabica Coffee in Two Planting Patterns

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SUMMARY

Arabica coffee is a shade-loving plant, which grows wild under three or four canopy strata of different forest tree species in its natural habitat in the south-western Ethiopia. It is also extensively cultivated in small gardens and large scale plantations under variety of shade trees in the country. In order to determine the effects of shade on coffee productivity, 13 known and widely used shade tree species were collected and established in strip blocks at Jima Research Center some 25 years ago. Selected coffee trees of arabica coffee were planted in two patterns (intercropped within and strip planted between the shade tree plots). Coffee yield differences due to shade trees and planting patterns were highly significant, where striped plots out yielded those intercropped with shades. Among the shade trees, *Millettia ferruginea, Acacia abyssinica, Albizia sp., Erythrina abyssinica, Calpurnea subdecondra* and *Cordia africana* resulted in significantly higher coffee yields. These shade trees also produced higher amount of litter fall (mulch), and most are leguminous with feathery leaves, adequate canopy coverage and moderate light interception (26-60%). It is, therefore, concluded that productivity of coffee stands can be more improved by growing in strips between such prominent shade trees or by inter cropping with/planting under/ the shades as practiced in the traditional production systems in the country.

INTRODUCTION

Coffee (*Coffea arabica* L.) is shade-loving plant, which is naturally growing as an under-story shrub in its original ecology in the tropical high rain forests of south and south-western Ethiopia (Friend, 1984; Cambrony, 1992; Paulos and Tesfaye, 2000). Besides; its wild and semi-domesticated phases in the complex natural forests of the country, the crop is extensively cultivated in traditionally managed gardens and in modern plantations under a variety of shade trees (Tesfaye, 1995; Yacob et al., 1996; Paulos and Tesfaye, 2000; Workafes and Kassu, 2000).

In most cases, productivity of the crop is very low, probably because of excessive or inadequate shading the trees which form the upper canopy layers (Friend, 1984; Venkata Ramanan and Govindappa, 1987; Yacob et al., 1996). The irregular pattern of growing coffee with shade trees is also responsible for such unregulated shade/light levels in most coffee production systems in the country (Workafes and Kassu, 2000).

This experiment was, therefore, conducted with the objectives of determining the optimum pattern of planting coffee with various known shades, and identifying ideal shade trees that improve yield of the crop.

MATERIALS AND METHOD

Thirteen known secondary forest shade tree species (*Millettia ferruginea*, four *Albizia* spp., *Acacia abyssinica*, two *Erythrina* spp., *Calpurnea subdecondera*, *Leucaena lecosyphylla, Cordia africana, Tephrosia vogellii* and *Gravillea robusta*) were collected and established in systematic strip blocks at Jima Research Center (7046'N, 36°0'E, 1753 m.a.s.l.) of EARO, Ethiopia, some 25 years ago. High yielding and disease resistant plants of Arabica coffee were intercropped.

With individual shade trees and planted in strips between two shade tree plots. The experiment was conducted in split plot design in systematic block arrangement with four replications, where the systematic strip plots of shade trees and the two planting patterns (intercropping and strip planting) constituted the main and sub plot treatments, respectively.

Coffee yield response to the treatments and percent light interception, seasonal litter fall and mean canopy diameter of some prominent shade trees were measured for eight consecutive years until 1997. Data was analysed following the standard procedure (ANOVA) for split plot design.

RESULTS AND DISCUSSION

Coffee yield increased significantly when intercropped with *Millettia ferruginea, albizia* sp., *Acacia abyssinica, Erythrina abyssinica* and *Calpurnea subdecondra,* followed by *Leucaena lecosyphylla, Cordia africana* and T*ephrosia volgellii*. However, plots under *Millettia* sp. and *Albizia* sp. out yielded those maintained under *Acacia* sp., *Erythrina* sp. and *Calurnea* sp. On the other hand, some of the *Albizia* sp. *Erythrina indica* and *Gravillea robusta* significantly depressed coffee yields (Table 1).

Table 1. Mean yield of coffee planted under established shade trees in intercropping pattern

Shade tree species		Clean coffee yield (kg ha^{-1})
1.	Millettia ferruginea	1809 a
2.	Albizia maronguensis	1580 b
3.	Albizia tanganyka	1521 b
4.	Albizia schimperiana	916 ef
5.	Albizia gunifera	724 f
6.	Acacia abyssinica	1534 b
7.	Erythrina abyssinica	1485 bc
8.	Erythrina indica	1088 de
9.	Calpurnea subdecodra	1467 bc
10.	Leucaena lecosyphylla	1216 cd
11.	Cordia africana	1204 cd
12.	Tephrosia vogellii	1184 cd
13.	Gravillea robusta	395 g
Mean		1240.23

Figures followed by same letter within the column are not significantly different at P=0.05

In contrast, mean yields of the coffee plots striped between two shade trees were higher than those intercropped under individual shades. Among the variety of shade tree stripings, a

combination of two of the known types (*Millettia* sp, *Albizia* sp., *Acacia* sp., *leucaena* sp., and *Calpurnea* sp.) resulted in significantly higher yields of the striped coffee plots (Table 2).

Table 2. Mean yield of coffee planted in strips between two shade tree plots

Shade tree striped	Clean coffee yield (kg ha^{-1})
1 Millettia + Albizia	2158 a
2 Acacia + Leucaena	1896 b
3 Calpurnea + Acacia	1693 c
4 Millettia + Gravillea	1343 d
5 Albizia + Acacia	1255 de
6 Erythrina + Tephrosia	1136 def
7 Tephrosia + Millettia	968 f
Mean	1492.71

Figures followed by same letter within the column are not significantly different at P=0.05

Seasonal litter fall of the shade trees also varied. Higher rates of defoliation were recorded for *Albizia tanganyka*, *Cordia africana*, and *Millettia ferruginea*, followed by *Acacia abyssinica* and *Erythrina abyssinica*. Litter fall of some of the *albizia* spp., *Erythrina indica* and, *Calpurnea subdecondra* was lower. On the other hand, mean canopy diameter of most of the shade trees ranged between 16x16m and 20x20m, except for *Millettia ferruginea* (8x8 m) and *Calpurnea subdecondra* (6x6m). Percent light interception by the shade trees also varied from species to species. The highest level of light intensity has been intercepted by *Erythrina indica* (60%), while the lowest by *Erythrina abyssinica* (19%). Most of the known shade trees including *Millettia ferruginea, Albizia sp., Acacia abyssinica* and *Capurnea subdecondra* intercepted moderate light intensity, ranging between 26% and 50% (Table 3).

Table 3. Some characteristics (seasonal defoliation, mean canopy diameter (CD) and light interception (LI)) of prominent coffee shade trees

Shade tree species	Litter fall (kg ha^{-1} annum^{-1})	Mean CD (m)	% LI
Millettia ferruginea	4271.34	8 X 8	40
Albizia schimperiana	1022.33	20 X 20	29
Albizia gunifera	4751.33	18 X 18	32
Albizia tanganyka	1240.00	18 X 18	26
Erytrina abyssinica	1549.67	16 X 16	19
Erythrina indica	1293.67	18 X 18	60
Acacia abyssinica	2167.00	20 X 20	30
Calpurnea subdecodra	452.33	6 X 6	50
Cordia africana	4511.67	16 X 16	36
Mean	2362.15	15.6 X 15.6	35.78

These results confirm earlier findings, which indicate that growth and productivity of coffee plants is more improved under moderate shades, but significantly lower in full sun or under very low light intensity (Soto, 1986; Guridi et al., 1988; Napoles et al., 1990; Yacob et al., 1996). Increases in the yield of coffee plots both intercropped with (Table 1) and planted in strips between the shade trees (Table 2) could be attributed to higher rate of net photosynthesis, as photosynthetic rate is low in C_3 crops like coffee when grown under excessive light (with no shade) (Soto, 1986; Cambrony, 1992) or under deeply shaded

environments, but it is enhanced under moderate light regimes (25-75%) (Friend, 1984; Venkataramanan and Govindappa, 1987; Guridi et al., 1988).

In the present study, seasonal defoliation of the shade trees due to changes in climatic factors and senescence of the leaves resulted in considerable amount of litter fall or mulch (Table 3), which could be used as organic matter to improve soil fertility upon decomposition. It has been reported that, besides moderating light intensity and improving soil fertility, shades also improve soil moisture status and relative plant water content by moderating soil and canopy temperature and decreasing the rate of evapotranspiration, all of which sustain productivity and improve coffee yields (Friend, 1984; Venkataramanan and Govindappa, 1987; Napoles et al., 1990; Tesfaye, 1995).

In general, most shade trees in this experiment are leguminous (except *Gravillea robusta* and *Cordia africana*) and have feathery leaves (except *Erythrina* sp.) with adequate canopy coverage and moderate light interception (Table 3). Since these are desirable characters of a shade tree, it is concluded that productivity of coffee stands could be improved by strip planting or intercropping with shade trees such as *Millettia ferruginea, Albizia tanganyka* and *A. maronguensis, Acacia abysssinica, Erythrina abyssinica* and *Calpurnea subdecondra*. Moreover, among the deciduous and non-leguminous shades, *Cordia africna* is also a potential species in areas where it can adapt well with coffee. Results of this study, therefore, confirm that the traditional method of coffee cultivation under shade is an acceptable and economically feasible practice in Ethiopia.

REFERENCES

Cambrony H.R. 1992. Coffee growing- CTA Wageningen, the Netherlands, and Macmillan Press Ltd. London. 119pp.

Friend D.J.C. 1984. Shade adaptation of photosynthesis in *Coffea arabica.* Phtosynthesis Research 5(5): 325-334.

Guridi F., Morales D., Ssoto F., Valdes R. and Vento H. 1988. Some aspects of coffee physiology in cuba. Twelfth international scientific colloqium on coffee. Paris, France; 1988. pp. 52, 501-518.

Napoles S., Tabares G., Mendez E. and Cuba B. 1990. Effect of shade and sunlight modality, plant per punch and irrigation o ‘catura rajo’ production. I: Growth. Ciencia y Tecnica en la Agricultura y Café Cacao **12** (1-2): 41-49.

Paulos Dubale and Tesfaye shimber. 2000. Some Ecological Parameters Occurring In The Major Coffee Growing Areas Of southwestern And Southern Ethiopia. Proceedings of the Workshop on control of coffeeBerry Disease (CBD) in Ethiopia. Addis Ababa, 13-15 August 1999. pp 533-539.

Tesfaye Shimber. 1995. Influence of watering frequency, mulching and shading on seedling growth of Araica coffee. M.Sc. Thesis Alemay University of Agriculture, Ethiopia. 112 pp.

Venkataramanan D. and Govindappa D.A. 1987. Shade requirement and productivity of coffee. Journal of coffee research **17** (2): 16-39.

Workafes Woldetsadik and Kassu Kebede. 2000. Coffee production system In Ethiopia. Proceedings of the Workshop on Control of Coffee Berry Disease (CBD) in Ethiopia. Addis Ababa, 13-15 August 1999. pp. 99-106.

Yacob E., Tesfaye S., Alemseged Y., Taye K., Mohammed Nur A., Anteneh N., Takele N. and Bekele B. 1996. Advances in coffee agronomy research in Ethiopia. Proceedings of

IACO Workshop Kampala, Uganda, 4-6 Sept. 1995. African Crop Science Society. 1996. pp. 40-55.

Minimum Diurnal Temperature: An Important Parameter in the Deployment of Coffee Berry Disease Resistance Genes in *Coffea arabica* L.

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SUMMARY

Cultivar Ruiru 11 is an Arabica Coffee variety resistant to Coffee Berry Disease caused by *Colletotrichum kahawae*. Long term observation on field resistance of the variety revealed sporadic but transient occurrence of Coffee Berry Disease on the variety. An examination of possible pre-disposing weather parameters indicated strong negative correlations with mean minimum temperatures during the sixth week after blossoming. Strong negative correlations were also observed between minimum temperature/lowest temperature) and severity during the 4th and 6th week post flowering. Incidence of the disease showed strong negative correlations with the number of days for which minimum temperatures below 15°C was recorded.

In this paper detailed discussion on the conditions under which expressivity for CBD may be compromised are given. The implications of the present observation on screening and breeding strategies, gene deployment and management of resistance in the field are also discussed.

INTRODUCTION

Environment is important in determining phenotypic expression for a number of economically important traits. In Arabica coffee (*Coffea arabica* L.) for example, Genotype-by- Environment (GE) interactions have been shown to be important in determining traits such as yield and growth components (Srinivasan, 1978; Srinivasan et al., 1979; Reddy et al., 1986; Walyaro, 1983; Montes et al., 1987). Agro-ecological conditions are also known to determine disease prevalence with coffee leaf rust being important in low altitude zones characterised by warm temperatures and Coffee Berry Disease (CBD) caused by *Colletotrichum kahawae* being more important in the high altitude zones where low temperatures and high humidity is prevalnt. In Kenya, field observations on resistant cultivar, Ruiru 11, reveal transient occurrence of higher levels of CBD in years with unstable weather conditions, hence indicating the possible importance of diurnal weather conditions on field resistance to CBD. In this paper, the role of temperature on field expression of resistance to CBD in cultivar Ruiru 11 is examined. Ways of cushioning the resistance genes against unfavourable fluctuations in environmental conditions are also discussed.

MATERIALS AND METHODS

Data was collected on the field resistance of Ruiru 11 during a period of six years. Data on rainfall, humidity, mean minimum temperature and mean maximum temperature were also collected during the same period. Laboratory inoculation was conducted in accordance with Van der Vossen et al. (1976) with modification to accommodate the following:

- Cold treatment at 5°C for four hours during infection
- Cold treatment at 10°C during infection
- Cold treatment at 10°C for 12 hours during infection.
- Inoculation followed by incubation at 10°C.
- Inoculation followed by incubation of 15°C.
- Inoculation followed by incubation at 18°C.
- Incubation at room temperature (20-25°C).

Linear correlations were carried out to establish the degree of association between the level of infection and (1) mean minimum temperatures during various fruit development stages after blossoming (2) lowest recorded temperatures at various development stages (3) the number of days when the minimum temperatures fell below 15°C. Data were analysed using MSTAT and STATISTICA softwares.

RESULTS AND DISCUSSIONS

Field observations

Temperature fluctuations during the 2nd and 3rd months of berry development did not have significant effect on field resistance to CBD. This was probably because these stages of berry development are not ideal for CBD infection. Strong negative correlations were however observed between mean minimum temperature during the 4th and 6th months of berry development and the level of CBD infection on CV Ruiru 11 (Table 1). The lowest temperature recorded during these periods had similar effects to those observed for mean minimum temperature. The observations indicate that low diurnal temperatures during the berry expansion and bean filling stages may suppress the expression of CBD resistance genes leading to unusual levels of field susceptibility by resistant varieties such as Cultivar Ruiru 11. Low temperatures are known to affect the level of resistance to CBD during seedling stages (Van de Vossen and Waweru, 1976) by reducing the ability of the host to respond adequately to invasion by the pathogen (Masaba and Van der Vossen, 1982).

Table 1. Correlations between various temperature variables and field resistance to CBD in cultivar Ruiru 11

Variables (°C)	Months after flowering								
	4			5			6		
	Incidence	Tree Severity	Field severity	Incidence	Tree Severity	Field severity	Incidence	Tree Severity	Field severity
Mean Minimum	-0.968**	-0.871	-0.924*	-0.656	-0.714	-0.834	-0.777	-0.960**	-0.995**
Days below 15°C	0.902*	0.587	0.716	-0.290	-0.068	0.054	0.947**	0.663	0.773
Lowest temp	-0.884	-0.970**	-0.965**	-0.829	-0.490	-0.641	-0.928*	-0.929*	-0.967**

Laboratory observations

From seedling inoculation experiments, it was shown that exposure to low temperatures for duration as short as 5 hours during the infection period was enough to completely depress the expression of seedling resistance to CBD (Figures 1 and 2). Observations made during this study indicated that temperatures below 10°C are quite common during the periods of the year when berries are either rapidly expanding or filling. Temperature induced field susceptibility may thus be a major problem when deploying CBD resistance genes in Arabica coffee in some coffee growing districts in Kenya. Under such circumstances, two possibilities could be considered as

means of alleviating problems related to environmental influence on CBD resistance. The first approach would be to find resistance genes which could be expressed at low temperatures. This is however feasible only on a long-term basis since so far no report exist of CBD resistance genes which effectively express themselves under low temperatures exist. An alternative approach with more short-term prospects would be to formulate ways of supporting the available genes. To this end, limited use of fungicides during 4th to 6th months when the adverse temperatures are expected to occur, use of irrigation (Large Scale farmers) to encourage off-season flowering so that the periods of adverse temperatures coincide with the hardening stage of berry development and the use of shade trees to stabilise diurnal fluctuations in temperature.

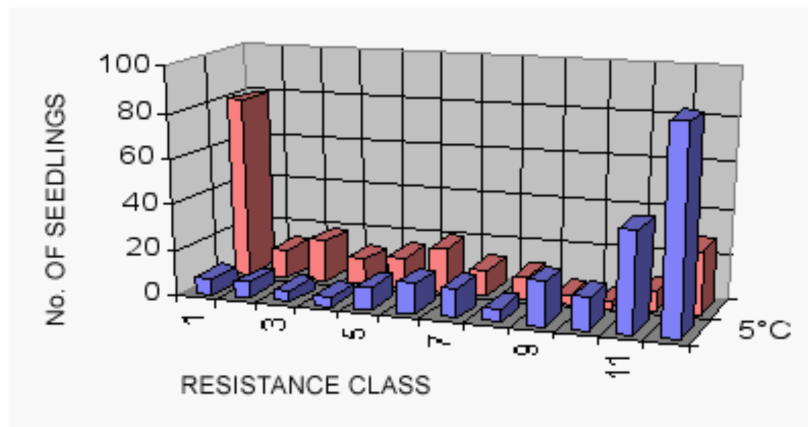

Figure 1. Determination of CBD resistance level for cross 8 based on two temperature regemes

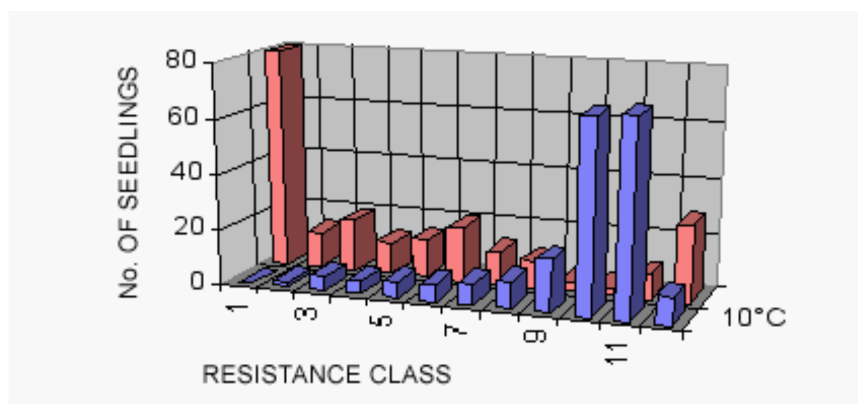

Figure 2. Determination of CBD resistance level for cross 8 based on two temperature regemes

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REFERENCES

Masaba D M and Van der Vossen H A M, 1982. Evidence of cork barrier formation as a resistance mechanism to berry disease (*Colletotrichum coffeanum*) in arabica coffee. Neth. J. Pl. Path. **88**: 19-23

Montes S., Cornide M.T., Sigarroa A. and Martin J.V., 1987. Etude de l'interaction génotype-environnement de onze lignées sélectionnées de *Coffea arabica* L.. In: 125th Int. Scient. Coll. On coffee, Montreux, pp. 813-827. ASIC, Paris.

Reddy A.G.S., Sreenivasan M.S. and Ramaiah P.K., 1986. Adaptation of coffee selection in non-traditional areas. India coffee **50**: 10-16.

Srinivasan C.S., Vishveshwara S. and Subramanya H., 1979. Genotype-environment interaction and heritability of yield in *Coffea arabica* L. J. Coffee Res. **9** (3): 69-73.

Van der Vossen H.A.M., Cook R.T. and Murakaru G.N.W., 1976. Breeding for resistance to coffee berry disease caused by *Colletotrichum coffeanum* Noack (Sensu Hindorf) in *Coffea Arabica* L. I. Methods of preselection for resistance. Euphytica **25**: 733-745.

Walyaro D.J.A., 1983. Considerations in breeding for improved yield and quality in arabica coffee (Coffea arabica L.) Doctoral Thesis. Wageningen Agricultural University, the Netherlands. 119 p.

Optimisation of Hybrid Seed Production in Arabica Coffee *Coffea arabica* L.

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SUMMARY

Hybrid varieties have become an integral part of coffee farming since the introduction of Cv. Ruiru 11, a Coffee Berry Disease and rust resistant variety, in 1985. More hybrid varieties are expected to appear in the coffee farming scene as various breeding programmes endeavour to introgress resistance to a number of diseases into the commercial cultivars. While procedures for artificial cross pollination at experimental levels exist, experience at the Coffee Research Foundation indicate that the availability of hybrid seed can be the main constraint to the rapid adoption of hybrid Arabica coffee varieties. Labour availability, climate factors such as rain and temperature, fruit set, and synchronised phenological changes have been identified as major bottleneck to efficient large scale hybrid seed production. In this paper, models for optimising the interrelationships between the various constraining factors and hence maximise seed yield are proposed. Data based on ten years of commercial hybrid seed production at the Coffee Research Foundation Ruiru are given in light of the proposed optimisation regime. Guidelines for making production decisions based on agro-climatic factors are proposed.

INTRODUCTION

The main objectives of Arabica coffee breeding include increased productivity, resistance to diseases and improved quality (Charrier and Eskes, 1997). Due to the long generation cycle characteristic of the species, incorporation of all the traits into a single commercial cultivar using conventional breeding techniques require many years of breeding on the possibility of shortening the process is through the development of hybrid varieties. The main challenge to the use of hybrid varieties is the production of sufficient seed for use by the farmers. Experience at the Coffee Research Foundation Ruiru, indicate that the procedures for small-scale artificial cross pollination exists (Carvalho and Monaco, 1969; Walyaro and Van der Vossen, 1977) have severe limitations when it comes to large scale seed production programmes due to a number of reasons. Firstly, Arabica coffee is gregarious in flowering habit, with individual plants flowering simultaneously and within short period of time in response to rainfall or irrigation. The gregarious nature makes hybrid seed production highly dependent on rainfall pattern since once flower initiation occurs all the emasculation and pollination activities are supposed to be completed within a period of four to seven days.

Related to the phenological changes are the issues of weather and labour availability and efficiency. Rainfall patterns determine the period and duration during which artificial emasculation and pollination can be conducted whereas labour availability and efficiency has a bearing on the seed output and cost of production. In this paper, a method is proposed for improving hybrid seed production efficiency in Arabica coffee through appropriate selection of seed production environments, efficient planning and use of labour.

CONSIDERATIONS FOR ESTABLISHING AN EFFICIENT HYBRID SEED PRODUCTION PROGRAMME

Choice of environment

The pattern of rain experienced determines the suitability of a site for hybrid seed production purposes where there is a bi-modal rainfall pattern, the two rainy periods should be separated by at least three months of dry spell. In the case of uni-modal pattern, at least four months of dry spell would be required. Environments receiving rainfall throughout most parts of the year are unsuitable for hybrid seed production unless special treatments such as the use of green house is used.

Labour availability & efficiency

Labour is an important component of hybrid seed production. On the average, an individual working for eight hours per day is capable of emasculating 1500 flowers. Figures as high as 6000 flowers per day can be realised depending on the level of training of the individual and the stage of development of the flower. Men tend to emasculate less flowers with poor stigma survival. The labour needs (MD) could be worked out based on the number of flowers per man-day (ER), realised fruit set (FS), the number of days available for emasculation (ED) and the expected seed output can be related according to the equation :

Seed Yield = [MD * ER * FS%* ED]/ 2

In principle therefore, a change in any of the factors could be compensated by shifts in other levels of the other factors. Figure 1 gives an example of how casual labour use could be projected depending on the length of emasculation period and the level of fruit-set.

Table 1. Rotational stumping for efficient hybrid seed production

Clean stumping Suckers selection	Suckers Fly crop	Fly crop 1st main crop	1st main crop 2nd main crop	2nd main crop Clean stumping
Fly crop	1st main crop	2nd main crop	Clean stumping	Sucker selection
1st main crop	2nd main crop	Clean stumping	Suckers selection	Fly crop
2nd main crop	Clean stumping	Suckers selection	Fly crop	1st main crop
Clean stumping	Suckers selection	Fly crop	1st main crop	2nd main crop

Canopy Management

Although older larger trees provide higher surface area for flowering and fruit formation, they are highly undesirable for seed production purposes. This is because they make emasculation isolation and pollination activities difficult due to plant height, increased interlocking of branches and non uniform branching. Ideally mother trees in a hybrid seed orchard should be compact in growth habit, posses few or no secondary branches and should be raised on a single head. The seed field should preferably be managed on a five year cycle of rotation in which a bearing head is allowed to have two main bearings before change of cycle (Table 1).

Unlike in commercial fields, change of cycle should exclusively be through clean stumping to encourage uniform production of strong suckers.

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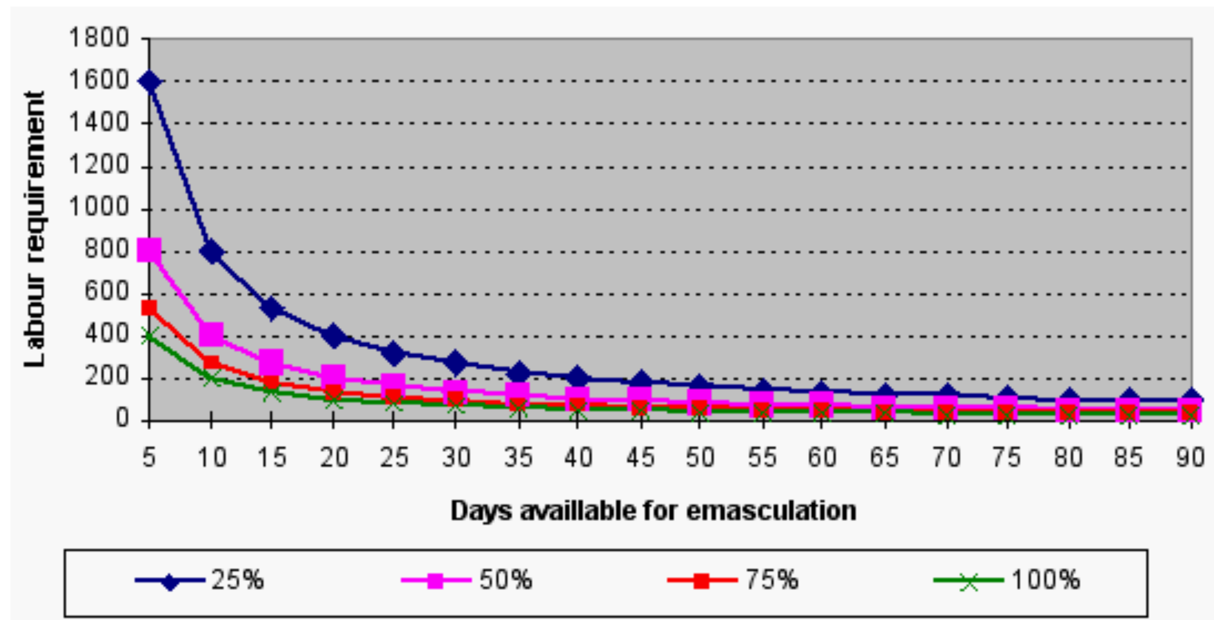

Figure 1. Effect of fruit set level and days available for emasculation on labour requirement for the production of 6 000 000 Ruiru 11 seed

REFERENCES

Carvalho A. and Monaco L.C., 1969. The breeding of arabica coffee. In Ferwerda F. P. and Wit F. (Eds.). Outlines of perennial crop breeding in the tropics. Misc. Paper 4. Landbouwh. Wageningen pp 198-216.

Charrier A. and Eskes A.B., 1997. Les caféiers. In. Charrier A., Jacquot M. and Hamon S. L'amélioration des plantes tropicales. 623 p. CIRAD & ORSTOM.

Pesticide Residues in Coffee Beans and their Impact on Coffee Quality in Kenya – A Review

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SUMMARY

The review points to the implications of high pesticide residue levels in coffee beans and their possible impact on the coffee quality. It stresses the need to stick to maximum Residue Levels (MRLs) of various pesticides in coffee beans set out by world bodies like WHO and FAO. High pesticide residue levels in coffee beans may lead to their rejection in the coffee market. A number of research gaps have been proposed that if addressed can alleviate the possible dangers of pesticide residues in coffee tissues.

INTRODUCTION

Kenya is renown for its high quality coffee processed by the wet method. The quality of coffee beans and more so the coffee liquor is an important aspect in determining coffee prices in the market. Unsafe levels of contaminants in the coffee beans pose a potential threat to the marketability of Kenya coffee as the European and North American consumers continue to be wary of the chemical residues in foods and beverages.

Copper-based biocides and organic-based pesticides are used to control diseases and pests in well managed coffee farms (Park and Burderkin, 1964). The pesticides which are frequently used in coffee farming in Kenya are copper based. Therefore, much of the pesticide residue work in Kenya has concentrated itself on copper biocides. However, research into organic pesticides is underway.

Coffee beans are consumed by humans in form of coffee liquor. High pesticide residue levels in coffee beans are of major concern. This is because of the toxic nature of the pesticides used in coffee protection. Though the use of these chemicals in protecting coffee cannot be done away with at the moment, they have to be regulated and their levels monitored. Frequent monitoring of the residue levels in the coffee beans, will lead to formulation of interventions to avoid reaching toxic proportions.

ROUTE OF PESTICIDE RESIDUES INTO THE COFFEE BEANS

During spray, pesticide residues land on coffee berries, leaves and branches. Some amount that misses the target land on the soil. Part of the pesticide residues on the tissues is washed down to the soil when it rains or during irrigation. However, some of the residues get strongly adsorbed onto the coffee pulp and part of these residues can get dislodged into processing water. Therefore, coffee beans get contaminated with pesticide residues from this same processing water.

It has been reported that roots take up nutrients along with pesticide residues from the soil and once in the plants' roots, the residues get translocated to other parts of the plant (Finlayson and McCarthy, 1965). With continuous application of pesticides, the uptake of the pesticide

residues from the soil increases the level of the residues in especially coffee pulp, leaves and beans. For instance, the prolonged usage of copper based fungicides, has been reported to increase copper to high levels in coffee leaf (Aduayi, 1973; Spencer, 1966).

PESTICIDE LEVELS OBSERVED IN COFFEE BEANS IN KENYA

Coffee beans from sprayed areas in Kenya recorded an average concentration of 19 μg/g of copper (Maroko, 1987 and 1989). Despite the status of copper in soils and coffee plant materials in coffee growing areas in Kenya being low or high (Lunt, 1983; Maroko, 1987 and 1989), Kairu (1988) reported that the crop yields have not been affected. However there is need to avoid the trace element copper attaining levels toxic to the coffee plant and more so to the consumers of the coffee beans.

DANGERS OF HIGH PESTICIDE RESIDUE LEVELS IN COFFEE BEANS

If the pesticide residues in beans go beyond the Maximum Residue Level (MRL) allowed, the coffee beans can easily be rejected in the coffee market. Consumption of such coffee beans may lead to toxic effects on the side of consumers.

FACTORS THAT LEAD TO REDUCED PESTICIDE RESIDUE LEVELS IN COFFEE BEANS

Supplementary evidence has been provided by research workers who have demonstrated that the uptake of pesticides by plants is inversely related to the amount of organic matter in soil (Beestman et al., 1969; Getzin, 1958; Patterson, 1962). Aduayi (1973) and Thuo et al., (1994) noted that copper is less available in soils with high organic matter concentration, high pH levels and high concentration of phosphates.

Reduced availability of pesticide residues to coffee beans lead to lower levels of the residues in the beans. Therefore use of such factors in coffee plantations can help in reducing chances of the residues in coffee beans going beyond maximum safety limits as set out by the consumer world bodies like WHO and FAO.

RESEARCH GAPS TO BE FILLED

1. Current levels of the recommended organic based pesticides in coffee beans and pulp. This will help in monitoring of the quality of coffee beans and pulp produced in terms of toxic chemical levels to see if the MRLs have been exceeded or not so that appropriate action is taken.

2. The percentages of the recommended pesticides transferred from coffee beans into the coffee liquor. How high pesticide residue levels do affect the coffee liquor probably in terms of flavour. This knowledge will help in coming up with safe daily intake limits of the coffee liquor depending on the levels and toxicities of the residues in the liquor. The levels of the pesticide residues in the coffee liquor are very important because it is actually the liquor from the beans which is consumed.

3. The rate of dissipation, degradation and accumulation in the coffee beans of the recommended pesticides in coffee production. This will help in isolating and rejecting the pesticides which accumulate at a high rate while dissipating and degrading at a low rate. Some of the pesticides are known to degrade to more toxic products. Identification of

these by-products of degradation will throw light onto the positive steps that can be taken to maintain the high quality of the Kenya coffee.

4. The degree of pesticide residue pollution of the surrounding environment emanating from the wet method used in processing coffee cherry. There is a possibility of coffee pulp and effluent polluting processing water. This assessment will help in maintaining the high quality of coffee beans and not polluting the surrounding water.

5. The effect of high copper levels in soils and coffee tissues on the size or grade of coffee beans and how the liquor flavour is affected.

6. Biodegradation of pesticide residues in coffee pulp with a view to improving its quality as a possible economic material for production of animal feed supplement and wines in Kenya. High organic pesticide residue levels in pulp are likely to be toxic to the consumers due to the toxic nature of these chemicals. Therefore, any biodegradation process of the chemicals into harmless substances is most welcome not only to the consumers but also the environment. This will greatly help in that, the factories downstream will use less contaminated water in processing their coffee and hence result to better quality beans.

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REFERENCES

Aduyi. E.A (1973). Effects of increasing levels of copper on the mineral nutrition and growth of *Coffea Arabica* L. in Kenya

Beestman G.B., Keeney D.R and Chesters G. (1969). Agron. J. 61, 247

Burderkin 1964 Calle H. (1977). Subproductos Del Cafe' Finlayson, D.G and MacCarthy, H.R. (1965). Res. Rev. 9, 114

Getzin L.W. (1958). Diss. Abstr. 19; 625

Kairu G.M. (1988). Coffee Research Foundation, Annual Report 1988/1989.

Lunt O.R. (1983). Radioisotopes in Agriculture. A report to the government of Kenya KEN/5/008 International Atomic Energy Agency.

Maroko J.B. (1987). Copper levels in soils and coffee plant material from Bahati-Solai, Nakuru, Kenya. Coffee **52** (613): 215-517

Maroko J.B. (1989). Copper levels in soils and coffee plant materials in Kenya – part II, Kenya Coffee, Vol. **54** NQ. 630 pg. 585-587.

Park P .0 and Buderkin, D.A. (1964). Studies on the application of a copper fungicide for the control of coffee leaf rust. Annals of Applied Biology **53**, 133-150.

Patterson R.S (1962). Diss. Abstr. 23, 1471

Spencer W.F. (1966). Effects of copper on yield and uptake of phosphorus and iron by citrus seedlings grown at various phosphorus levels. Soil Sci. **102**: 296-299.

Thuo J.T, Mburu J.N and Kamau H.M. (1994). Dynamics of Accumulated Copper in the soil and leaves of Coffea arabica L. Ken~a Coffee Vol. **59** No. 689 pg 1739-1742 j

Agronomic Performance and Economic Benefits of Coffee-fruit Tree Intercropping Systems in the Upper Coffee Zone of Kenya

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SUMMARY

The influence of intercropped fruit trees on two Arabica coffee cultivars - Ruiru 11 and SL 28 were studied at Kitale, Kenya between 1991 and 1997. The coffee plants were planted at densities of 2500 and 1330 plants/ha for Ruiru 11 and SL 28 respectively. The trial was laid out in a split plot design with the coffee varieties as main plots and the fruit trees as subplots. The intercropped fruit trees except guavas did not significantly affect the clean coffee yields. Ruiru 11 significantly outyielded SL 28 regardless of the intercrop. Intercropping both coffee varieties with the fruit trees resulted in positive net benefit except where SL 28 was intercropped with quavas and avocadoes. The screened fruit trees except guavas may are to be considered for intercropping in Arabica coffee.

INTRODUCTION

In Kenya, coffee farmers especially the small scale farmers, have for the past years intercropped their coffee with annual foodcrops and/or, fruit and timber trees despite an official ban. The main expectation from such intercropping systems is that the overall return from a unit of land is increased without adversely affecting the current or the long-term productivity of the coffee crop (Liyanage et al., 1984). The main fruit trees intercropped with coffee in Kenya include macadamia (*Macadamia ternifolia*), oranges (*Citrus sinesis*), bananas (*Musa sapientum*), mangoes (*Mangifera indica*) and pawpaws (*Carica papaya*) (Whitaker, 1986). Coffee has also been intercropped with bananas in Papua New Guinea (Bourke, 1985), with oranges (*Citrus sinesis*) and avocadoes in the Andes mountains (Escalante, 1985) and with bananas in Uganda (Oduol and Aluma, 1990). Despite this, most research in Kenya on intercropping systems based on coffee have focused on annual crops (Njoroge and Kimemia, 1993). The objective of this trial was therefore to study the effect of the fruit tree intercrops on the yields of arabica coffee grown at high altitude area (upper coffee zone). In Kenya, coffee plantings are being expanded into these areas which were formerly maize growing areas. Due to the subsistence nature of their farming systems, intercropping is expected to be prevalent.

MATERIALS AND METHODS

The study was conducted at Kitale from May 1991 to December 1997. The site is located at 1°00'N,35°12'E and 1890 m above sea mean level. At Kitale the rainy season spreads between April and September (and averages 1120mm per year. The soils are humic nitosols with a deep profile and reddish brown to dusky brown clays (Jaetzold and Schimdt, 1983). They are moderate in bases, low in phosphorus and slightly acidic with a pH range of 4.0 - 5.4 $CaCl_2$ (Siderius and Muchena, 1977). Arabica coffee cultivars-`Ruiru 11' and `SL 28' and nine fruit crops: - custard apple (*Anona squamosa L.),* papaws (*Carica papaya*), guava (*Psidium guajava*), loquats (*Eriobotrya japonica*), avocadoes (*Persea americana*), oranges(*Citrus sinesis*), passion fruit (*Passiflora edulis*), macadamia (*Macadamia ternifolia*), apples (*Malus pumila*) and mulberry (*Morus alba L.*,) were used in the study. The treatments were laid out in a split-plot

design with three replicates. The coffee varieties formed the main plots while the fruit tree intercrops the subplots. The coffee and fruit trees were managed as recommended (Mwangi, 1983; Anon 1981). At the end of each season ripe coffee cherries were picked from the six central plants. Net benefits from both the coffee and fruit trees were determined as described by Perrin et al. (1986).

RESULTS

Throughout the study period, Ruiru 11 outyielded SL 28. Intercropping both Ruiru 11 and SL 28 with guavas resulted in significantly lower clean coffee yields (Figure 1).

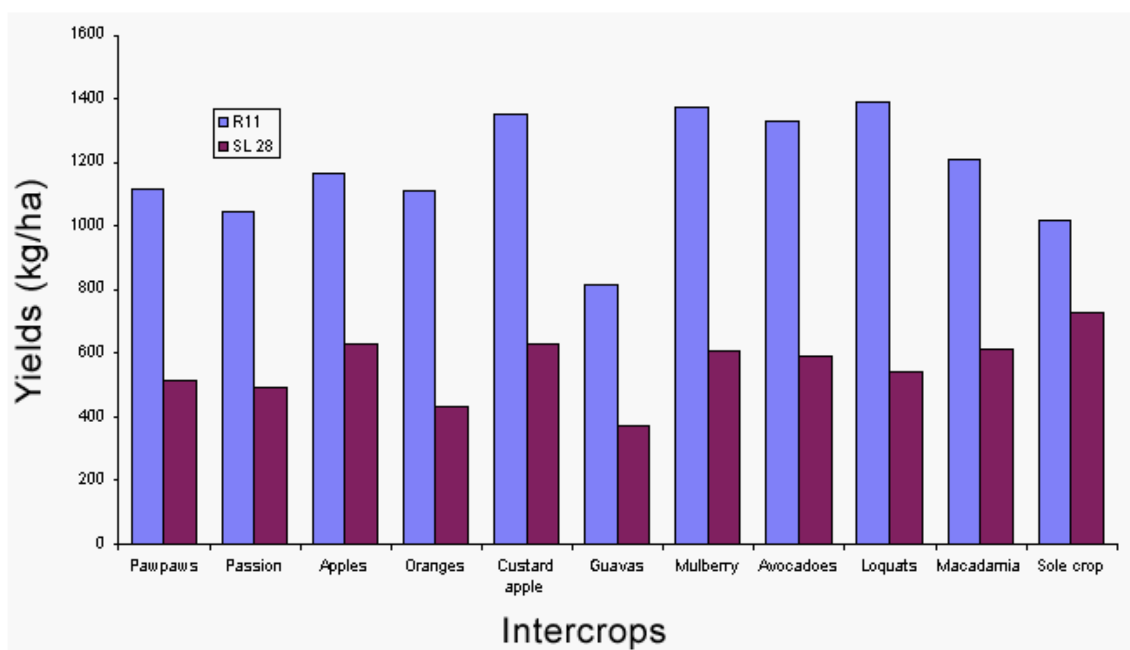

Figure 1. Effect of intercropping arabica coffeewith fruit trees on clean coffee yields at Kitale, Kenya

Mulberry and avocadoes had not produced by the sixth year. The fruit yields averaged 3.95, 1.46, 2.86, 13.03, 6.96, 97.29, 16.47, 22.96 and 1.36 tons ha^{-1} for pawpaws, passion fruit, apples, oranges, custard apple, guavas, bananas, loquats and macadamia respectively. Intercropping young coffee plants with fruit trees except intercropping SL28 with guavas and avocadoes resulted in higher net benefits than sole coffee (Figure 2).

Intercropping with Ruiru 11 was more profitable than intercropping with SL 28. Intercropping systems involving pawpaws, passion fruit and macadamia had the highest net benefits as compared to sole coffee.

DISCUSSION

The fruit crops intercropped with coffee except guavas did not depress significantly the yields of clean coffee. Similar results have been reported by Kimemia (1998) that intercropping coffee with fruit trees except guava and bananas did not adversely affect clean the coffee yields. Most tree crops take many years to establish full canopy thus leaving plenty of space between them (Jackson, 1983). In this study, it was observed that the fruit tree intercrops increased the net monetary benefits. This concurs with the findings of Bheemiah and Shariff (1989) who reported that intercropping coffee with oranges and bananas in India resulted in high and stable incomes. Njoroge and Kimemia (1995), Kimemia (1998) also found intercropping annual crops and fruit

with young coffee also increased monetary benefits. The inclusion of these trees into coffee cropping systems will encourage the farmers to take care of their coffee even in periods of low coffee prices. The Kitale area has large tracts of land suitable for coffee production but is prone to Bacterial Blight of Coffee (BBC) cased by *Pseudomonas syringae* which limits coffee production. The incidence of this disease has been observed to be reduced by shade (Kimemia and Njoroge, 1988). Intercropping with the perennial fruit trees will provide shade and hence may assist in reducing the incidence of BBC and the costs associated thereof. This aspect coupled with the improved returns from intercropping will be an encouragement for the coffee farmers in the locality. It was therefore concluded that, pawpaws, passion fruit, apples, oranges, avocadoes, loquats and macadamia trees can be intercropped with both the yields of clean coffee in the upper coffee zones in Kenya.

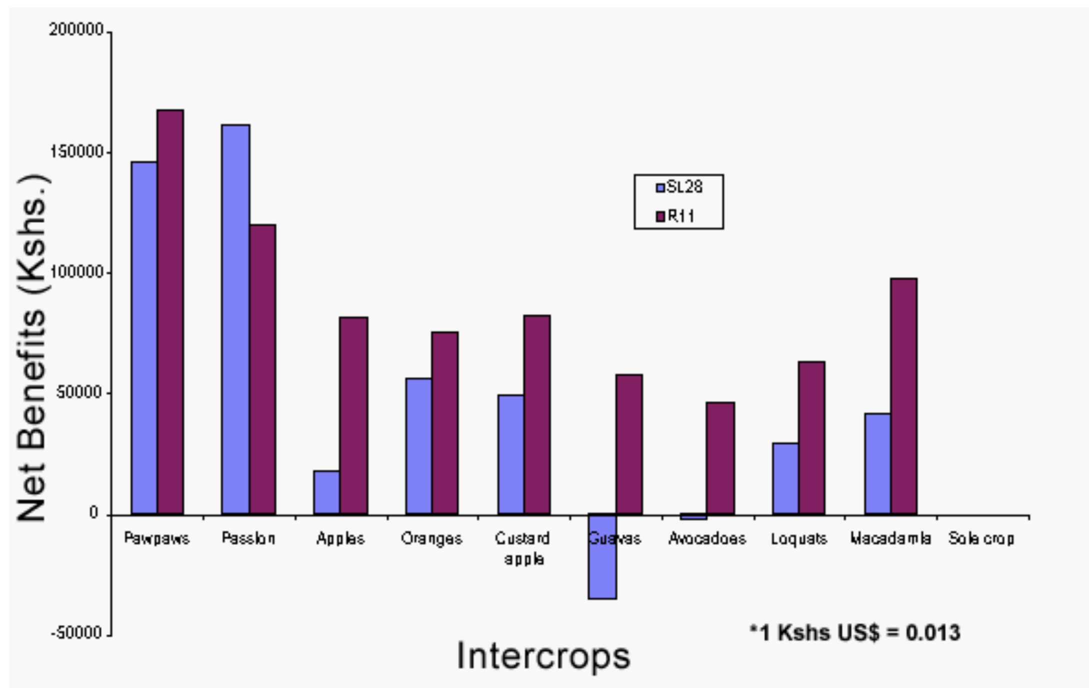

Figure 2. Incremental net benefits of intercropping arabica coffee with fruit trees at Kitale, Kenya

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REFERENCES

Anonymous, 1981. Major crops. Technical Handbook. Agricultural Information Centre. Nairobi, Kenya.

Bheemaiah M.M. and Shariff M., 1989. Multiple cropping in coffee. Indian Coffee **53**: 9-13.

Bourke M.R., 1985. Food, coffee and casuarina. An agroforestry system from the Papua New Guinea highlands Agroforestry systems **2**: 273-79.

Escalante E.E., 1985. Promising agroforestry systems in Venezuela. Agroforestry systems **3**: 209-21.

Jackson J.E., 1983. Light climate and crop - tree mixtures. In. Plant Research and Agroforestry (Huxley Ed.) International Centre for Research in Agroforestry Nairobi, Kenya pp 365-378

Jaetzold R. and Schmidt H., 1983. Farm Management Handbook. Vol II/B: Central Kenya. Ministry of Agriculture, Nairobi, Kenya

Kimemia J.K., 1998. Studies on green manure application and intercropping in *Coffea arabica*. PhD Thesis, University of Nairobi

Kimemia J.K. and Njoroge J.M., 1988. The effect of shade on coffee, - a review. Kenya Coffee **53**: 387-391

Liyanange M. de S, Tejwani K.G. and Niar P. K. R., 1984. Intercropping under coconut in Sri Lanka. Agroforestry Systems **2**: 215-28.

Mwangi C.N., 1983. Coffee Growers Handbook. Coffee Research Foundation, Ruiru Kenya. 182 pp.

Njoroge J.M. and Kimemia J.K., 1993. Current observations and future trends in coffee intercropping studies in Kenya. Outlook in Agriculture. **22**: 43-48

Njoroge J.M. and Kimemia J.K., 1995. Economic benefits of intercropping young arabica and robusta coffee with food crops in Kenya. Outlook in Agriculture **24**: 27-34

Oduol P. A. and Aluma J.R.W., 1990. The banana (Musa Spp) - Coffee robusta: traditional agroforestry system of Uganda. Agroforestry Systems **11**: 213-6.

Perrin, Winkelmann R. K., Moscardi E.R. and Anderson J.A., 1976. From Agronomic Data to Farmers Recommendations. An Economics Training Manual. Information Bulletin 27 CIMMYT, Mexico 51 pp.

Siderius W. and Muchena F.N., 1977. Soils and environmental conditions of agricultural research stations in Kenya. Ministry of Agriculture, National Agricultural Laboratories. Kenya soil survey miscellaneous soil paper No M5. 13 pp.

Whitaker M. J., 1986. Some characteristics of agricultural production on coffee small holdings in the main coffee producing districts of Kenya. Kenya coffee **51**: 99-131.

Efficiency of Early Selection in the Icatu Coffee*

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SUMMARY

Early selection of coffee has been studied in straight *Coffea arabica* cultivars but not in cultivars developed by interspecific hybridization like the Icatu, originally derived from a *C. canephora* x *C. arabica* cross. Seven selection fields of F_3 and F_4 of BC_2 generation located in Mococa, Campinas, Garça and Pindorama in State of São Paulo, Brazil were studied, being the yield recorded for seven to ten consecutive years. The *a posteriori* study of yearly selection was performed by comparing coefficients of phenotypic correlation between total yield of progenies and of individual trees with their annual and cummulative yields in each experiment. Cummulative data of 4 to 6 consecutive harvests were very effective in identifying the best progenies of Icatu coffee. For individual trees 100% efficiency was only attained in the 4 localities after the 5th harvest when the selection intensity was 50%. If intensity decreases to 25%, efficiency became much lower in all selection fields. Correlation of total yields and cummulative yields in high yielding years was very high. This information indicates that selection of best progenies and trees can be securely done on the basis of such years only. Although this does not save time, it however reduces considerably the costs of the coffee breeding program.

INTRODUCTION

Coffee fields in Brazil usually last around 20 years being then substituted by a new planting. The new varieties of arabica coffee start flowering in the very first year and in the second year they iniciate the production that usually increases up to the 11th to 14th consecutive crop. After the 4th or 5th crop, cicles of high and low yields is the common pattern of coffee production in Brazil and elsewhere (Mendes, 1949; Carvalho et al., 1957; 1973; Castillo and Quiceno, 1968; Vicente-Chandler et al., 1969; Fazuoli, 1977).

The best selection of individual plants or progenies is made with certainty only after recording the yields for 15 or more years. However this is a too long time of evaluation, reason why a number of investigations were carried out in order to study the possibilities and effectiveness of shortening this time (Mendes, 1951; Carvalho, 1952 and 1989; Krug, 1953; Antunes-Filho, and Carvalho, 1957; Carvalho et al., 1961 and 1973; 1975; Antunes, 1962; Carvalho and Mônaco, 1972; Fazuoli, 1977; Fazuoli and Carvalho, 1979; Walyaro and van der Vossen, 1979; Srinivasan, 1982; Walyaro, 1983; Cera, 1987). Obviously, as the number of sucessive yields are recorded, the more efficient the selection process becames. This efficiency is greatly influenced by the type of plants under selection. Vigor, longevity, capacity to withstand continuous high yields are usually correlated. In addition, these characteristics also

interact with crop sistems, spacings, fertilizer levels and occurence of occasional stress conditions. Evidently, among those, the major factor is the genetic constitution of the germplasm under selection. Several attempts have been successfull in correlating specific characteristics of the plants with the efficiency of selection.

The general pattern observed in such studies is that the associated or correlated characteristics are as good as they are one of the limiting yield components of the population under selection. Therefore, the effectiveness of evaluations could be improved if selections were additionally made in specific situations for trunk or canopy diameter, tree height, number of nodes and plagiotropic branches, number of flowers and inflorescenses per leaf axil, percentage of taking, of outurn and many others. Also, on the basis of chance alone it is obvious that in the attempts to early select the future best performing lines or individual plants, the less intensive the selection pressure is, the more efficient it eventually becomes. Another aspect is that the efficiency is usually much higher for progenies than for individual plants as the latter are statistically subjected to much more variation then the former.

In such investigations only *Coffea arabica* germplasm have been studied. This paper deals with a similar attempt made with a genetically different coffee named Icatu, wich was derived from the interspecific cross of *C. canephora* x *C. arabica* (Mônaco et al., 1974).

MATERIAL AND METHODS

The Icatu coffee originated from a cross made in 1950 in the Instituto Agronômico de Campinas of a tetraploid *C. canephora* cv. Robusta with a doubled haploid *C. arabica* cv. Bourbon Vermelho. The tetraploid F_1 hybrid was further backcrossed to *C. arabica* cv. Mundo Novo followed by variable number of selfings and pedigree selections. The Icatu coffee corresponds to lines from the second or third backcross to Mundo Novo, occasionally to Bourbon Amarelo, with 3 or 4 generations of selfings coupled with selections for yield, rust (*Hemileia vastatrix*) resistance, vigor, ripening time, percentage of flat seeds and general resemblance to *C. arabica*.

The experiments analysed had been set up in 4 localities (Mococa, Campinas, Garça, Pindorama) of state of São Paulo, corresponding to F_3 and F_4 of BC_2, grown and harvested from 7 to 10 years in 7 experimental plots.

The *a posteriori* study of early selection was performed in those Icatu populations comparing in each experiment the coefficient of phenotypic correlation between the total yield of the selections with their annual and cummulative yields. The efficiency of early selection was evaluated according to the formula of Hamblin and Zimmerman (1986) ES = B-C/A-C, where ES is the selection efficiency (%), A is the number of selected progenies, B is the number of progenies common to the final selections and C, the expected number of progenies. Single plant selections were simulated among the best progenies and the efficiency of early selection also evaluated for them.

RESULTS AND DISCUSSION

The correlation coefficients and the efficiency of early selection of progenies are shown in Table 1 and Table 2, respectively. The selection based on cummulative harvests are much more dependable than the ones based on specific years. This can be seen by the correlation coefficients between the total yield and the yield of each year in 3 localities (Table 1 and Table 2).

Table 1. Correlation coefficients between the total yield and yield of each harvest as well as between total yield and cummulative partial harvests of Icatu coffee progenies evaluated in Mococa, Campinas and Garça in São Paulo, Brazil

Harvest	Correlation coefficient			Cummulative Havests	Correlation coefficient		
	Mococa	Campinas	Garça		Mococa	Campinas	Garça
1st	0.23	0.42	0.41	2	0.74	0.52	0.58
2nd	0.74	0.49	0.59	3	0.79	0.90	0.79
3rd	0.55	0.93	0.41	4	0.92	0.88	0.83
4th	0.94	0.63	0.74	5	0.95	0.89	0.98
5th	0.52	0.18	0.65	6	0.97	0.99	0.98
6th	0.96	0.74	0.88	7	0.98	0.99	1.00
7th	0.86.	0.40	0.74	8	1.00	1.00	-
8th	0.92	0.89	-	9	-	1.00	-
9th	-	0.78	-				

Table 2. Efficiency (%) of early selection of Icatu coffee progenies in several localities according to Hamblin and Zimmermann (1986), with selection intensity of 25%

Cummulative Havests	Correlation Coefficient			
	Mococa	Campinas	Garça	Pindorama
1	33.3	50.6	77.8	33.3
2	55.6	75.3	77.8	56.6
3	77.8	87.7	77.8	77.8
4	100.0	87.7	77.8	77.8
5	100.0	100.0	100.0	77.8
6	100.0	100.0	100.0	100.0
7	100.0	100.0	100.0	100.0
8	100.0	100.0	-	100.0
9	100.0	-	-	100.0
10	100.0	-	-	100.0

Data of the first 4 to 6 cummulative harvests were very effective for identifying the best progenies of Icatu coffee as shown by the high values observed. However, the coefficients between total yield and each harvest were much smaller in the 3 locaties. The highest values were observed in years of high yields as previously observed by Mendes (1951), Carvalho (1952) and several others with regular *C. arabica* lines. So, these observations seem to be a general feature of the yield potential of coffee lines irrespective of their origin provide this was previously seen in progenies of Bourbon Vermelho, Caturra Vermelho, Mundo Novo, Acaiá, Kenia and India cultivars wich are all straight arabicas and, in the present investigation, in many Icatu lines derived from an interspecific cross.

Evaluation of the efficiency of the early selection of individual plants within the best progeny of each experiment indicated lower values than the ones obtained in the selection of progenies. Data evaluated according to Hamblin and Zimmermann (1986) formula indicate that 100% efficiency of selections of individual Icatu plants was obtained only after the 5th year of cummulative harvests when the selection intensity was 50%, Again, the observation that efficient identification of the best progenies is made in the years of high yields is equally valid for selecting individual trees. As to the selection intensity, when it was 25% instead of 50%, the efficiency was considerably lowered in the four localities (Table 3).

Table 3. Efficiency (%) of early selection of individual plants within the best progeny of Icatu coffee of the experiments in Mococa, Campinas, Garça and Pindorama subjected to selection intensities of 25 and 50%

Cummulative Harvests	Correlation Coefficient							
	Mococa		Campinas		Garça		Pindorama	
	25%	50%	25%	50%	25%	50%	25%	50%
1	0	63	45	58	58	56	26	63
2	26	63	45	58	58	63	26	63
3	26	63	72	72	58	70	26	63
4	26	63	72	72	85	70	26	63
5	63	63	72	72	85	100	63	82
6	63	63	100	86	85	100	63	82
7	63	100	100	86	100	100	63	82
8	100	100	100	100	-	-	100	100
9	100	100	-	-	-	-	100	100
10	100	100	-	-	-	-	100	100

It was observed also that a very high correlation exists between the total yield and cummulative yields of years of high production. This information although does not improve the efficacy of selection in time it is, nevertheless, a very interesting information from a practical point of view because on the basis of this information the controlled harvest in years of low yields does not need to be done resulting in considerable savings. Harvesting operation in regular coffee fields accounts for more than 40% of the production cost. In experimental situations, costs of controlled harvests are much higher.

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REFERENCES

Antunes Filho H. and Carvalho A. Análise de produção de progênies e híbridos de café Bourbon. Bragantia, **16**: 175-195, 1957.

Antunes C. S. N. Melhoramento do cafeeiro. XXV – Produtividade de progênies e híbridos de café. Bragantia, **21**: 591-615, 1962.

Carvalho A. and Mônaco L. C. Adaptação e produtividade de cafeeiros portadores de fatores para resistência a *Hemileia vastatrix*. Ciência e Cultura, **24**: 924-932, 1972.

Carvalho A. Melhoramento do cafeeiro. VI. Estudo e interpretações para fins de seleção, de produções individuais na variedade Bourbon. Bragantia, **20**: 179-200, 1952.

Carvalho A., Mônaco L. C. and Fazuoli L. C. Melhoramento do cafeeiro. XXXV – Altura e produtividade das plantas e características das sementes de progênies e híbridos de café plantados a uma e quatro plantas por cova. Bragantia, **34**: 295-308, 1975.

Carvalho A., Antunes Filho H., Mendes J. E. T., Lazzarini W., Reis A. J., Aloísio Sobrinho J., Moraes M. V., Nogueira R. K. E Rocha T. R. Melhoramento do cafeeiro. XIII – Café Bourbon Amarelo. Bragantia, **16**: 411-454, 1957.

Carvalho A., Krug C. A., Mendes J. E. T., Antunes Filho H., Junqueira Reis A., Aloísio Sobrinho J., Rocha T. R. E Moraes M. V. Melhoramento do cafeeiro. XXI – Comportamento regional de variedades, linhagens e progênies de café ao sol e à sombra. Bragantia, **20**: 1045-1142, 1961.

Carvalho A., Mônaco L. C., Fazuoli L. C. Melhoramento do cafeeiro: XXXIII. Produtividade o outras características de vários cultivares em Monte Alegre do Sul. Bragantia, **32**: 245-260, 1973.

Carvalho S. P. Metodologias de avaliação do desenvolvimento de progênies do cafeeiro (*Coffea arabica* L.). Lavras, 63p., 1989.

Castillo-Z. J. and Quiceno G. Estudo de la producción de seis variedades comerciales de café. Cenicafé. Chinchiná, **19**: 18-39, 1968.

Fazuoli L. C. and Carvalho A. Estudo de avaliação precoce de progênies de café do cultivar Mundo Novo. In: Reunião Anual a Sociedade Brasileira para o Progresso da Ciência. 31. Fortaleza, 1979. Resumos. São Paulo, SBPC, p. 575-576, 1979.

Fazuoli L. C. Avaliação de progênies do café Mundo Novo (*Coffea arabica* L.). Piracicaba. Ms. Thesis – Escola Superior de Agricultura "Luiz de Queiroz". USP 146 p., 1977.

Hamblin J. and Zimmermann M. J. O. Breeding common bean for yield in mixtures. Plant Breeding Reviews, **4**: 245-272, 1986.

Krug C. A. Aspectos teóricos y prácticos de un programa de mejoramiento del cafeto. Suelo Tico, San José, **7**: 40-47, 1953.

Mendes J. E. T. Ensaio de variedades de cafeeiros II. Bragantia, **9**: 81-101, 1949

Mendes J. E. T. Ensaio de variedades de cafeeiros III. Bragantia, **11**: 29-43, 1951.

Monaco L. C., Carvalho A., Fazuoli L. C. Melhoramento do cafeeiro. Germoplasma do café Icatu e seu potencial no melhoramento. In: Congresso Brasileiro de Pesquisas Cafeeiras, 2. Poços de Caldas, 1974. Resumos: Rio de Janeiro, IBC, 1974. p. 103

Sera T. Possibilidades de emprego de seleção nas colheitas iniciais de café (*Coffea arabica* L. cv. Acaiá). Piracicaba. PhD Thesis – Escola Superior de Agricultura "Luiz de Queiroz". USP 147 p., 1987.

Srinivasan C. S. Pre-selection for yield in coffee. Indian Journal of Genetics, **42**: 15-19, 1982.

Vicente-Chandler J., Abruna F., Bosque-Lugo R., Silva S. El cultivo intensivo de cafe em Puerto Rico. Universidad de Puerto Rico, Estación Experimental Agrícola, Rio Piedras, P.R., Boletin, 218, 99 p. 1969.

Walyaro D. J. and van der Vossen H. A. M. Early determination of yield potencial in arabica coffee by applying index selection. Euphytica. **28**: 465-472, 1979.

Walyaro D. J. Considerations in breeding for improved yield and quality in arabica coffee (*Coffea arabica* L.). PhD Thesis. Wageningen. Agricultural University. 119 p., 1983.

Possibilities of Modifying Height, Architecture and Cup Quality of Arabica Coffee by Monitoring the Segregation of Caturra (*Ct*), Erecta (*Er*) and Mokka (*Mo*) Alleles*

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SUMMARY

Recent trends of the international market demand coffees produced under specific circunstances, pocessing unique atributes or with superior cup quality. The Ibairi cultivar although having the best flavor among the arabicas was never cultivated in Brazil because its low yieds and small seeds. From a cross of Ibairi (originally developed from var. *mokka* x Bourbon Vermelho) with a Caturra Erecta plant, a F_1 hybrid heterozygous for *mo* (mokka – determining good cup quality and modifying several morphologycal characteristics), *Ct* (caturra - short stature and compact growth) and *Er* (erecta – upright plagiotropic branches) was selfed, 362 F_2 plants and a seed sample from them were classified, yield in three consecutive harvests were recorded and corresponding F_3 progenies additionally scored in the nursery for *mo*, *Ct, Er* and vigor at seedling stage. Statistically significant, the homozygous *mo mo* F2 individuals yielded an average of 34,5% less than the normal (*Mo Mo*) segregants and 19,7% less than the heterozygous. The vigor of the F3 was increased by 4% on *mo mo progenies*. Yield decrease and vigor increase was observed irrespective of the genotypes at the caturra or erecta loci. *Ct* and *Er* singly or together did not have any effect on yield of F_2 but a 3,3% decrease in vigor was detected in the *Ct Ct* F_3 progenies. *Ct* and *mo* shorten the internodes of both ortho and plagiotropic branches, but unfortunately their jointly effect on the final architecture was not completely favorable as they were not sufficient to prevent the branches of erecta plants (*Er*) with a heavy load of cherries from bending over the rows or from frequently breaking off during the harvesting operation. However, it looks quite promising several short and compact caturra mokka plants with normal branches (*mo mo Ct Ct er er*). They might be further selected aiming at dense plantings that might compensate for the lower individual yields. The several traits that characterize mokka are not pleiotropic effects of the *mo* allele, provide they occasionally dissociate in F_2 plants and F_3 progenies. It is discussed the compelling evidences pointing out mokka as a trait derived from a species other than *C. arabica*.

INTRODUCTION

A modern trend of the international coffee market is the increasingly demand for products of unique characteristics or of high cup quality. Indeed, the explosion of gourmet and espresso coffee shops and the general betterment and diversification of available products in the major consumer centers is a well known fact. Prices paid for special coffees are gradually rising, investments in all areas of the gourmet sectors are steadily increasing and in Brazil – the world's largest producer – two nationwide contests of cup quality linked with premium prices stimulates farmers to improve the quality of their green coffee.

Many factors influence the final quality of coffee such as region, altitude, care during harvest, procesing type, storage conditions and several others. However, another important component of the final quality is the variety grown. Certainly, the genetic constitution of the variety determines the upper limits of the quality provided other influencing factors are the same. In other terms, for a specific region, if the microenvironment, cultural pratices, care and the processing system are all highly favorable, the final quality will ultimately depend on the genetic make up of the cultivar grown.

Years ago, many different items of the germplasm bank of the Instituto Agronômico de Campinas were subjectly but comparably cup tested for overall aroma, flavor and body revealing conspicuous differences among them that could be alloted to their genetic constitution provided they were grown, harvested, processed and tested with the same care, place and conditions. Some of such lines were further tested in great detail by the ICO in London (Cari, P. & Medina-Filho, H.P.; personal communication) confirming the genetic diversity for quality. Repeated assessment of the Campinas germplasm have indicated the superior cup quality of the cultivar Ibairi (IAC 4761) developed from the genetic recombination of the alleles *lr lr* (laurina) and *mo mo* (mokka) present in the original acession (Carvalho et al., 1990; Krug & Carvalho, 1951; Krug et al., 1939). Except for some hectares in Hawaii, where it is grown, Ibairi has never attainned commercial production, due to its unfavorable architecture and consistent low yields. Since the Ibairi besides *mo mo*, has the genetic background of the cultivar Bourbon Vermelho which itself has low yields, it is unknown whether the low yields are due to the Bourbon background or to a pleiotropic effect of *mo*. The cup quality of Ibairi, superior to Bourbon Vermelho is likely due to the pleiotropy of the *mo mo* genotype.

The Bourbon Vermelho is a regular, tall cultivar. In the last decades, incorporation of dominant short stature alleles like *Ct* (caturra) (Novaes Antunes et al., 1964; Carvalho & Monaco, 1972; Carvalho et al., 1984) in high yielding varieties has resulted in cultivars with short stature, short lateral internodes and high yields, preferred by farmers in plantings at high densities (Fazuoli et al., 1996; 1999).

Normal coffee varieties have the plagiotropic branches developing from the orthotropic branches at an angle of 25 degrees (Figure 3A). A completely dominant mutation named erecta (Carvalho and Krug, 1950; Carvalho, 1959), changes this angle to 65 degrees (Figure 3B), causing an upright growth of the plant which would theoretically allow further reduction in the spacings. However, there is not any commercial variety with the erecta characteristic. Investigators have claimed that branches of erecta plants on regular backgrounds bend over as they grow longer in adult plants (Figure 3C).

On the basis of above considerations, a breeding project was started in order to investigate the possibilities of developing lines with good yields, better cup quality, compact and upright growth through the recombination of alleles *mo*, *Ct* and *Er* and to critically evaluate the different combinations of them.

Grouth habits, yield results of three consecutive harvests of F_2 trees and seedling vigor of F_3 progenies are reported in this paper.

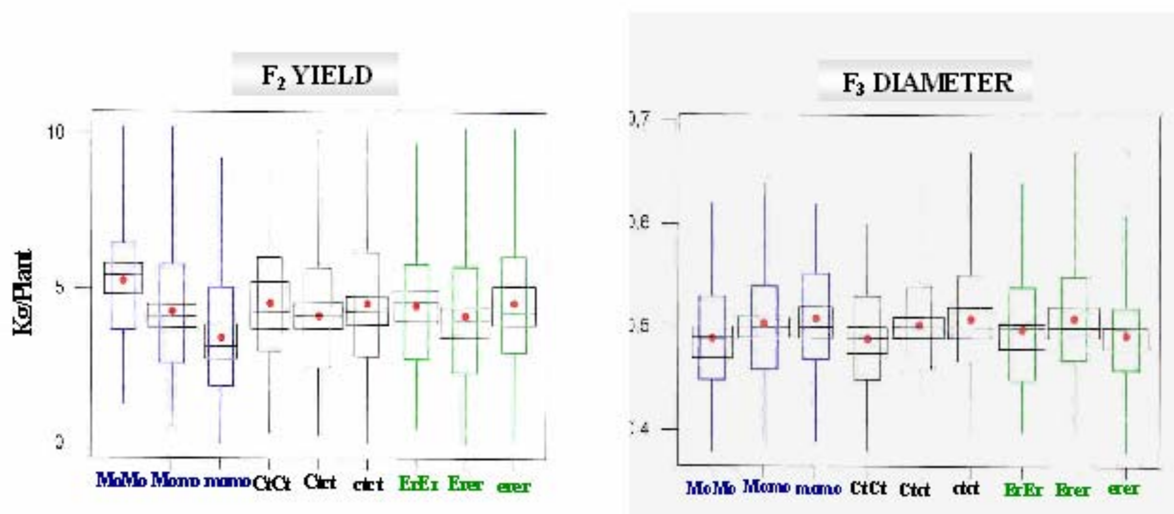

Figure 1. Yield of F_2 plants and vigor (mean stem diameter) of F_3 progenies according to their genotype at mokka, caturra and erecta loci. Box plots despict means (red dots), medians, first and fourth quartiles (whiskers) and 95% confidence intervals for the median (wider boxes)

MATERIAL AND METHODS

F_2 seeds were sown and seedlings raised in nursery at the Centro de Café in Campinas and randomly transplanted to the field (Figure 3D) at 4 x 2m spacing at the Estação Experimental in Mococa (21º28'S, 47º1'W, 665m altitude, loamy clayey Udals soil) where they were subjected to normal cultural pratices. Individual plants were harvested and yields recorded in clean coffee for the first three consecutive years. Individual bean samples of patio sun dried cherries were used for partially classifying the plants for mokka (Figure 3E) in the second year. Additional classification of each plant was done in the field for the general aspects characterizing mokka (Figures 3F, 2A, 2B), as wel as for caturra (Figure 3C) and erecta (Figure 3B). For 362 F_2 segregants, an F_3 progeny of 28 plants was raised in regular plastic bags in the nursery for one year, checked for the previous phenotypic classification of the parent F_2 plant and seeds and the heterozygous genotypes ascertained by the segregation of corresponding F_3 progeny. Since mokka and caturra affect leaf size and height, general vigor of the F_3 progeny was estimated by the mean diameter of the main stem of one year old seedlings measured at 5 cm above the soil. Single and joint effects of genotypes on yield of F_2 plants and vigor of F_3 progenies were evaluated by one-way ANOVA and 95% confidence intervals for mean of each genotype based on pooled standard deviation of each analysis. ANOVA and boxplot despicting descriptive statistics of the segregant population were performed with MINITAB 13 statistical software.

RESULTS AND DISCUSSION

The possible effect of the mokka allele on yield can be evaluated by investigating the yield of F_2 plants of genotypes *MO MO*, *Mo mo* and *mo mo* separately. The ANOVA was highly significant (F=17,8; p=0,000) being also different at 95%, the confidence intervals (CI) of the means of normal *Mo Mo* plants (5,3 Kg), heterozygous *Mo mo* (4,3 kg) and *mo mo* mokka individuals (3,5 kg). Thus, the mokka allele *mo* decreases the yield to a considerable extent. For this characteristics there is no recessiveness as additivity is evident. Homozygous mokka *mo mo* plants yielded an average of 34,5% less than the normal *Mo Mo* and 19,7% less than the heterozygous segregants. The heterozygous *Mo mo* population yielded 18,5% less than the

normal plants (Figure 1). Evidence of this effect is also provided by the analysis of plants considering the joint segregation of mokka with caturra and of mokka with erecta. In both cases the average yield is decreased in *mo mo* plants by 36,2% and the difference of the heterozygous to both homozygous from 17-23%. For those cases, the ANOVA was also highly significant with F=5,5 and 5,8 being p < 0,000 for both. The 95% confidence intervals for all the 9 genotypes clearly showed three groups of genotypes with decreased yields ascribed to *Mo Mo*, *Mo mo* and *mo mo* genotypes.

When the F_2 segregant population was grouped according to the three alternative genotypes of caturra (*Ct Ct, Ct ct, ct ct*) or erecta (*Er Er, Er er, er er*) (Figure 1) or even their joint segregation, no effect on yields were observed (F=1,51,p=0,22; F=1,29, p=0,28; F=0,81, p=0,60 respectively).

As to the effect on the vigor of the F_3 progenies, homozygous and heterozygous mokka plants showed a slight increase (4%) on the diameter (Figure 1) that were almost significant at 95% by the CI's, although the ANOVA was significant (F=3,43, p=0,033). The same statistical situation was observed with the homozigius Caturra (*Ct Ct*) decreasing an average of 3,3% the diameter in the F_3 progenies as compared with the heterozygous and homozugous genotypes. The major differences on the vigor of progenies were between those of the *mo mo ct ct* parents and *Mo Mo Ct Ct* or *Mo Mo Ct ct*, again showing the influence of both *Mo* and *ct* alleles, both decreasing vigor. As to erecta, the F=2,92 was not significant by itself or tested jointly with caturra. So the erecta does not affect the yield or the vigor of the progeny.

Erecta plants homozygous or heterozygous, which are totally indistinguishable between themselves, have a quite interesting shape in the first two years after being transplanted to the field. However upon bearing a heavy crop, several plagiotropic branches bent over the rows and frequently were broken off during the harvest operation. The new growth at the end of those branches normally resumes the upright orientation (Figure 3D), resulting in a quite disorganized and unfavorable architecture (Figure 3E). This was observed in all erecta plants, irrespective of its genotype at the caturra and/or mokka locus. Caturra erecta plants had, as one would expected, shorter internodes in the branches, a more compact architecture than the normal erecta, but unfortunately not compact enough to avoid branches from bending over. Actually in such genotypes, they do it at a less extent, but not sufficiently to overcome the problem. The effect of mokka in normal background segregants was observed as predicted, reducing leaf size, internodes length and increasing secondary and terciary lateral branching. Those branches however are thinner and more brittle than in normal plants, reason why it did not resulted either in a favorable combination with erecta.

Among the array of phenotypes produced by recombining six alleles at three loci, a group that looks promising so far is the mokka caturra plants (*mo mo Ct er er*). With normal angled branches shortened by caturra and further up by mokka with additional secondary laterals, the plants of such genotypes display besides the general mokka phenotype, a very compact aspect, shorter than normal caturra, with more conical shape (Figure 3F) that probably would withstand closer spacings than normal caturra. Although such plants are in general not very productive, the possibility exists that planting at closer spacings might compensate for the lower individual yields. Definitive interest in pedigree selection of those genotypes would thus await the realization of this possibility and the confirmation of its superior cup quality. Another feasible avenue of its exploitation is to use such lines in crosses with modern short stature cultivars described in Fazuoli et al. (1996 and 1999) in order to evaluate the same genotype in other genetic backgrounds.

Figure 2. Normal insertion of (A) plagiotropic branches and of (B) the erecta mutant, (C) bending over the row. (D) Field of F_2 plants segregating for mokka, caturra and erecta. (E) Bean sample of normal (above) plants and mokka (below). (F) Cherries of normal (left) and mokka (right) plants

Figure 3. (A) Leaf of normal (left) and mokka plants (right); (B) Typical dense lateral branching of mokka; (C) Normal caturra plant of genotype *MoMo CtCt erer*; (D) New growth of bended over erecta plant reasuming upright orientation; (E) Unfavorable architecture of erecta (Er) plant; (F) Promissing architecture of a mokka caturra plant with normal branches (*momo CtCt erer*)

On the course of scoring the F_3 seedling progenies in the nursery, it called the attention the fact that among progenies of homozygous or heterozygous mokka plants, seedlings with aneuploid phenotype (Cruz, 1972) appeared much more frequently (25%) than in lines not carrying *mo* (8%). Another aspect is that the typical mokka characteristics as classically described (Krug et al., 1939; Krug and Carvalho, 1951), is not always a totally constant phenotype. Suposely, homozygous *mo mo* plants have small and narrow leaves with large domatias, seedlings first branching at the 25-42nd internode and small round fruits bearing small dark green hemispherical flat beans. Although not investigated in detail, it was quite evident in some F_2 plants in the field and in the F_3 progenies in the nursery, that not all these characteristics were always associated and inherited as an invariable syndrome as some of this characteristics were lacking in some individuals classified as mokka. For instance, in some individuals the characteristic hemispheric seeds or the large domatias or the very narrow leaves was not seen. This observation suggests that the gene nature of mokka might be more complex, conjecturally composed of a gene block normally inherited as a single genetic unit but subjected, not rarely, to be genetically dissociated.

The general performance, characteristics and the aforementioned considerations on mokka lead to a further discussion of the subject. The data here presented indicated that the mokka allele causes a marked reduction on yield of adult plants and that homozygous seedlings are slightly but significantly more vigorous. The several characteristics of mokka seems not to be conditioned by a single gene with many pleiotropic effects, but likely a gene block most frequently, but not always, transmitted as a unit. Aneuploidy is three times more frequent in its progenies than in the progenies of normal (*Mo Mo*) genotypes. An extensive search of leaf miner attack in the living collection of Campinas indicated that in natural field conditions, all cultivars, botanical varieties and mutants of *Coffea arabica* were highly attacked by leaf miner except mokka, which showed reduced attack (Medina-Filho et al., 1977). Probably this was due to a general field non-preference of the plants by the insects, provide further test in closed boxes in lab conditions (Guerreiro-Filho et al., 1992), indicated that mokka is as susceptible as other arabicas.

The aforementioned facts about mokka constitute a sufficient array of evidences to consider the possibility that the mokka syndrome is not a single gene mutation but it has its genetic origin in other species than *C. arabica*. Other unique characteristics of mokka, the smallest flowers, leaves and smallest and most hemispheric seeds, the proportionally largest domatia, seedlings first branching after the 25th node instead of in the 12th, the highest oil content in the seeds, and the most flavorfull beverage found in the species adds to the list of compelling evidences pointing out mokka as of interspecific origin. In support of the uniqueness of characteristics of mokka, it is noteworthy to mention the keen statement of P. J. S. Cramer (1957) from Java that "*C. arabica* var. Mokka is so different from *C. arabica* typica ... that might well be considered another species." Although this refers to the true original mokka (*lr lr mo mo*) (Rauol, 1897; Krug & Carvalho, 1951; Carvalho et al., 1991) having a more extreme phenotype than the Ibairi (*Lr Lr mo mo*) which was developed from it, the general consideration seems to be equally valid converging toward the same direction. The subject surely deserves more detailed investigations.

If this conjecture turns to be true, it would be a quite interesting candidate to be addressed in a molecular assisted selection project in coffee. It would be an extreme favorable and attractive situation for the breeding point of view if a complex trait such flavor could be mostly inherited as a simple gene block conditioning superior cup profile and be molecularly monitored in a classical breeding program or for cloning specific sequences for using in genetic transformation.

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REFERENCES

Carvalho A., 1959. Genética de *Coffea*. Mutantes de *Coffea arabica* procedentes da Etiópia. Bragantia **18** (25): 353-370.

Carvalho A. & Krug C.A., 1950. Genética de *Coffea*: Hereditariedade da características erecta em *Coffea arabica* L. Bragantia. **10**: 321-328.

Carvalho A. & Mônaco L.C., 1972. Transferência do fator Caturra para o cultivar Mundo Novo de *Coffea arabica*. Bragantia **31** (31): 379-399.

Carvalho A., Medina-Filho H.P. & Fazuoli L.C., 1990. Recombinação genética derivada do café mokka com boa qualidade de bebida. 16º Congresso Brasileiro de Pesquisas Cafeeiras. Anais. Espírito Santo do Pinhal. p. 57-58

Carvalho A., Medina-Filho H.P., Fazuoli L.C. & Costa W.M., 1984. Números de locos e ação de fatores para porte pequeno em *Coffea arabica*. Bragantia **43** (2): 421-442.

Carvalho A., Medina-Filho H.P., Fazuoli L.C., Guerreiro-Filho O. & Lima M.M.A., 1991. Aspectos genéticos do cafeeiro (Genetic aspects of the coffee tree). Revista Brasileira Genetica **14** (1): 135-183.

Cramer P.J.S., 1957. A review of literature of coffee research in Indonesia. SIC Editional. Inter-American Institute of Agricultural Sciences. Turrialba, Costa Rica. 262 p.

Cruz M.D., 1972. Aneuplóides de café. Aspectos morfológicos e citológicos na análise de duas progênies de café Mundo Novo (*Coffea arabica* L.). Tese de Doutorado. ESALQ-USP, Piracicaba.

Fazuoli L.C., Medina-Filho H.P., Guerreiro-Filho O., Gonçalves W., Silvarolla M.B. & Lima M.M.A., 1999. Coffee cultivars in Brazil. Association Scientifique International du Café, 18 Colloque. Helsinki p. 396-404.

Fazuoli L.C., Medina-Filho H.P., Guerreiro-Filho O., Lima M.M.A., Silvarolla M.B., Gallo P.B. & Costa, W.M., 1996. Obatã (IAC 1669-20) e Tupi (IAC 1669-33), cultivares de café de porte baixo e resistentes a ferrugem. Congresso Brasileiro de Pesquisas Cafeeiras, 22º, Águas de Lindóia. p. 149-150.

Guerreiro-Filho O., Medina-FIlho H.P. & Carvalho A., 1992. Método para seleção precoces de cafeeiros resistentes ao bicho mineiro *Perileucoptera coffeella.* Turrialba, **42** (3): 348-358.

Krug C.A. & Carvalho A., 1951. The genetics of *Coffea*. In: Advances in genetics . Vol. IV, (DEMEREC, M, ed.). Academic Press, N.Y.

Krug C.A., Mendes J.E.T. & Carvalho A., 1939. Taxonomia de *Coffea arabica* L. Descrição das variedades e formas encontradas no Estado de São Paulo. Boletim Técnico nº 62. Instituto Agronômico, Campinas. 57 p.

Medina-Filho H.P., Carvalho A. & Mônaco L.C., 1977. Melhoramento do cafeeiro XXXVII. Observações sobre a resistência do cafeeiro ao bicho mineiro. Bragantia **36**: 131-137.

Novaes Antunes C.S., Mônaco L.C & Carvalho A., 1964. Melhoramento do cafeeiro. Produtividade de cafeeiros de porte reduzido. Bragantia **23** (32): 387-394.

Raoul E., 1897. Culture du caféier. 10 ed. Paris. A. Chalamel. p. 237-238.

Influence of Media Mixture and Watering Frequency on Seed Germination and Seedling Growth of Arabica Coffee

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SUMMARY

A split plot design with three replicates was used to arrange potting media and watering as main- and sub-plot treatments, respectively. The media treatments included topsoil (TS), forest soil (FS), coffee compost (C), and fine sand (S), which were blended in various ratios. The watering frequency consisted of four watering intervals applied at an interval of every 1, 2, 3 and 4 days/week starting from seed sowing up to the appearance of first pair of true leaf. Thereafter, these watering intervals were respectively extended and applied at intervals of every 2, 4, 6 and 8 days within a week up to field transplanting stage with six pair of true leaves. Germination count was made at every week intervals and subsequent extension and destructive growth parameters were recorded at the two growth stages. The results show significant effects of the main treatments on seed emergence and seedling growth parameters recorded at both stages. The use of compost as a media ingredient shortened mean days to emergence and resulted in maximum germination per cent and early seedling vigor. In other words, TS alone and media composition without compost (2TS: 0C:1S) resulted in significantly inferior seedling growth with the least shoot, root and total dry matter yields, particularly at the last growth stage. In contrast, seedling growth was more favored due to the use of FS and all other media types blended with composts and the results followed follow the order: FS > 3TS:1C:0S > 2TS:1C:1S > 2TS:1C:0S > 6TS:3C:2S > 3TS:2C:1TS > 1TS:1C:1S > 2TS:2C:1S, suggesting diverse potting media options in coffee nurseries. Both more frequent and infrequent watering delayed mean days to emergence while the fastest seed emergence was noticed on pots that received water at an intervals of every 3 days per week. Seedling growth data measured at the first pair of true leaf stage depict significantly lower results due to frequent watering at an interval of every other day. In other words, those seedlings that received water at an interval of every 2 (first stage) and 4 days (second stage) gave significantly higher results for most of the growth parameters, including shoot, root and total dry matter production. In general, media prepared without compost and delayed watering resulted in a stunted growth, with short plant height, thin girth and lower total dry matter yields noticed at both growth stages. On the other hand, treatment interaction had no marked effect on the growth responses at both growth stages. However, most parameters recorded at the first stage were maximum when water was applied at an interval of every 2 days on most media types. Similarly, interaction impact was not significant with increased seedling age, though maximum shoot and root growth parameters were noticed on most media that received water at a moderate interval of every 4 days/week.

INTRODUCTION

In Ethiopia, coffee is used as a cash source to the subsistent farmers and foreign exchange earnings to the country. Despite its decisive roles, the national average yield level is very low. This could be attributed to several production constraints: among which are poor and irregular stand establishments in the newly established modern coffee plantations. In this case, the

horizontal expansion of the improved disease resistant and high yielding coffee cultivars relies, among others, on the continuos production of large number of desirable coffee seedlings produced under optimum nursery management conditions. This is because the early growth potential of coffee seedlings is believed to put the most imprints on the chance of survival, uniform stand establishment and yield performances.

According to Mesfin (1982) one of the major problems, which accounts for the large percentage of tree death are the use of undesirable coffee seedlings with twisted and forked root. This could possibly be due to improper growing media compositions with inadequate physical and chemical status at the nursery critical growth stage (Chane, 1991; Taye, 1998). In this regard, the report of Yacob (1986) emphasised on the contributions of nursery soil media, mainly forest soils, to produce vigorous and healthy coffee seedlings. However, there is not only a diminished accessibility to the sources, but the accelerated deforestation practices would also call for alternative potting media prepared from the locally available organic sources with due consideration into both physical and chemical properties.

The establishment of successful coffee plantation depends, among others, on planting of vigorous and healthy seedlings raised in well-prepared soil media composition with optimum physical and chemical conditions. The soil mixtures used for germinating coffee seedlings in pots may be heterogeneous and determine the external factors affecting seed germination and seedling establishment (Veerendra and Raju, 1988). The physical properties of the soil influence the availability and uptake of water, aeration, the mineralization of organic matter, the emergence of seedlings and the establishment of root (Mayer and Mayber, 1975). The ripe coffee seeds may require a minimum of 25 to 30 days to germinate depending on the existence of optimum temperature and moisture, the aeration of the soil and initial pH (Veerendra and Raju, 1988).

Experiments in India indicate that soil mixture containing forest soil; well rotted farmyard manure and fine sand in 6:2:1 ration has been found to be desirable foe coffee seed germination and early growth (Veerendra and Raju, 1988). On the other hand, disinfected well-pulverized forest soil has been reported to be the best medium for coffee seed germination in Ethiopia (Yacob, 1986). Accordingly, the use of forest soil alone was common in the major coffee growing areas. The access to get forest soil is, however, becoming difficult because of the escalating rate of deforestation. The need to prepare potting media from locally available organic materials that can mimic forest soils becomes imperative to reduce the reliance on forest soils and expand modern coffee plantations.

In addition, conventional seedbed watering is widely practiced at most nursery sites without much considering the soil types, media compositions, techniques of sowing, stages of seedlings and prevailing climatic conditions of the area. The limited available information (Tesfaye, 1995) indicate that watering frequency at an every 4 to 8 days per week has been found to be advantageous for coffee seedlings grown on a medium composed of soil, compost and sand in a ratio of 6:3:1, respectively. However, the influence of different media mixtures and watering intervals on seed germination and subsequent growth of coffee seedlings has not been studied. This experiment was, therefore, aimed to bridge this gap and the specific objective of the study was, therefore, to select and determine optimum proportions of topsoil, coffee husk compost and sand in association with different watering frequency for successful seed emergence and growth of Arabica coffee seedlings.

MATERIALS AND METHODS

The study was conducted at Jimma Agricultural Research Center of the Ethiopia Agricultural Research Organization. The center is found in the southwestern parts at an altitude of 1750 meters above sea level. Geographically, it is the located at coordinates of 7° 46' N latitude and 36° 0' E longitude. The average annual rainfall recorded over the last 30 years is 1500 mm with the mean maximum and minimum temperatures of 25.0 and 11.2°C, respectively.

A factorial experiment arranged in a split plot design with three treatment replicates was used to assign ten media types and four watering intervals as main-and sub–plot treatments, respectively. Topsoil, decomposed coffee husk compost and fine sand were brought to the nursery site, dried, crushed and sieved through sieve size of 2 mm. The media ingredients were blended in various ratios and firmly filled in black diothene 200-gauge polythene tube of 12 cm wide 24 cm long.

Ripe red cherries from a known coffee berry disease resistant coffee selection (7440) were selectively harvested and the seeds were sorted out for uniform size and sown at the rate of two seeds per bag. This is to reduce risks of germination and to obtain enough sample seedlings to measure agronomic data at true leaf and transplanting stages. The watering treatments varied vis-a-vis the growth stages of coffee seedlings. That means, water was applied at an interval of every 1, 2, 3 and 4/week starting from sowing up to the appearance of the first pair of true leaves and the interval were reduced and doubled to the respective intervals of 2,4,6 and 8 days/week thereafter. The plots were covered with plastic sheet to protect from rains. All nursery management activities were as adhered to the recommendations of the center (Yacob, 1986; Tesfaye, 1995). Emergence count and extension growth parameters were recorded at every week intervals within a month to calculate mean days to emergence and emergency rate. In addition, destructive parameters were carefully recorded from those coffee seedlings thinned out at true leaf stage with a view to determine early seedling vigor indices. Finally, both non-destructive and destructive growth parameters were measured from the remaining sample seedlings at normal transplanting stage with six pairs of true leaves. Intact leaf area was estimated using a prototype developed by Yacob et al. (1993). The different plant parts that included leaf, stem, branch and roots were separated and weighted using sensitive balance. Here, soil intact roots were immersed in clean water to remove the soil. Square paper was put under clean glass to count the total number of squares covered by lateral roots and calculate its length. Subsequent to fresh weight measurement, the samples were kept in an oven at 100°C for 24 hours to record dry weights. Finally, the relevant data were subjected to statistical analysis according to the procedures described by Gomez and Gomez (1984) and treatment mean separation was undertaken using DMRT at 5% probability level.

RESULTS AND DISCUSSION

Media composition

Influenced mean days to emergence, emergence rate and seedling vigor. As a result, the least values were recorded from media without compost. In other words, the incorporation of organic compost shortened days to seed emergence and ensured more germination per cent and early seedling vigor (Table 1).

Table 1. Influence of media composition and watering interval on seed emergence and seedling vigor of Arabica coffee

Treatment	Mean days to emergence	Germination rate (%)			Seedling vigor indices		
		60 days	90 days	Over all mean	60 days	90 days	Over all mean
Media							
TS	81.28	3.12	44.80	68.75 b	9.46	67.83	547.89 c
FS	79.70	10.40	69.77	87.50 a	33.62	226.06	1848.36 a
6TS:3C:2S	72.53	30.20	82.30	83.33 ab	117.79	321.22	1349.14 ab
3TS:2C:1S	72.07	31.25	85.42	87.50 a	117.12	320.36	1190.76 ab
3TS:1C:0S	72.20	25.00	78.12	80.21 ab	97.52	305.33	1451.19 ab
2TS:1C:1S	73.59	27.05	72.92	75.00 ab	110.10	296.61	1279.30 ab
2TS:1C:0S	72.27	30.20	80.20	83.33 ab	125.21	311.17	1315.23 ab
2TS:0C:1S	83.05	4.17	42.70	68.7 5b	9.79	107.84	540.13 c
1TS:1C:1S	70.33	31.22	80.20	81.25 ab	125.18	321.68	1021.32 bc
2TS:2C:1S	73.78	26.02	76.07	82.29 ab	109.07	318.82	990.29 bc
Watering frequency (day/week)							
Every 1	62.62	32.04	73.30	82.08	95.07	217.50	1180.84 ab
Every 2	63.95	19.21	70.36	77.92	69.55	254.08	1366.92 a
Every 3	60.79	19.96	67.45	78.33	77.25	261.04	1044.82 b
Every 4	67.07	17.44	72.89	80.83	68.40	285.88	1020.86 b

This could be associated with maximum contact between seed and soil as well as optimum temperature and aeration within the media that enhance seed water imbibition and ensure fast and maximum germination as Devlin and Witham (1983) has indicated it. Media treatment had also significant impact on most growth parameters taken at the first pair of true leaf stage. In this case, except girth and shoot dry weight, most growth parameters: plant height, tap root length, leaf dry weights and total dry matter yield were significantly higher for coffee seedlings grown on forest soil and media types blended with topsoil, compost and sand in various ratios (Table 2, Figure 1).

This could probably be attributed to the improved soil conditions due to compost and this concurs with the works of Chane (1991) and Taye (1998) who have reported the benefits of coffee composts to improve soil physico-chemical conditions and growth performance of coffee seedlings.

Extension and destructive growth results recorded at normal transplanting stage depict more pronounced impact of media treatment. All growth parameters, except number and length of lateral roots were significantly differed among media types where the use of topsoil alone or in combination with sand alone resulted in the most inferior values for all parameters. The highest shoot to root value was recorded on compost blended media with high organic matter content and the values that ranged between 2.09 and 4.72 for TS and 3TS: 2C:1S, respectively (Table 3).

Similarly, most seedling growth parameters were more favored by the use of media treatments in the descending order of FS > 3TS: 1C: 0S > 2TS: 1C: 0S> 3TS: 2C: 1S (Table 3, Figure 2).

This is in line with the data taken at the first pairs of true leaf stage, indicating the need to incorporate coffee compost in nursery media, mimicking forest soils. The incorporation of sand didn't bring significant variation on the parameters measured before and at the appearance of the first pair of true leaf and thereafter. This shows that the dominant coffee soil has little problem as far as its physical condition is concerned. This is in agreement with (Coste, 1992; Paulos, 1994) who reported that coffee soil is more friable and deeper, but it is deficient in major plant nutrients. In general, the results depict that the addition of compost greatly improved seed emergence and subsequent growth performances, suggesting the need to blend topsoil with organic materials to prepare diverse types of alternative media types with ideal chemical conditions like forest soils. (Yacob, 1986) has recommended forest soil that is widely in use at most nurseries. The limited access to obtain forest soil and the possibility to simulate it by using organic composts available in the area has been elaborated in our previous findings (Chane, 1991; Taye 1998).

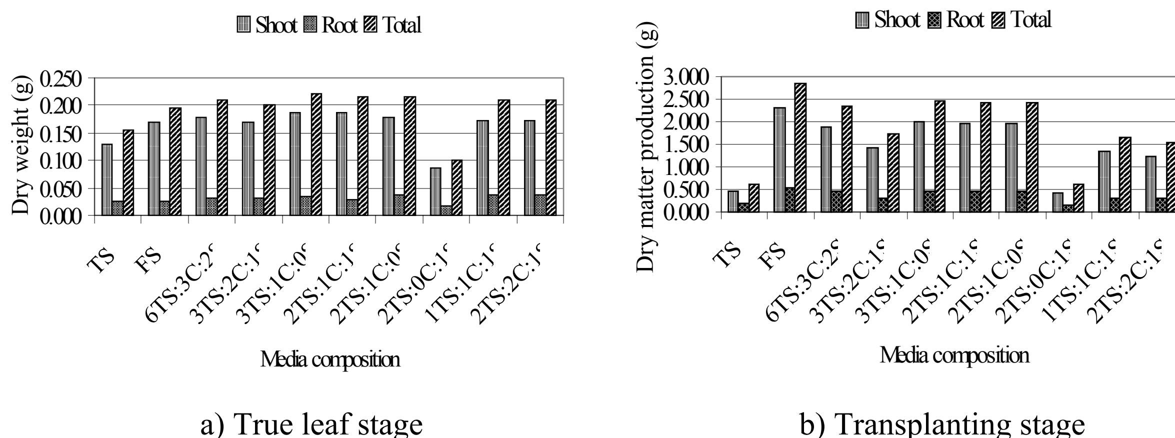
Table 2. Effect of media composition and watering frequency on the early growth of Arabica coffee seedlings (harvest at first pair of true leaf stage)

Treatment	Plant height (cm)	Girth (cm)	Leaf dry wt (g)	Stem + branch dry wt (g)	Shoot dry wt (g)	Tap root length (cm)	Root dry wt (g)	Total dry wt (g)
	Media							
	**	NS	*	NS	*	**	**	*
TS	6.97 b	0.22	0.09	0.04	0.13ab	6.89 c	0.026 b	0.16 ab
FS	7.49 ab	0.21	0.12	0.05	0.17a	7.95 b	0.025 b	0.19 a
6TS:3C:2S	7.66 ab	0.23	0.13	0.05	0.18a	9.32 a	0.033 ab	0.21 a
3TS:2C:1S	7.55 ab	0.23	0.12	0.05	0.17a	8.83 ab	0.031 ab	0.20 a
3TS:1C:0S	7.91 ab	0.22	0.13	0.05	0.19a	9.09 ab	0.035 ab	0.22 a
2TS:1C:1S	8.06 a	0.24	0.13	0.06	0.19a	8.91 ab	0.029 ab	0.22 a
2TS:1C:0S	7.95 ab	0.23	0.13	0.05	0.18a	8.93 ab	0.037 a	0.22 a
2TS:0C:1S	5.75 c	0.20	0.06	0.03	0.09b	5.87 c	0.016 c	0.10 b
1TS:1C:1S	8.16 a	0.23	0.12	0.05	0.17a	9.30 a	0.036 a	0.21 a
2TS:2C:1S	7.91 ab	0.24	0.12	0.05	0.17a	8.90 ab	0.037 a	0.21 a
SEM(df=18)	0.31	0.01	0.01	0.05	0.02	0.35 b	0.003	0.02
CV (%)	14.03	14.05	47.22	21.28	43.38	14.48	32.26	40.13
	Watering frequency (day/week)							
	**	**	**	**	**	**	NS	**
Every 1	6.76 b	0.21 b	0.09 b	0.04 b	0.13 b	7.37 b	0.029	0.16 b
Every 2	7.64 a	0.23 ab	0.12 a	0.05 a	0.17 a	8.41 a	0.031	0.20 a
Every 3	7.95 a	0.23 a	0.12 a	0.05 a	0.17 a	8.95 a	0.030	0.20 a
Every 4	7.81 a	0.24 a	0.13 a	0.05 a	0.18 a	8.88 a	0.033	0.21 a
Mean	7.54	0.23	0.12	0.05	0.16	8.40	0.031	0.19
SEM (df=60	0.15	0.003	0.01	0.002	0.01	0.35	0.002	0.01
CV (%)	10.64	7.51	27.31	26.25	26.37	22.67	27.15	25.44

Watering frequency

Watering intervals had no clear marked pattern on mean days of emergence, emergence rate and seedling vigor. However, frequent watering at every other day interval gave maximum germination per cent and seedling vigor up to 60 days after sowing. Maximum seedling vigor results were noticed with decreased watering frequency and the highest result was recorded

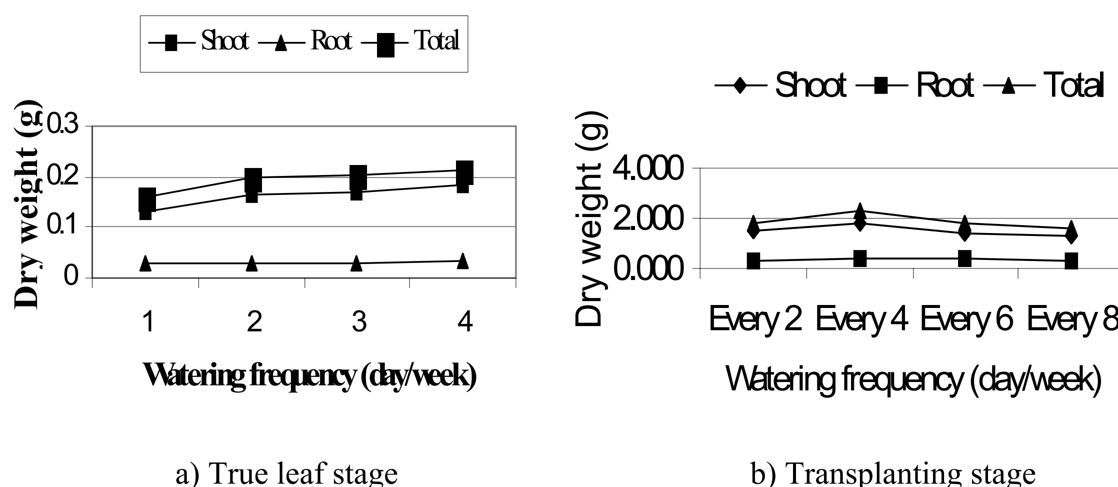
from seedlings, which received water at 4 days interval per week (Table 1). This indicates the importance to consider coffee seedbed watering intervals in relation with the growth stages of coffee seedlings and this is in line with the report of Tesfaye (1995).



a) True leaf stage

b) Transplanting stage

Figure 1. Effect of different media compositions on dry matter production of coffee seedlings at two growth stages



a) True leaf stage

b) Transplanting stage

Figure 2. Dry weight of coffee seedlings as influenced by watering intervals applied at two growth stages

Similarly, all the agronomic data taken at transplanting stage of six pairs of true leaves, except taproot length and shoot to root ratio, were significantly influenced due to watering treatments, which was delayed from the initial watering intervals. Accordingly, seedlings that received water every 4 days had significantly maximum responses as compared with those seedlings receiving more frequent and delayed water (Table 3). Non-destructive (Table 3) and destructive (Table 4) seedling parameters were significantly influenced by various media composition and watering treatment. Shoot, root and total dry matter yield were significantly influenced by watering treatment where maximum values were measured at a watering interval of every 4 days and reduced results were noticed with more delayed watering (Figure 2). This was more evidenced on the above ground shoot growth as compared with roots. Consequently, decreased shoot to root ratio was observed with reduced watering intervals and the results ranged from 4.10 to 3.58 for watering intervals of 2 and 8 days, respectively (Table 4).

Table 3. Non-destructive growth parameters of coffee seedlings grown under different media composition and watering frequency

Treatment	Plant Ht (cm)	Girth (cm)	No. of Nodes	Internode Length (cm)	Leaf		Tap root Length (cm)	No of Lateral roots	Lateral root Length (cm)
					Number	Area (cm^2)			
	Media								
	**	**	**	**	**	**	*	NS	NS
Top soil	11.73 e	0.23 e	5.38 c	2.23 bc	5.96 c	5.41 d	23.00 bc	56.83	167.90
Forest soil	24.20 a	0.37 a	8.83 a	2.72 a	14.25 a	18.54 a	31.18 a	74.38	274.71
6TS:3C:2S	20.12	0.34 abc	8.00 ab	2.47 abc	12.88 ab	14.39 bc	27.44 ab	54.88	174.08
3TS:2C:1S	18.86 cd	0.32 abc	7.88 ab	2.34 bc	13.13 ab	13.90 bc	22.11 bc	56.67	154.77
3TS:1C:0S	22.60 ab	0.37 a	8.38 ab	2.71 a	13.88 a	16.12 ab	24.80 bc	57.46	169.50
2TS:1C:1S	21.51	0.36 ab	8.13 ab	2.55 ab	13.17 ab	16.73 ab	25.72 bc	53.96	208.85
2TS:1C:0S	21.50	0.34 abc	8.25 ab	2.57 ab	13.58 a	16.18 ab	23.93 bc	59.17	222.33
2TS:0C:1S	11.74 e	0.24 de	5.38 c	2.19 c	6.67 c	5.84 d	21.81 c	56.08	169.17
1TS:1C:1S	17.84 d	0.30 bc	7.67 ab	2.29 bc	12.08 ab	13.79 bc	24.65 bc	53.33	132.75
2TS:2C:1S	16.88 d	0.29 cd	7.46 b	2.18 c	11.08 b	11.83 c	24.47 bc	55.25	169.77
SEM	1.10	0.02	0.37	0.10	0.72	1.15	1.58	7.81	32.09
C.V (%)	20.32	20.08	16.98	14.87	21.24	30.04	21.98	46.83	60.28
	Wateringfrequency (day/week)								
	**	**	**	**	**	**	NS	*	**
Every 2	18.85 b	0.322 b	7.77 ab	2.37 b	12.03 ab	14.20 ab	23.99	53.57 b	158.42 b
Every 4	21.50 a	0.351 a	8.00 a	2.62 a	12.78 a	15.90 a	26.19	63.88 a	216.22 a
Every 6	17.57 b	0.299 c	7.37 bc	2.33 b	11.15 bc	12.03 bc	25.64	56.34 b	184.87 b
Every 8	16.88b	0.289 c	7.00 c	2.38 b	10.70 c	10.96 c	23.83	57.42 ab	178.03 b
Mean	18.70	0.32	7.53	2.42	11.67	13.27	24.91	57.80	183.38
SEM (df=	0.67	0.01	0.16	0.62	0.42	0.89	0.83	2.51	9.70
C.V (%)	19.75	14.20	11.80	14.10	19.73	36.59	18.16	23.77	28.80

*Figures followed by the same letter(s) within a column are not significantly different at 0.05 probability. NS = Non-significant, *,** = significant at P≤0.05 and P≤0.01, respectively*

In other words, frequent watering resulted in luxurious vegetative growth with succulent stem and thin leaves. Whereas, more delayed watering that exceeding may cause moisture deficit of the media and stunted growth is an inevitable phenomenon. In general, the findings indicate the importance of water application at moderate intervals to favor vegetative growth with higher total dry matter production of coffee seedlings. The result also reveals the importance to manipulate soil media to grow coffee seedlings with the desired shoot and root growths with more efficient use of soil moisture and ensured performances both under nursery and field conditions. Tesfaye and Berga (1997) have reported similar findings to produce high quality transplantable coffee seedlings.

Treatment interaction effect

The interaction effect of media by watering frequency was not significant for most of the growth parameters recorded at both growth stages. However, seedling growth response was more favored when most of the media treatments receive water at intervals of every 2 days/week during the first stage and at 3 to 4 days interval/week during the latter stage. Accordingly, relatively maximum total dry weight was noticed due to water application at

interval of every 4 days and the results tended to decline thereafter. In other words, treatment interaction impact had not significant impact with increased seedling aging, thought the application of water at an interval of every 4 days had maximum total dry matter of coffee seedlings grown on most media types. Hence, maximum response was noted on such media treatment as FS, 6TS:3C:2S and 2TS:2C:1S in that order when water was applied at moderate intervals. This was in contrast to the results recorded on TS and 1TS:1C:1S where reduced total dry matter was noticed with more delayed watering. However, with the exception of topsoil, the other media treatments in interaction with watering frequency at intervals of 2 days per week showed maximum results for most growth parameters studied. This probably comes from the benefits of composts with rich organic matter to conserve soil moisture as it has been reported in previous findings (Chane, 1991; Taye, 1998). Our present findings generally suggest the importance to consider watering intervals in relation with media compositions and growth stages of coffee seedlings.

Table 4. Destructive parameters of coffee seedlings as influenced by media and watering interval

Treatment	Leaf weight (g)		Stem + branch weight (g)		Shoot weight (g)		Root dry weight (g)	Shoot to root ratio	Total dry matter (g)
	Fresh	Dry	Fresh	Dry	Fresh	Dry			
	Media								
	**	**	**	**	**	**	**	**	**
Topsoil	0.91 d	0.25 d	0.53 d	0.20 dc	1.44 d	0.45 d	0.18c	2.09 c	0.63 d
Forest soil	5.93 a	1.50 a	2.31 a	0.81 a	8.25 a	2.30 a	0.55a	4.40 ab	2.85 a
6TS:3C:2S	4.67 ab	1.21 abc	1.86 abc	0.68 abc	6.53 ab	1.89 abc	0.43ab	4.15 ab	2.34 abc
3TS:2C:1S	3.80 bc	0.95 bc	1.44 bc	0.49 bcd	5.24 bc	1.44 bc	0.31bc	4.72 a	1.75 bc
3TS:1C:0S	4.92 ab	1.27 ab	2.05 ab	0.73 ab	6.97 ab	2.00 ab	0.48ab	4.16 ab	2.47 ab
2TS:1C:1S	4.73 ab	1.25 ab	1.94 ab	0.70 abc	6.67 ab	1.95 ab	0.47ab	4.32 ab	2.42 abc
2TS:1C:0S	4.85 ab	1.29 ab	1.80 abc	0.66 abc	6.65 ab	1.95 ab	0.47ab	4.40 ab	2.43 abc
2TS :0C:1S	0.93 d	0.24 d	0.48 d	0.19^{e}	1.41 d	0.43 d	0.17c	2.36 c	0.60 d
1TS:1C:1S	3.45 c	0.88 bc	1.33 bc	0.46 bcde	4.78 bc	1.35 bc	0.30bc	4.37 ab	1.65 bc
2TS:2C:1S	3.20 c	0.81 c	1.10 cd	0.41 cde	4.29 c	1.22 c	0.32bc	3.75 b	1.54 c
SEM (df=18)	0.45	0.13	0.25	0.09	0.68	0.22	0.07	0.27	0.28
C.V. (%)	41.30	45.60	57.10	60.5	44.96	50.30	61.00	23.92	51.50
	Watering frequency (day/week)								
	**	**	**	*	**	**	**	NS	**
Every ½	3.87 b	0.96 b	1.45 b	0.50 b	5.32 b	1.45 b	0.34 b	4.10	1.79 b
Every 2/4	4.78 a	1.20 a	1.91 a	0.65 a	6.69 a	1.85 a	0.45 a	4.03	2.30 a
Every 3/6	3.33 bc	0.89 b	1.35 b	0.52 b	4.68 b	1.41 b	0.35 b	3.78	1.76 b
Every 4/8	2.98 c	0.81 b	1.23 b	0.46 b	4.21 b	1.28 b	0.34 b	3.58	1.62 b
Mean	3.78	0.96	1.49	0.53	5.22	1.50	0.37	3.87	1.87
SEM (df=60)	0.28	0.07	0.13	0.04	0.40	0.12	0.02	0.17	0.14
C.V. (%)	41.10	41.95	46.93	45.60	41.99	42.69	31.60	24.67	39.71

*Figures followed by the same letter(s) within a column are not significantly different at 0.05 probability. NS =Non-significant, *, ** = significant at P≤ 0.05 and P≤ 0.01, respectively*

CONCLUSION

Topsoil and media composition without compost had the most inferior growth responses at both stages. Hence, different alternative potting media, mimicking forest soils, with better physical and chemical conditions can be prepared by blending decomposed coffee composts. More frequent watering prolonged mean days to germinate and inhibit subsequent growth stages whereas delayed watering resulted in stunted seedling growth. The finding also show the advantages of water application to coffee nursery seedbeds at moderate intervals to fasten germination, favor subsequent growth responses of coffee seedlings. It can be, therefore, suggested that watering frequency should consider media composition and growth stages of coffee seedlings so as to produce high quality coffee seedlings with the right proportion of shoot and root growth that can ensure maximum field establishments. However, the current findings should be repeated under diverse coffee growing agro-ecology and edaphic conditions.

REFERENCES

Chane Abate (1991). Effect of media composition, volume and nutrition on *Coffea arabica* L. Seedlings M.Sc thesis, Alemaya University of Agriculture, School of Graduate Studies, Pp 146.

Coste R. 1992. Coffee: The plant and the product. Macmillian, London.

Devlin R.M and Witham F.H.(1983). Plant physiology (4th ed.). New Delhi: CBS.

Gomez K. A. and Gomez A.A. (1984). Statistical Procedures For Agricultural Research. (2nd.). A Wiley-International Publication John Wiley and Sons., Inc., New York.

Veerendra Kumar M.M and Ruaju T. (1988). Seed germination and seedling growth of Robusta Coffea Canephora Pierre ex Froehner, as influenced by nursery soil mixture. Indian Coffee, **52** (4).

Mayer A.M. and Pojakoff-Mayber A. (1975). The germination of seeds. Second ed., vol. **5**, Perganon press, New York.

Mesfin Ameha (1982). A review of coffee nursery research in Ethiopia. Papers presented at Coffee Agronomy Workshop, Addis Ababa, Ethiopia.

Paulos Dubale (ed.). (1994). Mineral fertilization of coffee in Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia.

Taye Kufa (1998). Response of Arabica coffee to various soil fertility management. MSc thesis, School of Graduates Studies, Alemaya University of Agriculture, pp 137.

Tesfaye Shimber (1995). Influence of nursery watering frequency, moisture status of rooting media and media moisture conservation on growth of coffee (*Coffea arabica* L.) seedlings. M.Sc Thesis, School of Graduates Studies, Alemaya University of Agriculture.

Tesfaye Shimber and Berga Lemaga (1997). Influence of watering frequency on seedling growth of arabica coffee. Pp 52-63. In: Weldeyesus Sinebo (ed.). 1997. Crop management research for sustainable production: status and potentials; proceedings of the 2nd Annual Conference of the Agronomy and Crop Physiology Society of Ethiopia (ACPSE). 30-31May 1996, Addis Ababa, Ethiopia.

Yacob Edjamo (1986). Review of coffee nursery at Jimma Research Centre (Melko). In: Proceeding of the first coffee symposium in Ethiopia, 21-26, August, 1986, Addis Abeba, Ethiopia (in press).

Yacob Edjamo (1993). Relative performance of three CBD resistant coffee cultivars under varying light regimes. Fifth International Scientific Colloquium on Coffee, Montpelier, France.

Organic Coffee Production: Hope for Small-Scale Farmers in Ethiopia

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SUMMARY

In Ethiopia, coffee is grown under four main production systems. These include, forest, semi-forest, garden and plantation coffee, which account for 10, 35, 50 and 5% of the total volume produced in the country, respectively. In other words, smallholder, rain-fed and generally low input-output oriented cropping system, predominantly characterizes coffee based farming system. That means ninety-five percent of the coffee production system is based on traditional practices under natural forest canopy and can be considered as bird friendly or shade grown organic coffee, although not yet officially documented and certified. Besides the traditional farming, the immense genetic potential in Ethiopia helped breeding works to select coffee types adaptable, tolerant to diseases and insect pests in the local climates. Th use of proper management practices, including maintenance of tree vigor through pruning and rejuvenation, optimum planting pattern and shade regulations to disfavor diseases, insects and weeds and recycling of organic materials to maintain soil fertility greatly helped to circumvent yield losses and curtailed the use of agro-chemical inputs. In other words, organic coffee production with minimum use of non-renewable resources and minimum destruction to the environment deserved closer attention at the whole range coffee production. *In-situ* and *ex-situ* conservation of coffee gene pool and complementing farmer's indigenous knowledge also become important research aspects. In light of the existing agro-ecological and socio-economic opportunities, it is therefore imperative to formulate a platform for the key stakeholders involved in coffee productions with the interest to create awareness and provide efficient record keeping taking into account the standard criteria for quality control of smallholder farmers'. On the whole, maximum exploitation of the existing untapped potentials to produce organic coffee in Ethiopia requires the assistance of global organic coffee consumers as well to support and facilitate the certification process. This would ensure not only to export accredited demand led fully organic coffee but also it is the only hope for small-scale coffee farmers to sustain coffee cultivation and gain from the premium prices offered for it.

INTRODUCTION

Organic farming may be defined as the growing of crops without the application of harmful chemical inputs. These include inorganic fertilizers, pesticides and herbicides (Schmidt, 1998). It relies more on the use of organic and green manure, bio-fertilizers and biological control of diseases and insect pests. One of the benefits of organic farming is that it may reduce costs, although yields tend to decrease (Kotchi, 1990). This is particularly important to the subsistent Ethiopian farmers who have limited access, can not afford to purchase agro-chemicals due to the escalating prices and low profitability of the chemicals. Consequently, most smallholder farmers in Ethiopia remain to produce low crop yields using traditional means of production systems.

In Ethiopia, smallholders posses less land size, not exceeding on an average one to two hectares of coffee farms. This is largely attributed, among others, to the increasing population pressure at

an alarming rate. On the other hand, coffee market potential and prices is not as such encouraging to expand or intensify coffee cultivation, unless the low coffee yield produced under traditional management practices is certified and sold at premium price. In other words, farmers are market sensitive and there is a fear that they may cut the land race coffee types and replace by other cash crops, causing genetic loss of Arabica coffee materials. This will become a great challenge to Ethiopia in particular and to the international coffee market in general and calls for closer attention to look for efficient means to set up a national certifier for coffee in Ethiopia and secure coffee farmers to remain in the system.

The traditional low-input and -output oriented coffee production systems offered our subsistent coffee farmers the scope for the production of organic coffee. This has been documented in the recent report of Scholer (2000) and Taye et al. (2000a) who reported that more than 90% of the coffee grown in Ethiopia is organic, but there is no certification system. In addition to the traditional production system, there are no enzymes used in the processing and marketing stations or in the fertilization process. As a result, Ethiopia is one of the few countries with several opportunities to produce organic coffee to the international market, thought there is no certifying body in the country. However, the government of Ethiopia is encouraging farmers to continue growing coffee organically and promoting high quality coffee on the world market in line with the desire of the consumers, particularly to the west where organic products are highly desired. For this, formal designation is very relevant to adopt IFOAM's accreditation programme criteria for smallholder certification (Heid, 1999) and to apply the various methods of inspection described in IFOAM and IOAS (1998). This is with the purpose to enhance the export of accredited organic coffee and hence improve the marketing potential and prices for small-scale farmers. This paper is, therefore, an attempt to share experiences on and to highlight the current status and prospects of organic coffee production in Ethiopia with fellow conference participants, who hail from both coffee producing and consuming countries with the aims to seek for assistance to expedite the certification process.

METHODOLOGY

This paper is a culmination of work done on organic coffee production mainly by Jimma Agricultural Research Center, which holds the national mandate to co-ordinate Coffee Research in Ethiopia, and the Coffee and Tea Authority. The existing enormous prospects, current traditional organic coffee production systems, which takes in to account plating materials, soil fertility management, crop protection and quality control are highlighted. Furthermore, future areas of focus and options were suggested to improve the traditional organic coffee farming systems and enhance the export of accredited organic coffee to the global market.

Production potentials

Arabica coffee of high genetic diversity originated in the forest ecosystem of the south and southwestern Ethiopia where the ideal climate and soils offer the characteristic features for its sustainable cultivation as semi-wild or spontaneous and naturalized coffee plantation. That is why, traditional smallholder coffee production farms provide 95 to 97% of the national production. In more specific terms, forest, semi-forest, garden and plantation coffee account for 10, 35, 50 and 5% of the total volume produced in the country, respectively (Workafes and Kassu, 2000). Coffee is considered the most important single commodity of the country since it contributes 60 to 70% of the foreign exchange earnings and 30% to direct government revenue. The crop is, thus, of cardinal importance with 8.4% output to the agricultural sector and on which 25% of the population depends for livelihood in production, processing and marketing.

With regard to climate, in Ethiopia coffee grows at various altitudes, ranging from 550-2,750 meters above sea level (m.a.s.l). However, the bulk of Arabica coffee is produced in the eastern, southern and western parts of Ethiopia, which have altitudes ranging from 1,300-1,800 m.a.s.l. The annual rainfall in the coffee growing regions of the country varies from 1,500-2,500 mm. Where production is less, as in the eastern part of the country, which has only 1,000mm per annum, it is supplemented with irrigation. It is not only the total rainfall that is important for good coffee production but also its distribution. Rainfall distribution in the southern and eastern parts of the country is bimodal, and in the western part is mono-modal. These distribution patterns enable the country to harvest coffee at different times of the year, ensuring a supply of fresh beans all year round. Arabica coffee grows best in the cool, shady environment of the forests of the Ethiopian highlands. The ideal temperature is considered 15-25°C and it prevails in the most coffee growing areas of the country. In essence, altitude, temperature, soil and rainfall are among the factors, which determine the suitability of an area for coffee production in Ethiopia and Admassu (1992) reported their level of suitability.

The diversified types of Arabica coffee in its homeland country with ideal climatic and soil conditions contributed to produce high quality coffee with unique high land Moka flavor and aroma types and consumers know Ethiopian coffee as highland coffee. It has a great deal to offer in the way of gourmet, specialty and organic coffees. Ethiopian coffee is rich in acidity and body. It possesses an aromatic and sweet flavor and is characterized by winey, spicy notes and the world famous mocha tastes so highly prized by connoisseurs. Because it has so much to offer, it can be enjoyed as a single variety and it can also be blended with coffees from other origins to upgrade them. The existence of EU-member states involved in rural development projects in general and coffee improvement projects in particular is another opportunity to assist and facilitate the certification process and enhance complete conversion of the current traditionally produced organic coffee into organic farming system with an organic management plan (Scmidt, 1998).

Planting materials

In Ethiopia, wild coffee types are grown spontaneously in the humid hot forests of south and southwestern parts where the natural forest cover is more or less intact. This forest grown coffee is heterogeneous and offers a wide diversity for selection and breeding so as to have plant stock selected for disease resistance, high yields and top quality. Consequently, about 800 arabica coffee accessions have been collected in the national collection program and are at various research stages. In light with the current deforestation practices, limited forest coffee areas in southwestern parts of the country were delineated for *in-situ* conservation (Paulos and Demel, 2000). In garden coffee production system, the planting materials could be local and/or improved, adaptable and disease resistant varieties. The disease resistant cultivars have been selected and released to the users and are under production. Most of these coffee selections (74110, 74112, 74140, 74148 and 74158) are of compact canopy types (IAR, 1996) and are suitable for organic coffee production system. Because, cultivars with compact canopy spatial arrangement are more suitable for close planting and more productive during the early cropping stages as there can be suppressed weed growth and more efficient use of available soil resources such as manure, moisture and light (Yacob et al., 1996).

Soil management

The soils in the southern and western parts of the coffee growing regions of the country are volcanic origin, with a high nutrient-holding capacity for clay minerals. The Mesozoic layer, made up of sandstone and calcium carbonate, is found in the eastern part of the coffee-growing

region. All the coffee-growing regions have fertile, friable, loamy soils, with a depth of at least 1.5 m. The topsoil is dominantly dark brown or brownish in colour, with a pH ranging mostly from 5-6.8. One outstanding characteristics of the coffee soil is that its fertility is maintained by organic recycling i.e., through litter fall, pruning and root residue from the perennial coffee and shade trees (Paulos, 1994).

In the wild and semi-wild production systems, coffee grows under natural forest ecology with more fertile soil. The decomposition of litters and self-mulching maintained the fertility of the soil and protected the soil from erosion. This has been documented by Paulos (1994) who found no significant coffee yield responses under forest conditions. In garden and modern coffee plantations, coffee trees are mostly fertilized with locally available organic materials and off-farm disposals. That means, the smallhoder coffee farmers, who are the major producers, use organic fertilisers to supplement the natural fertility of the soil. This is because, mixed farming of crops and livestock is an inseparable farmers' practice in the major coffee growing agro-ecology of Ethiopia, indicating organic matter is easily recycled into the soil and according to the rules of organic farming (Schmidt, 1998) there is no need to apply agrochemical. The bulk of coffee processing by-products from unwashed and washing coffee processing stations are commonly recycled in coffee farms where it is used as mulch and organic fertilizer sources. The advantages of using organic farmyard manure and composts in coffee has been documented in our previous report (Taye et al., 2000b). These include improved soil physico-chemical conditions and better growth performances of young and mature coffee trees.

Cognizant of the ability of leguminous plants to fix atmospheric nitrogen, focused research attention is given to investigate the roles of temporary and permanent leguminous trees and forage legumes in association with coffee trees in different cropping patters are under way in the major coffee growing agro-ecology of Ethiopia. In most cases, *Albizia spp, Acacia spp, Cordia africana, Milletia spp, Erytherinia spp, Sesbania sesban* and *Leucenia lecocephala* are widely used as coffee shade trees (Yacob et al., 1996; Tsegaye and Taye, 2000). Crop residue management, crop rotation between coffee plots, inter-cropping coffee with fruits and other horticultural crops, alley cropping and strip cropping coffee with leguminous trees and other traditional agro-forestry systems are practiced by the farmers, largely to provide organic matter and humus to the soil and sustained soil fertility status. Suitable land preparation techniques, including banding, tied and untied ridges, planting vetivar grass are also used to control soil erosion and conserve soil moisture and plant nutrients. Integrated soil fertility management practices is practiced in large-scale coffee farms where there is also naturally grown shade trees.

Weed control

In Ethiopia, coffee grows under natural forest ecology where the growth of noxious weeds is suppressed. Accordingly, little or no management is practiced, often limited slashing the undergrowth prior to harvesting. In other words, farmers slash the weeds once a year to facilitate harvesting of the coffee beans. In newly planted modern coffee plantations, grassy weeds are common and different cultural practices, including slashing and digging, hoeing, cover crops, mulch and shading (Tadesse et al., 1998). Due to shortage of land area, farmers often use close planting that can offer a high degree of ground cover in early stage to suppress weed growth. In addition, almost all coffee farmers grow coffee under shade conditions where cultural practices can efficiently combat the annual broad leaf weeds (Kassahun and Taye, 1994; Tsegaye and Taye, 2000). The feasibility of integrated weed management in the diverse coffee production systems is under investigation.

Disease and insect control

The use of land race coffee types well adapted to the environment and disease resistant cultivars helped coffee growers not to depend on agro-chemicals. In addition, due to the high cost of the chemicals or their scarcity in the local market farmers don't spray their coffee farms. In this regard, several agronomic practices that included rejuvenation, pruning, soil and moisture conservation, population adjustment, shade regulation were recommended to manipulate the micro-climate of coffee trees in a way to disfavor disease causing pathogens and insect pests and improve tree vigor for sustainable yields (Yacob et al., 1996). Currently, integrated pest management approach and natural/botanical pest and disease control methods are underway to draw recommendations in line with the basic standards of IFOAM (1992).

Harvesting and processing

Coffee harvesting is done by human labor to selectively pick only the fully ripe cherries. Such a labor-intensive process contributes to the high cost of coffee. In Ethiopia, coffee is processed by two widely known methods: dry and wet. Ethiopia exports 80-85 per cent natural or sun-dried coffee. Normally, cherries are dried on mats on drying table of local material and raised a meter high, or on concrete or cement drying floors. The basic raw materials required from successful hulling are full dried cherries of reasonable and even size. The ripe cherries are spread out to dry out on mats. They are turned frequently for 2-3 weeks to ensure even drying, and they are then placed in the hullers to remove the dried pulp and the inner skin.

The country is well known for this high quality wet-processed coffee because of a well-established and linked structure that connects coffee farmers, processing plant owners, governmental organization and coffee purchasing enterprises, leading to effective quality control and efficient marketing. The pulped wet parchment coffee goes to the different fermentation tanks to ferment naturally. The process is carefully supervised to avoid under or over fermentation. The wet parchment coffee is dried in morning and afternoon sun on raised drying tables and stored at 11 to 12 per cent moisture content. In this method about 15-20 per cent of the total coffee is processed.

Quality control

In Ethiopia, coffee grading and quality control are effected at the producer, regional and central level. This integrated control system helps to grade coffee before auction and export, which is very important for all those involved in the production, collection, export and consumption of coffee. The present coffee grading and quality control system is based on both green and cup-quality assessments, which include the cleanliness of the cup and the origin, taste and character of the coffee. Because, coffee grading and quality control are very useful in encouraging good quality coffee production and ensuring dependable and competent exporters and in increasing lasting business friendships with overseas clients.

CONCLUSION

Ethiopia is the primary center of origin and genetic diversity for the highland Arabica coffee, which contributes more than 60% to the country’s foreign exchange earnings. The livelihood of about a quarter of the total population directly or indirectly depends on its production, processing and marketing. Thus, as well as being an important export crop, coffee plays a vital role in both the cultural and the socio-economic life of the country.

It is known that, Ethiopia is endowed with several opportunities to produce true organically grown Arabica coffee. These include, among others, suitable altitude, ample rainfall, optimum temperatures, fertile soil and untapped resources of coffee germplasm. The current production systems where the highest proportion (95%) of the national coffee yield comes from smallholders, who relay on the traditional low-input and-output oriented- production systems, remain maintain traditionally organic coffee. Above all, maintaining and planting of adaptable coffee types under natural forest strata and suitable agro-forestry system with leguminous shade trees, soil organic matter amendment through recycling of plant materials and the low economic level of the farmers not to afford expensive inputs also contributed to grow agro-chemical free coffee farms. In addition, selective picking of fully ripe red cherries by hand and natural wet processing and sun drying as well as integrated quality control systems enabled to export the unique high quality coffee types, thought there is no certification systems in Ethiopia.

Full exploitation of the existing potentials of organic coffee farming systems calls for efficient collaboration between Ethiopia and importer countries to promote and sustain high quality organic coffee production and thus ensure the health situation of the consumers and upgrade the economic status of the rural subsistent coffee farmers in Ethiopia. Assessing farmers' indigenous knowledge and their attitudes to be organized in a way to enhance organic coffee and identifying the extent, constraints and opportunities of organic coffee farming systems are some of the areas that need closer attention. For this, training to local research and development staff is of a paramount important to increase awareness and provide proper information at the whole range of the production, processing, packing and marketing. Above all, the roles of institutions involved in organic product certification and labeling and government policy supporting and pushing the adoption of the guidelines and basic standards of IFOAM (1992) are very crucial. In addition, establishment of local, national and international certifying agencies become crucial to ensure the success of developing consumer led organic Arabica coffee production in its homeland country and for the international recognition and for the benefits of Ethiopia in particular and the international markets in general.

REFERENCES

Admassu Shiferaw (1992). Coffee Arabica L. suitable areas in Ethiopia. Coffee and Tea Authority (CTA) "Bunachin" Megazine, February 1992, Addis Ababa, Ethiopia.

Coffee and Tea Authority, CTA (1999). Ethiopia Cradle of the wonder bean coffee Arabica (Abssinica), Coffee and Tea Authority megazine, Addis Ababa, Ethiopia, Pp 36.

Gast H. and Richter (eds.) (1995). Ecologically appropriate agri-culture: A manual of ecological agriculture development for smallholders in development areas, DSE and ZEL, Fedafing, Germany.

Heid P. (1998). The weakest go to the wall. LEISA, ILEIA Newsletter for low external input and sustainable agriculture, **14** (2): 14-15.

IAR (1996). Recommended production technologies for coffee and associated crops. Research extension task force of Jima Agricultural Research Center, Institute of Agricultural Research (IAR), Addis Ababa, Ethiopia.

IFOAM (1992). Coffee and tea guide lines. International Federation of Organic Agriculture Movements (IFOAM), Thorey-Theley, Geramny.

IFOAM and IOAS (1998). Building trust in organic: A guide to setting up organic certification programmes, complied by Gunner Rundren with the support of Sida.

Kotchi J. (ed.) (1990). Eco-farming practices for tropical smallholdings. GTZ Tropical

Agroecology[5].

Kassahun Siyoum and Taye Kufa (1997). Farmer's weed management in coffee based farming systems of Jima areas. Pp 35-44. In: Fasil Reda and D.G. Tanner (eds.). Proceedings of the Second Annual Conference of Ethiopian Weed Science Society (EWSS), Arem, Vol **2** and **3**, Addis Ababa, Ethiopia.

Paulos Dubale (ed.) (1994). Mineral Fertilization of Coffee in Ethiopia. Institute of Agricultural Research, Addis Ababa, Ethiopia.

Paulos Dubale and Demel Teketay (2000). The need for forest coffee germaplasm conservation in Ethiopia and its significance on the control of coffee berry disease (CBD) in Ethiopia. Pp 125-135. In: proceedings of the workshop on the control of coffee berry disease (CBD) Ethiopia, Addis Ababa (Ghion Hotel), 13-15 August 1999.

Tadesse Eshetu, Paulos Dubale and Bayissa Mormonne (1998). Weed control in the western coffee growing areas of Ethiopia. Pp 22-28. In: Beyene Seboka and Aberra Deressa (eds.). Agricultural Research and Achievements in Western Ethiopia. Proceedings of the Third Technology Generation, Transfer and Gap Analysis Workshop, 12-14 November 1996, Nekemt, Ethiopia.

Taye Kufa (1998). Response of Arabica coffee to various soil fertility management. MSc thesis, School of Graduates Studies, Alemaya University of Agriculture, 137Pp.

Taye Kufa, Tesfaye Shimber and Yehasab Aschalew (2000a). An overview of organic coffee production in Ethiopia. P 278. In: Thomas Alfoldi, William Lockeretz and Urs Niggli (eds.). Proceedings of 13th International IFOAM 2000 Scientific Conference, 28-31 August 2000, Basel, Switzerland, vdf Hochschulverlag AG an der ETH Zurich.

Taye Kufa, Mesfin Abebe and Paulos Dubale (2000b). Effects of NP and organic fertilizers on growth and developments of coffee (*Coffea arabica* L.) seedlings. Fourth All African Crop Science Conference, 11-14 October 1999, Casablanca, Morocco, African Crop Science Conference Proceeding (in press).

Tsegaye Yilma and Taye Kufa (2000). Informal survey on the farming systems of Haru wereda: Western Wellega Zone. A Research Report, Pp 32 (in press).

Schmidt H. (ed.) (1998). EU Regulation "Organic Farming": A legal and agro-ecological commentary on the UE's Council Regulation (EEC) Number 2092/91/Hanspeter Schmidt; Manon Haccius: Margraf, GTZ, Germany.

Scholer M. (2000). Coffee-organic certification and labeling. P280. In: Thomas Alfoldi, William Lockeretz and Urs Niggli (eds.). Proceedings of 13th International IFOAM 2000 Scientific Conference, 28-31 August 2000, Basel, Switzerland, vdf Hochschulverlag AG an der ETH Zurich.

Workafes WeldeTsadik and Kassu Kebede (2000). Coffee production systems in Ethiopia. Pp 99-106. In: proceedings of the workshop on the control of coffee berry disease (CBD) in Ethiopia. Addis Ababa (Ghion Hotel), 13-15 August 1999.

Yacob Edjamo, Tesfaye Shimber, Taye Kufa, Alemseged Yilma, Takele Negewo, Anteneh Netsere and Bekele Bogale (1996). Advances in coffee agronomy research in Ethiopia. Pp 40-45. In: Tenywa J.S., Adipala Ekwamu, M.W. Ogenge-Latigo (eds.). Proceedings of Inter-African Coffee Organization (IACO) workshop, 4-6 September 1995, Kampala, Uganda.

Technological and Morphological Characterization of Coffee Commercial Lines Developed by IAC – Brazil*

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SUMMARY

The Instituto Agronômico de Campinas (IAC) has developed a large number of coffee inbredlines. Although the IAC cultivars are already commercially in use, they are constantly beeing characterized and this characterization is essentially accomplished by analysis of their genetic origin and agronomic descriptors. We investigated if quantitative variables could be useful for the establishment of a Clusters Analysis, allowing the identification of coffee inbredlines of the reported cultivars.

According to the analyses the quantitative variables evaluated are efficient to identify the different coffee cultivars, but are insufficient to identify correctly the inbredlines from those cultivars. Molecular markers may represent an alternative tool to identify these inbredlines.

INTRODUCTION

Brazil is responsible for about 27% of World coffee production (Anuário Estatístico do Café, 2000). Coffee always occupied a high rank in the Brazilian commercial balance and nowdays about 4% of all exportations are due by this product.

Since 1932, the Instituto Agronômico de Campinas (IAC) has developed an extensive Program of Coffee Breeding. During this period, a large number of coffee inbredlines was developed and many of those were introduced and recommended for plantation in different regions of Brazil.

In respect to variability, these cultivars of *C. arabica* L. have a very narrow genetic origin, beeing derived from basically two cultivars: *C. arabica* L. var. Typica and *C. arabica* L. var. Bourbon.

The cultivars were developed to attend producers demand for specific agronomic traits, such as higher productivity, *C. arabica* L. var. Bourbon Amarelo (Carvalho et al., 1957), *C. arabica* L. var. Mundo Novo and *C. arabica* L. var. Acaiá (Fazuoli, 1977); management, *C. arabica* L. var. Catuaí Amarelo, *C. arabica* L. var. Catuaí Vermelho (Carvalho and Monaco, 1972), and *C. arabica* L. var. Ouro Verde (Fazuoli, 2000); resistance to diseases, *C. arabica* L. var. Icatu Amarelo, *C. arabica* L. var. Icatu Precoce, *C. arabica* L. var. Icatu Vermelho (Fazuoli, 1991), *C. arabica* L. var. Tupi and *C. arabica* L. var. Obatã (Fazuoli, 2000); resistance to nematodes, *C. canephora* Pierre var. Apoatã (Fazuoli, 1986); and cup quality, *C. arabica* L. var. Bourbon Amarelo (Fazuoli, 1986) and *C. arabica* L. var Ibairi.

Although the IAC cultivars are already commercially in use, they are constantly beeing characterized and this characterization is essentially accomplished by analysis of their genetic origin and agronomic descriptors (Carvalho and Fazuoli, 1993).

These analyses aim not only the evaluation of developed cultivars, but also a precise technological and morphological characterization that will allow future identification of an unknown cultivar.

So far, the utilization of a reduced number of qualitative variables such as fruit color, resistance to *Hemileia vastatrix*, uniformity during maturation, young leaf colour and plant stature, allow the identification of all cultivars selected by IAC. However, these same variables are insufficient for the identification of different inbredlines of these cultivars.

In this work we investigated if quantitative variables could be effective for setting up a Clusters Analysis, allowing the identification of coffee inbredlines of the reported cultivars.

MATERIALS AND METHODS

A total of 30 plants were selected from each inbredline, based on their general appearance and productivity (Table 1).

Table 1. Coffee Inbredlines and Cultivars developed by IAC

Cultivars	Inbredlines	Cultivars	Inbredlines	Cultivars	Inbredlines
Catuaí Amarelo	IAC 47	Icatu Amarelo	IAC 2944-6	Icatu Vermelho	IAC 2945
Catuaí Amarelo	IAC 62	Icatu Precoce	IAC 3282	Icatu Vermelho	IAC 4040
Catuaí Amarelo	IAC 74	Bourbon Amarelo	IAC 18	Icatu Vermelho	IAC 4042
Catuaí Amarelo	IAC 86	Obatã	IAC 1669-20	Icatu Vermelho	IAC 4045
Catuaí Amarelo	IAC 100	Tupi	IAC 1669-33	Icatu Vermelho	IAC 4046
Catuaí Vermelho	IAC 44	Acaiá	IAC 474-4	Mundo Novo	IAC 376-4
Catuaí Vermelho	IAC 46	Acaiá	IAC 474-16	Mundo Novo	IAC 379-19
Catuaí Vermelho	IAC 81	Acaiá	IAC 474-19	Mundo Novo	IAC 388-17
Catuaí Vermelho	IAC 99	Ouro Verde	IAC 4395	Mundo Novo	IAC 501
Catuaí Vermelho	IAC 144			Mundo Novo	IAC 515

The following variables were evaluated in this work:

- Morphologic variables: plant (height and diameter); leaf (length, width and petiole length); flower (corolla tube length, stamen length, style-stigma length, number of stamens and number of petals); fruit (length and width); seed (length, width and thickness).

- Technologic variables: fruit (empty fruit rate); seed (out-turn rate, thousand-bean weight, bean grade, flat, peaberry and elephant-bean rate); cup quality (fragrance of ground coffee, aroma, defects, acidity, bitterness, flavor, aftertaste, body and overall).

RESULTS AND DISCUSSION

The results regarding the analysis of variables used for morphologic characterization, technologic characterization of fruits and seeds and technologic characterization of cup quality were submit to a Principal Component Analysis (ACP). The distribution of inbredlines in respect to the axis projected are illustrated respectively in Figures 1, 2 and 3.

The morphologic characteristics (Figure 1) can separate with relative efficiency the cultivars with high stature from the short stature ones. The variables plant height and plant diameter, correlated to the axis 2, have an important role in this aspect. The axis 1 is composed basically by leaf characteristics. It can be remarked that at experimental conditions, the cultivars Mundo Novo, Acaiá and Bourbon Amarelo have smaller leaves than the others cultivars. Important divergences among inbredlines are observed on the cultivars Catuaí (short stature) and Icatu (high stature).

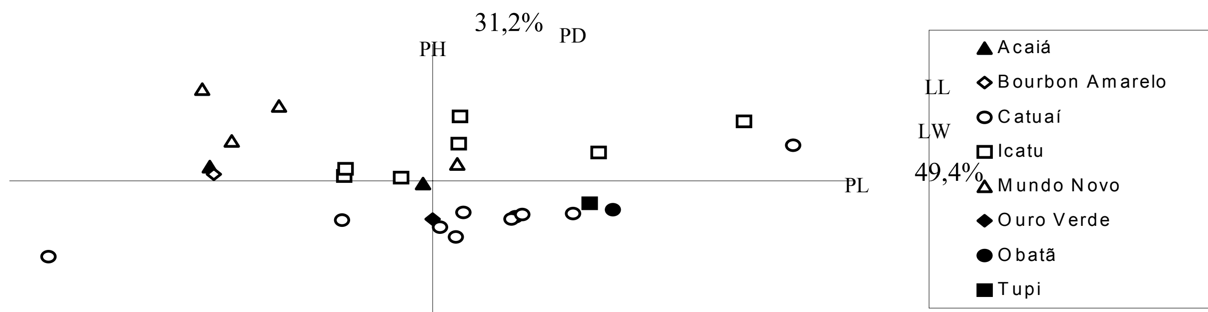

Figure 1. Principal Component Analysis. Relationship of variables plant height (PH), plant diameter (PD), leaf length (LL), leaf width (LW) and petiole length (PL). Representation on plan 1-2 for cultivars of *C. arabica* selected by IAC. Distribution of the cultivars regarding morphologic characteristics

In respect to technologic characteristics of fruits and seeds (Figure 2), the plan 1-2 can separate with efficiency two inbredlines of Acaiá cultivar from the others inbreds. They show highest values for the characteristics analyzed, while the cultivar Icatu Amarelo shows the smallest values for the same characteristics. The variables fruit length, seed length, width and thickness are highly correlated by the axis 1, demonstrating to be efficient for the differentiation of cultivars. It was observed that, at experimental conditions, the cultivars Icatu Vermelho, Obatã and Mundo Novo have the highest values for the variables empty fruit rate and peaberry plus elephant bean rate. These high values for the cultivars Icatu Vermelho and Obatã may be correlated with their interespecific origin (*C. arabica* x *C. canephora*). This origin could lead to cytological incompatibilities during meiosis, resulting in aborted embryos. Regarding the variable flat bean rate it can be observed that cultivars Catuaí Vermelho and Catuaí Amarelo showed the highest values.

The distribution of inbredlines from different cultivars of *C. arabica* on the plan 1-2 evidence the uniformity existent among the cultivars, based on the technologic characteristics of cup quality (Figure 3). Generally the data analyzed show that the cultivars are closely related and eventual differences observed can be explained by environment effects concerning management conditions. The non-significant distance observed among inbredlines from a cultivar, like Catuaí Amarelo or Catuaí Vermelho, seems to confirm this affirmative. It can be observed that variables such as flavor, aroma and aftertaste display high correlation among themselves, and also in respect to the overall quality.

CONCLUSIONS

According to the analysis carried out in this work the quantitative variables evaluated are effective to identify different coffee cultivars. However, the same parameters are insufficient to identify correctly inbredlines from those cultivars. Then it becomes necessary the use of other parameters, that could access the genetic and phenotypic differences observed among

the inbredlines studied. Molecular markers, such as SSR and AFLP, may represent alternative tools to identify these inbredlines.

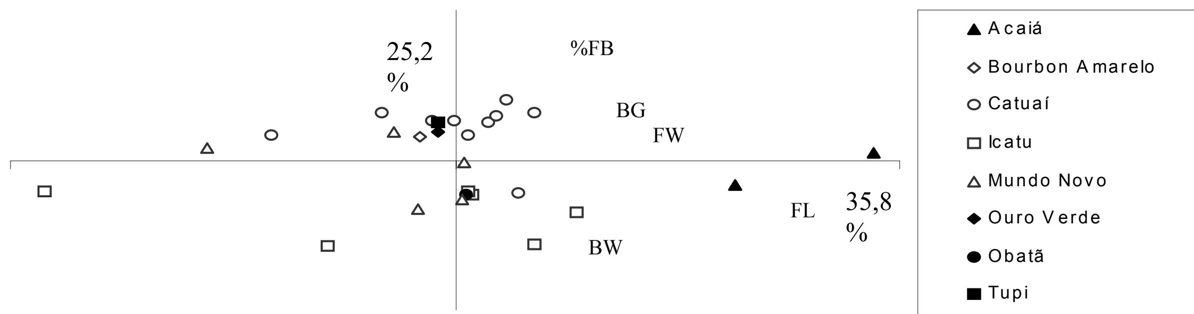

Figure 2. Principal Component Analysis. Relationship of variables empty fruit rate (% EF); fruit length (FL); fruit width (FW); out-turn rate (% OT); thousand bean weight (BW); bean grade (BG); flat-bean rate (% FB); peaberry and elephant-bean rate (% PE); seed length (SL); seed width (SW) and seed thickness (ST). Representation on plan 1-2 for cultivars of *C. arabica* selected by IAC. Distribution of the cultivars regarding technologic characteristics of fruits and seeds

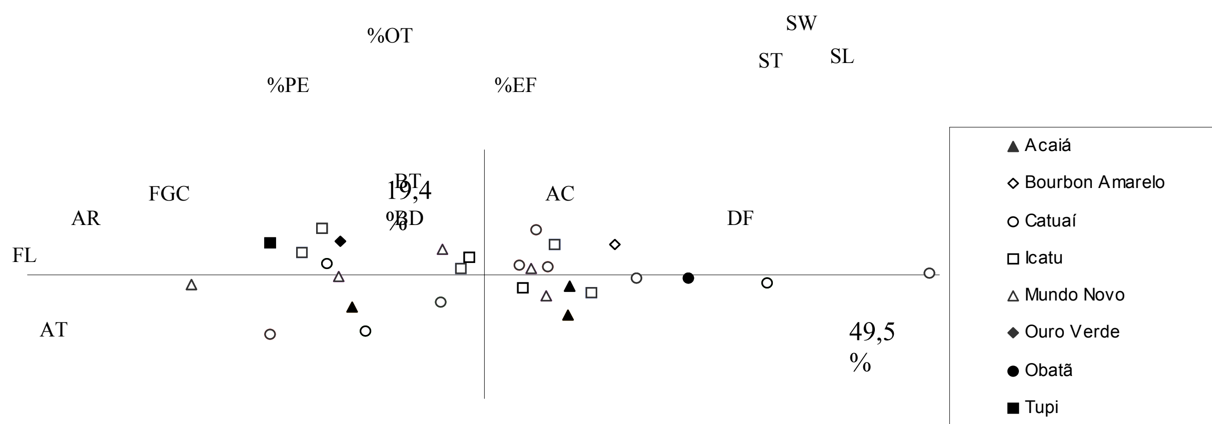

Figure 3. Principal Component Analysis. Relationship of variables fragrance of ground coffee (FGC); aroma (AR); defects (DF); acidity (AC); bitterness (BT); flavor (FL); aftertaste (AT); body (BD) and overall (OA). Representation on plan 1-2 for cultivars of *C. arabica* selected by IAC. Distribution of the cultivars regarding technologic characteristics of cup quality

REFERENCES

Anuário Estatístico do Café, 6º edição, 2000/2001.

Carvalho A., Antunes Filho H., Mendes J. E. T., Lazzarini W., Junqueira Reis A., Aloisi Sobrinho J., Moraes M. V. de, Nogueira R. K. & Rocha T. R. Melhoramento do cafeeiro. XIII - Café Bourbon Amarelo. Bragantia, Campinas, **16** (28): 411-454, 1957.

Carvalho A. and Fazuoli L. C. In: Café. O melhoramento de plantas no Instituto Agronômico. Ed. Furlani A. M. C. and Viégas G. P. Campinas, 1993, Cap. 2, p. 29-76.

Carvalho A. and Monaco L. C.. Transferência do fator Caturra para o cultivar Mundo Novo de *Coffea arabica*. Bragantia, Campinas, **31** (31): 379-399, 1972.

Fazuoli L. C. Avaliação de progênies do café Mundo Novo (*Coffea arabica* L.). Piracicaba, 1977. 146 p. (Mestrado-Escola Superior de Agricultura "Luiz de Queiroz").

Fazuoli L. C. Genética e melhoramento do cafeeiro. In: Rena A. B., Malavolta E., Rocha M., Yamada T., org. Cultura do cafeeiro. Piracicaba, Potafós, 1986. p. 87-113.

Fazuoli L. C. Metodologia, critérios e resultados da seleção em progênies do café Icatu com resistência a *Hemileia vastatrix*. Campinas, 1991. 322 p (Doutorado-Universidade Estadual de Campinas).

Effects of Overhead Irrigation with and without Fungicide Sprays on Colletotrichum Kahawae Sporulation and Coffee Yield

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SUMMARY

Production of spores of *Colletotrichum kahawae* the Coffee berry disease (CBD) pathogen on disease lesions on berries from overhead irrigated an unirrigated plot with and without fungicide treatments was studied. The results indicated that irrigation did not significantly activate sporulation of the pathogen on the disease lesions. The implication of the results in relation to incidence and control of CBD are discussed.

INTRODUCTION

Overhead irrigation in coffee is usually applied to even out shortfalls in rainfall amounts and distribution. Its benefits include increased proportion of grade AA beans and decreased proportion of grade TT beans (Gathaara and Gitau, 1999). The wetting of the canopy by irrigation water has however, been suspected to activate production of spores of *Colletotrichum kahawae* resulting in increased incidence of coffee berry disease (Mukunya, et al*.,* 1988). The magnitude of reactivation of sporulation from berry lesions was therefore studied.

MATERIALS AND METHODS

The trial was on a randomized block design with three treatments plus control. Twenty five tree plots replicated 4 times formed a treatment. Coffee berry disease (CBD) and leaf rust control was by Daconil/Copper tank mixture. Irrigation was applied during daytime at 76 mm at 28 days interval at soil moisture deficit of 100 mm (Gathaara and Kiara, 1988). Incidence of CBD and coffee yield were assessed using the method of Masaba (1986). Diseased berries were sampled before and after irrigation and the number of spores produced determined by haemocytometer.

RESULTS AND DISCUSSION

Results are summarized in Tables 1, 2, 3 and 4 and Figures 1 and 2. Spore production, CBD incidence and yield were not significantly affected by irrigation alone during both years. The + fungicide/+ irrigation treatment showed a significantly higher spore production than – fungicide/+ irrigation treatment during 1994 (Table 2) but this could be attributed to the rain during the period between irrigation applications. CBD was significantly higher in unsprayed treatments during 1993 and early 1994 regardless of the irrigation treatments but not significantly different in all treatments during the disease peak at 11.02.94 (Table 3). Coffee yield from the relatively minor early crop was significantly higher in the – fungicide treatments but the main crop from both fungicide treatments was not significant different (Table 4). Coffee is usually irrigated when prevailing weather conditions are not suitable (Nutman and Roberts, 1960) for appreciable fungal sporulation, dispersal and infection to occur.

Table 1. Spore production from cbd lesions on berries from various treatments 1993 (,000 spores/ml)

Treatment.		21.08	28.08	31.08	03.09	17.09	24.09	27.09	03.10
A	+F +I	113.82 a	61.36 a	129.45 ab	135.63 ab	99.30 a	93.68 a	89.45ab	111.5 ab
B	-F +I	126.76 a	61.36 a	111.15 bc	119.30 ab	36.36 b	54.03 ab	54.03b	99.30 ab
C	+F -I	74.30 a	86.36 a	86.36 bc	81.36 b	71.71 ab	71.71 ab	43.68b	36.36 c
D	-F -I	61.36 a	71.71 a	54.03 c	64.03 b	61.36 ab	36.36 b	36.36	54.03 bc

Table 2. Spore production from cbd lesion on berries from various treatments 1994 (,000 spores/ml)

Treatment		21.01	24.01	10.02	20.02	02.03	07.03
A	+F +I	90.97 bc	148.80 a	155.24 a	441.13 a	446.94 a	524.59 a
B	-F +I	70.71 c	103.03 b	120.30 b	280.02 b	383.59 ab	414.24 ab
C	+F -I	21.01 ab	130.15 ab	135.14 ab	396.27 ab	380.03 ab	530.27 a
D	-F -I	23.92 ab	120.32 ab	125.72 b	243.91 b	283.19 b	452.38 a

Table 3. Percentage of cbd infection on marked branches during 1993 and 1994

Treatment		1993			1994		
		17.08	13.09	21.10	13.01	11.02	14.03
A	+F +I	5.36 c	5.55 c	5.78 c	9.49 e	21.95 abce	15.10 ab
B	-F +I	13.31 a	16.67 a	16.08 a	17.28 abc	23.97 ab	11.63 bc
C	+F -I	5.31 c	6.42 c	5.16 c	13.10 cde	22.50 abc	15.60 ab
D	-F -I	13.00 a	15.29 ab	13.30 ab	18.64 a	26.07 a	11.57 bc

Table 4. Clean coffee yield (kg/ha) early crop - 1994

TREATMENT		Early crop 11.05-17.06.94	Late crop 25.10-05.01.95
A	(+) Fungicide (+) Irrigation	30.50 b	72.53 ab
B	(-) Fungicide (+) Irrigation	56.49 a	94.07 ab
C	(-) Fungicide (+) Irrigation	22.21 b	70.48 ab
D	(-) Fungicide (-) Irrigation	56.55 a	95.15 a

The dry period proceeding the accumulation of the 100 mm SMD are likely to inhibit sporulation from disease lesions on berries. Very few spores may therefore, be dispersed during irrigation. The optimum overhead irrigation for the traditional arabica coffee varieties in Kenya is reported to be 76 mm of water at 28 day intervals (Gathaara 1988). One

application can be completed within 1.5 hrs which falls short of the 5 hrs of wetness needed for germination and infection to occur (Waller, 1971). The hot and dry conditions are in addition likely to hasten the drying of the coffee surfaces reducing further the period when free water is available to the sores of the pathogen. Overhead irrigation as applied in this study and in combination with fungicide sprays may not increase sporulation of *C. kahawae* on berry lesions or the incidence of CBD. There is however need to study the effect of other often practiced irrigation regimes including higher rates and night time application.

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REFERENCES

Gathaara M. P. H. and Gitau K. M. (1999). The influence of overhead irrigation on the yield and quality of arabica coffee cultivar Ruiru 11. Kenya Coffee Vol **64** No. 756.

Gathaara M. P. H. and Kiara J. M. (1988). Effects of irrigation rates and frequency on the growth and yield of arabica coffee. Kenya Coffee, April 1988, Pg 309-312.

Mukunya D. M., Ondieki J. J., Opile W. R. and Kairu G. M. (1988). The current status of CBD: Occurrence and control in Kenya and factors associated with its increased severity. Consultancy Report, CRF, 1988.

Nutman F. J. and Roberts F. M. (1960). Investigations on a disease of Coffea arabica caused by a form of Colletotrichum coffeanum Noack II. Some factors affecting germination and infection, and their relation to disease distribution. Trans. Br. Mycol. Soc. **43**: 643-59.

Waller J. M. (1971). Incidence of climatic conditions favorable to Coffee Berry Disease in Kenya. Expl. Agri. (1971), **7**: 303-314.

Wrigely (1988). Environment for growing coffee in “Coffee”. Longman Scientific and Technical. Pg 150.

Soil Moisture Dynamics in an Arabica Coffee – Fruit Tree Intercropping System in Kenya

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SUMMARY

In Kenya, about 55% of the coffee is grown by small scale farmers in monoculture. Due to declining world coffee prices and diminishing farm holdings, farmers have been intercropping their coffee with both annual and perennial crops. As most of the small scale coffee farming is rainfed, competition for moisture particularly with perennial crops will determine the success or failure of an intercropping system. The soil moisture profile in coffee intercropped with different fruit trees was studied from June 2000 to February 2001 at Ruiru Kenya.

There was no significant differences in residual soil moisture between the two coffee varieties. However there was significantly more soil moisture in the 50-100 cm zone than in the upper zone.

Intercropping coffee with passion fruit, oranges, guavas, avocadoes and macadamia resulted in lower soil moisture at both soil sampling zones, whereas there was higher soil moisture in plots intercropped with bananas regardless of the coffee variety.

INTRODUCTION

Coffee is an important crop cash crop in the Kenyan economy. It is grown by both small scale and large scale farmers contributing 55% and 45% of the total production respectively. Since its introduction in Kenya, coffee has been grown as a monoculture, but recently it has been observed to be intercropped with various annual and perennial crops (Njoroge and Kimemia, 1993), mainly due to declining world coffee prices and farm units. Intercropping hedges farmers against price fluctuation by diversifying farm income and hence militates against coffee uprooting. Intercropping systems are known for better utilization of resources mainly light, water and nutrients (Willey and Osiru, 1972). In the tropics, light is not a limiting resource but water is. Yield advantages under intercropping systems in the tropics can be attributed to higher water use efficiency (WUE) (Baker and Norman 1975). Where perennial crops are involved the situation requires more study as the crops are deeper roofed and thus exploit a bigger soil profile.

MATERIALS AND METHODS

The soil moisture determination was carried out from June 2000 to January 2001, at Coffee Research Station Ruiru. The trial was carried out in a plot where two coffee varieties Ruiru 11 (A disease resistant, arabica hybrid) and SL 28 where intercropped with *Carica papaya, Passiflora edulis, Musa sapientum, Psidium guajava*, and *Macadamia ternifolia*. The trial was laid in a split-split plot design, coffee varieties forming the main plots, perennial trees subplot and soil depth the sub-sub plot. The coffee and fruit trees were planted in 1991 and were managed as recommended (Mwangi 1983; Anon, 1984). The soil moisture was determined gravimetrically at depths of 0-50 cm and 50-100 cm. The soil samples were taken at the space

between the coffee and fruit tree using a 2” auger. Samples were taken at the different sampling depths. The samples were then weighed and oven dried at 105°C to constant weight. The difference between the wet weight and dry weight was the soil moisture content.

RESULTS

The results indicated no significant differences between the coffee varieties in terms of residual soil moisture.

There was significantly more residual soil moisture in the 50-100 cm soil depth except than in the topsoil (Figure 1). There was significantly lower soil moisture in the upper soil profile of the sole coffee as compared to the intercropped coffee (Figure 2). There was no significant difference in the lower soil profile (Figure 3).

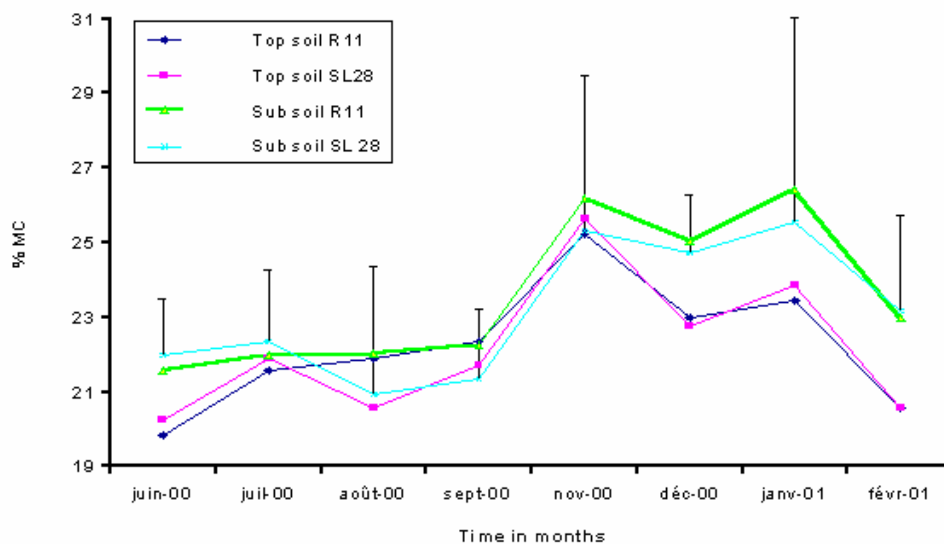

Figure 1. The influence of arabica coffee varieties on soil moisture content in a coffee – fruit tree intercropping system in Kenya

DISCUSSION

Water is crucial for plant growth and production, as it is the media through which nutrients are transported within the plant. The availability of water determines to a great extent the success of non-irrigated cropping systems.

Under intercropping systems, water competition is very crucial. The water use efficiency have been reported to be higher under intercrops particularly where the component crops exploit different layers of the soil (Steiner, 1982). Where the intercrops have similar growth habits like in the case of coffee and fruit trees, the scenario is expected to change. The higher soil moisture in th e upper soil profile where coffee was intercropped could be attributed to the shading effect and covering of the soil surface by litter, which all reduces evaporation.

The more soil moisture in the lower zone is not a new phenomenon as the lower soil layers are not exposed to evaporation (Michori, 1993). Also tree crops exhibit reverse flow or downward siphoning where tree roots transported water from the top soil profile to the lower profile (Smith et al., 1999). By transferring the water beyond the reach of shallow rooted neighbours, downward siphoning may enhance the competitiveness of deep-rooted perennials. In fact, a

positive correlation between soil moisture and soil depth was reported in a field planted with Arabica coffee hybrid Ruiru 11 (Gathaara, 1998).

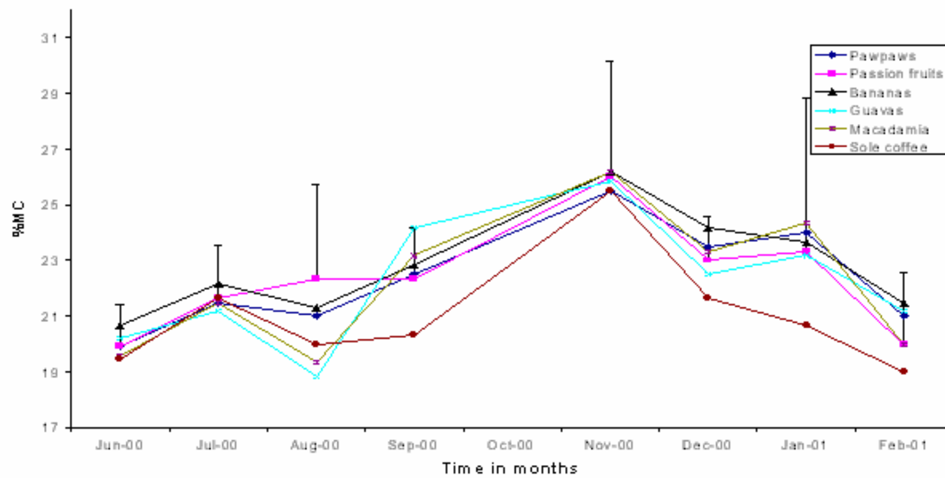

Figure 2. The effect of intercropped fruit trees in coffee on the top soil moisture content at Ruiru, Kenya

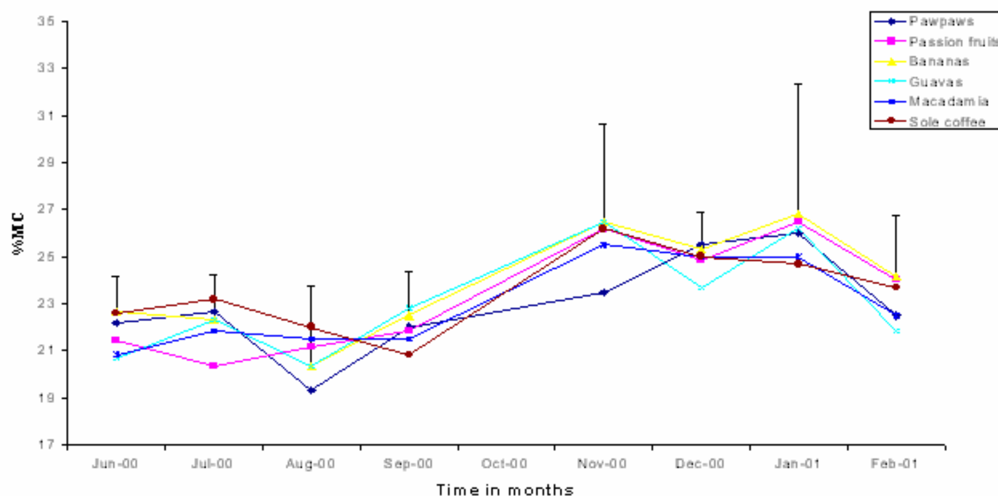

Figure 3. The effect of intercropped fruit trees in coffee on the subsoil moisture content at Ruiru, Kenya

CONCLUSION

It was observed from this study that the screened fruit trees did not significantly influence the soil moisture content and hence did not compete with coffee with soil moisture. It can therefore be concluded that the screened fruit trees may be recommended for intercropping with coffee.

ACKNOWLEDGEMENTS

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REFERENCES

Anonymous, 1981. Major crops. Technical Handbook Agricultural Information Centre, Nairobi, Kenya

Baker E.F.I and Yusuf Y., 1976. Research with mixed crops at Institute of Agriculture Research Samaru Conference paper No. **10**. 28 pp.

Gathaara M.P.H., 1998. The influence of drip irrigation on ecophysiology and yields of Arabica Coffee hybrid Ruiru 11 PhD Thesis. Kenyatta University

Kimemia J.K., 1998. Studies on green manure application and intercropping in *Coffea arabica* production. PhD Thesis, University of Nairobi

Michori P.K., 1993. Nitrogen budget under coffee PhD Thesis, University of Reading

Mwangi C.N., 1983. Coffee Growers Handbook. Coffee Research Foundation, Ruiru, Kenya 182 pp

Natarajan M and Willey R.W., 1980. Sorghum - Pigeon pea intercropping and the effects of plant population. 1. Growth and yield. Journal of Agricultural Science **95**: 51-58

Smith D.M, Jackson N.A, Roberts J.M. and Ong C.K., 1999. Reverse flow of sap in tree roots and downward siphoning of water by *Grevellea robusta*. Functional Ecology **13** (2): 256-264

Steiner K.G., 1982. Intercropping in Tropical small holder agriculture with special reference to West Africa. GTZ Germany 303 pp

Willey R.W. and Osiru D.S.O., 1972. Studies on the mixtures of maize and bean *Phaseolus vulgaris* with particular reference to plant population. Journal of Agriculture Science **79**: 519-29.

Efficiency of Close Spacing and Light Interception in Promoting Productivity of Arabica Coffee in Ethiopia

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SUMMARY

The impact of high density planting (close spacing) on vegetative growth and productivity of Arabica coffee on light interception by individual trees has been studied to determine the optimum plant population for the various coffee berry disease (CBD) resistant cultivars grown in the different agro-ecologies in the southern and south western parts of Ethiopia. The experiment was carried out in two sets at Jima (mid-altitude), Tepi (low land), Gera and Wonago (highlands) research centers. Set I was conducted in split plot design with four replications, using ten cultivars from the three canopy classes (open, intermediate and compact coffee types) as main plot and five spacing treatments (2500 to 10,000 trees ha^{-1}) as sub plot factors. In set II, a widely adaptable cultivar (an intermediate type, 7454) was planted at seven spacings representing 4,000 to 10,000 trees ha^{-1}) in randomized complete block design replicated four times. After ten years of experimentation, it was observed that close spacing significantly and consistently encouraged the rate of death of productive primary branches, but depressed the growth of potential crop bearing primaries at all the trial sites. As a result, eight years mean crop yield of individual coffee trees decreased, while yield ha^{1} significantly increased with increasing population density. Increases in the proportion of dead primary branches and, thus, the decline in crop bearing surface and yield $tree^{-1}$ were directly associated with the increased level of mutual shading or with the reduction in light interception by individual trees in high-density plantings. It is, therefore, suggested that depending on the agro-ecological condition and canopy spacial arrangement of coffee varieties the biological optima lies between 4000 and 6000 trees ha^{1}, while the economic optima assumes as high as 6,000 to 9,000 trees ha^{-1}.

INTRODUCTION

It is well known that growth and productivity of most crop plants depend on light intensity, moisture supply and availability of nutrients, and that the question of optimum spacing between plants is directly associated with the need for efficient utilization of these environmental inputs. Even if all other essential production inputs are optimal, excessive light or deep shade due to low or high plant population may adversely affect crop growth and yields through its effect on physiological processes, mainly photosynthesis and respiration (Jackson, 1980; Hipkins, 1984; Crisoto et al., 1990).

In tree crops like coffee, close spacing is not uncommon, as it has obvious yield advantage over low population density specially at early stages of plantation life. However, closer spacings induce overlapping of canopies and interlocking of branches in the later years of production. As a result of such an increased shade level and limited light penetration into the canopy, productivity of close-spaced coffee plants may considerably decrease unless effective pruning method is adopted (Gathaara and Kiara, 1984). Besides age, canopy special

arrangement of a crop or a variety also determines the optimum plant population per unit area of land (Friend, 1984; Yacob et al., 1996; Thompson, 1987; Tesfaye et al., 1998).

The objectives of this study were, therefore, to determine the optimum spacing and the corresponding level of light interception or shade that favours growth and development of individual coffee trees and promotes productivity of modern plantations with different varieties.

MATERIALS AND METHOD

An experiment consisting of ten CBD resistant coffee cultivars and twelve spacing treatments has been conducted in two sets (between 1989 and 2000) at four research centers, representing different coffee growing agro-ecological zones in south and southwest Ethiopia. Set I was laid down in split plot design with four replications at Jima (mid-altitude: 1750 m a.s.l.), where coffee cultivars from the three canopy classes (open types: 741 and 75227; intermediates: 7440, 7454 and 7487; and compacts: 74110, 74112, 74140, 74148 and 74165) assigned as main plots and five between plant and row spacings as sub plot factors. The spacing treatments were 2.00 m x 2.00 m, 1.75 m x 1.75 m, 1.50 m x 1.50 m, 1.25 m x 1.25 m and 1.00 m x 1.00 m with the respective plant population of 2,500; 3,265; 4,444; 6,400 and 10,000 trees ha^{-1}.

On the other hand, a widely adaptable coffee cultivar (7454) was planted at seven spacings (1.58 m x 1.58 m, 1.41 m x 1.41 m, 1.29 m x1.29 m, 1.19 m x 1.19 m, 1.12 m x 1.12 m, 1.05 m x 1.05 m, and 1.00 m x 1.00 m, making the population density 4,000; 5,000; 6,000; 7,000; 8,000; 9,000 and 10,000 trees ha^{-1}, respectively) in the second set of the experiment. It was conducted in randomized complete block design replicated four times at Gera (highland: 1940 m a.s.l.), Wonago (highland: 1880 m a.s.l.) and Tepi (lowland: 1200 m a.s.l.).

In both Set I and Set II, twelve representative sample trees from the central rows of each plot were randomly identified for data collection. Eight years mean crop yield ha^{-1} and $tree^{-1}$, productivity indices (proportion of bearing, non-bearing and dead primaries, and potential crop bearing surface) and mean percent light interception by individual trees were recorded and statistically analysed as per the design just ten years after planting.

Mean percent light interception by branches at various positions (top, middle and bottom) within a canopy of morphologically different cultivars (open, intermediate and compact) was estimated as the ratio of light incidence at a given position to direct light intensity measured under cloud-free sky using LI-1776 Quantum light meter. Conversely, the level of mutual and/or self-shading by individual trees was calculated as 100% minus mean percent light interception by the trees. Canopy volume (CV) and potential crop bearing surface (CBS) were estimated as follows :

$$CV = \frac{1}{2} (H_1 - H_2)\, \Pi\, (CD/2)^2$$
$$\% \; CBS = ((H_1 - H_2) / H_1) 100$$

Where, CD = mean canopy diameter, H_1 = total plant height, and H_2 = plant height up to the first lower primary branch.

RESULT AND DISCUSSION

Yield and yield components

Closer spacings significantly decreased the proportion of both crop bearing and young unproductive (non-bearing) primary branches (except at Jima) and increased the rate of branch death. As a result, mean crop yield of individual trees consistently decreased, while yield ha^{-1} increased with increasing population density (Table 1). The rate of branch death was higher in the lower canopy position and decreased in the upper part of the canopy.

Table 1. Factors associated with productivity of individual coffee trees as affected by spacing (population density) at various locations

Location	Spacing (m^2)	Density (trees ha^{-1})	% primary branches		Dead primaries		Clean coffee yield	
			Crop bearing	Non-bearing	Total No.	Total %	Kg $tree^{-1}$	Kg ha^{-1}
Jima (1750 m.a.s.l)	2.00	2500	47.39a	3.38 b	92.8 d	49.58 d	0.45 ab	1126 c
	1.75	3265	46.78a	4.22 b	96.5 d	49.13 d	0.49 a	1612 b
	1.50	4444	40.87b	5.90 a	110.3 c	53.34 c	0.42 b	1883 a
	1.25	6400	34.28c	6.60 a	127.5 b	58.87 b	0.29 c	1884 a
	1.00	10000	28.31d	6.90 a	142.4 a	64.78 a	0.16 d	1601 b
Tepi (1200m.a.s.l)	1.58	4000	12.14	33.58	112.69	54.27	0.22	876
	1.41	5000	12.10	33.55	110.85	54.38	0.23	1135
	1.29	6000	13.13	33.65	104.73	53.27	0.20	1229
	1.19	7000	7.25	37.75	112.19	55.00	0.18	1288
	1.12	8000	6.65	36.04	116.70	57.30	0.16	1311
	1.05	9000	9.77	27.34	116.96	62.89	0.15	1362
	1.00	10000	9.65	31.10	110.02	59.02	0.13	1349
Wonago (1880m.a.s.l)	1.58	4000	47.87	20.21	31.73	31.92	0.33	1322
	1.41	5000	41.81	19.73	39.83	38.36	0.30	1490
	1.29	6000	43.04	18.62	39.80	38.34	0.27	1596
	1.19	7000	39.86	23.75	36.07	36.38	0.24	1657
	1.12	8000	40.45	19.31	39.97	40.23	0.26	2058
	1.05	9000	39.42	16.79	48.17	44.33	0.24	2184
	1.00	10000	37.28	18.60	48.27	44.10	0.19	1936
Gera (1940m.a.s.l)	1.58	4000	66.72	14.21	18.80e	19.08e	0.43	1705c
	1.41	5000	57.83	11.83	27.10e	30.33d	0.43	2144b
	1.29	6000	56.06	4.45	45.20d	39.49c	0.38	2307ab
	1.19	7000	47.46	6.55	57.57c	45.99b	0.33	2329ab
	1.12	8000	46.01	4.04	70.00b	49.94ab	0.32	2584a
	1.05	9000	43.59	4.29	83.90a	52.12a	0.25	2266ab
	1.00	10000	43.40	4.72	81.80ab	51.88a	0.23	2270ab

Figures followed by same letter within a column at the respective location are not significantly different (P= 0.05)

This, in turn, increased plant height up to the first lower primary and decreased canopy volume and potential crop bearing surface of coffee trees grown at closer spacings (Table 2).

Table 2. Effect of spacing on percent daylight intercepted (LI) and level of mutual shading by coffee trees, death rate of branches and estimated crop bearing surface at Jima and Tepi

Location (trial site)	Spacing (m_2)	Density (trees ha^{-1})	Canopy volume (m^3)	Mean % L.I	Mean % shade	% Branch death		Height Upto 1st lower branch (m)	Estimated crop bearing surface (%)
						Upper canopy	Lower canopy		
Jima	2.00	2500	2.09a	38.61a	61.39 d	29.15 a	20.43 d	0.46 d	79.55 a
	1.75	3265	2.36a	32.53 b	67.47 c	28.56 a	20.57 d	0.46 d	79.38 a
	1.50	4444	2.22a	16.61 c	83.39 b	28.72 a	24.62 c	0.62 c	75.57 b
	1.25	6400	1.60b	10.89 d	89.11 a	22.19 b	36.68 b	0.95 b	63.65 c
	1.00	10000	0.92c	6.69 d	93.31 a	13.28 c	51.50 a	1.41 a	48.57 d
Tepi	1.58	4000	1.78	59.92 a	40.08 c	33.72	20.55	0.65	79.45
	1.41	5000	1.69	29.83 b	70.17 b	33.60	20.78	0.67	79.22
	1.29	6000	1.62	22.73 bc	77.27 ab	32.75	20.52	0.64	79.48
	1.19	7000	1.74	20.74 bc	79.26 ab	29.73	25.27	0.90	74.73
	1.12	8000	1.39	17.37 bc	82.63 ab	27.01	30.29	1.13	69.64
	1.05	9000	1.32	16.09 bc	83.91 ab	28.54	34.35	1.26	65.65
	1.00	10000	1.28	13.09 c	86.91 a	25.35	33.67	1.21	66.33

Figures followed by same letter within a column at the respective location are not significantly different (P= 0.05)

As observed at Jima and Tepi, increases in the proportion of dead primaries and, thus, decline in crop bearing surface and yield $tree^{-1}$ were directly associated with the increased level of shade or reduction in light interception (Table 2) as a result of interlocking net work of the branches (overlapping canopies) and increased mutual and self-shading of adjacent coffee trees in high density plantings. Besides higher rate of death of the older branches due to limited light penetration in to the lower part of the canopy (Table 3), increases in the proportion of young non-bearing primaries in the upper canopy position might have also accounted for a reduction in the yield of individual trees grown at closer spacings at Jima (Table 1). On the other hand, wider spacings (lower population densities) enhanced the rate of death of the upper (younger) branches (Table 2), probably because of exposure to excessive light intensity (direct sunlight), resulting in over bearing and early exhaustion.

In agreement with these results, some earlier findings on coffee indicate that this crop is a C_3 or an intermediate between C_3 and C_4, flourishing best under moderate light regimes, but its growth and yield decrease under no shade (in open sun) and under deep shades (Jackson, 1980; Friend, 1984; Tesfaye, 1995; Yacob et al., 1996). This is associated with the physiology of the crop and the balance between its net photosynthetic and respiration rates (Levitt, 1980b; Hawkins, 1982; Hipkins, 1984). As a C_3 plant, coffee is said to have efficient quantum requirement and can withstand limited light supply or adapt low light intensity (Friend, 1981; Cambrony, 1992). However, plants grown under heavy shade or reduced light intensity may spend much of their photosynthetic activities in maintenance and may use materials (Carbon compounds) which under normal conditions would have been utilized for the formation of plant parts as substrates for respiration (Jackson, 1980; Levitt, 1980b; Tompson, 1987; Crisosto et al., 1990). In line with this, the decrease in percent crop bearing primaries and yield per tree, and the increased rate of death of branches (Table 1) specially in the lower

canopy in this experiment may be associated with reduced level of light intercepted and increased level of mutual-shading by coffee trees in closer spacings (Table 2, Table 3).

Table 3. Mean percent light intercepted by individual coffee trees as influenced by spacing (plant population) and branch position within a canopy of morphologically different cultivars (canopy classes) grown at Jima

Spacing (m^2)	Plant pop n. ha^{-1}	Branch position			Coffee type/cultivar			Mean % light intercepted
		Top	Middle	Bottom	Open	Intermediate	Compact	
2.00	2500	62.19	33.82	19.83	38.05	35.33	42.46	38.61 a
1.75	3265	53.35	28.72	15.52	28.79	35.62	33.18	32.53 b
1.50	4444	25.00	17.11	7.71	17.39	12.09	20.34	16.61 c
1.25	6400	18.90	8.46	5.30	13.45	7.63	11.59	10.89 d
1.00	10000	12.31	5.09	2.67	8.93	4.84	6.30	6.69 d

Figures followed by same letter within a column at the respective location are not significantly different (P= 0.05)

On the other hand, the rate of branch death in the upper canopy part increased as the spacing between plants increased. This could be attributed to the increased level of light interception as a result of minimum mutual and self-shading by coffee trees grown at wider spacings (Table 2). Moreover, branches at the top of a tree canopy are more exposed to excessive light intensity and, thus, suffer from overbearing die-back because of absence of overhead shades, compared to branches in the lower (middle and bottom) canopy strata. In agreement with this finding, the works of Hawkins (1982), Hipkins (1984) and Crisosto et al. (1990) have confirmed that although C_3 plants are efficient users of minimum Quanta under deep shades will photorespire excessively in open sun. Under high light intensities, as observed for low population density and top canopy part in this experiment (Table 2, Table 3), there will be inadequate reaction centers to accommodate the light energy and convert it into biochemical energy (Hawkins, 1982) and the coffee plants may experience excessive photo inhibition/photo-oxidation and eventually the rate of photosynthesis becomes lower than respiration. Thus, most of the stored carbohydrates will be depleted and the plants may suffer from severe dieback and reduction in yield (Jackson, 1980; Levitt, 1980b; Hipkins, 1984; Thompson, 1987).

Light interception by coffee trees

As expected, mean percent sunlight intercepted by individual trees significantly decreased and the level of mutual shading consistently increased as the spacing between plants decreased and population density increased (Table 2). Obviously, leaves/branches at the top generally intercepted more light, compared to those at the middle and bottom part of the tree canopy. On the other hand, unlike the open and intermediate coffee types (canopy classes), trees of compact cultivars with drooping branches were found to be capable of intercepting more light, particularly at wider spacings and in the upper canopy positions (Table 3).

The reduced level of light interception with closer spacings and/or at lower canopy parts might have accounted for higher rate of death of the lower older branches, and thus, increased plant height up to the first lower primary branch and decreased crop-bearing surface of coffee trees (Table 2). On the other hand, the increase in shade level in the lower part of tree canopy was associated with formation of interlocking net-work of the upper primaries and increased

level of both self-and mutual-shadings of adjacent coffee trees in high density plantings. In this line, it has been reported that trees grown at closer spacings or those with very large leaf area (foliage mass) and overlapping canopies may experience increased mutual or self shading, leading to lower rate of net photosynthesis (Jackson, 1980; Gathaara and Kiara, 1984; Crisosto et al., 1990), excessive respiration and subsequent death of plant parts from starvation (Levitt, 1980b; Hipkins, 1984; Thompson, 1987).

These conditions might have resulted in higher rate of branch death specially in the lower canopy part, increased plant height up to the first lower primary branch and reduced canopy diameter and volume and estimated crop bearing surface of coffee trees grown at higher densities in this experiment. As observed at Jima, the proportion of dead branches and, thus the rate of dark respiration significantly increased from 20.43% to 51.50% as the population density increased from 2,500 to 10,000 trees ha^{-1}, and it was 20.55% and 33.67% for 4,000 and 10,000 trees ha^{-1}, respectively, at Tepi. This was so because the average level of light intercepted by individual plants was significantly lower in high density plantings (6.69% at Jima and 13.09% at Tepi for 10,000 trees ha^{-1}) than that intercepted by wide-spaced coffee trees (38.61% for 2,500 trees ha^{-1} at Jima, and 59.92% for 4,000 trees ha^{-1} at Tepi (Table 2).

With regard to this, studies on plant growth and development indicate that the synthesis of biochemical constituents, dry matter production and crop yield decline under heavy shades because of decreased photosynthetic rates as dark respiration proceeds normally (Jackson, 1980; Hawkins, 1982; Hipkins, 1984). These findings may explain the present results, indicating that the decline in crop bearing surface and yield of individual coffee trees and increases in the proportion of non-bearing primaries and dead branches in the lower canopy layer (Table 1, Table 2) could be attributed to the increased level of mutual shading (reduced light intensity) with increasing plant population (Table 2, Table 3).

REFERENCES

Cambrony H.r. 1992. Coffee growing. CTA Wageningen, the Netherlands, and Macmillan Press Ltd., London. 119 p.

Crisosto C.H., Grantz D.A., Perry M.H. and Luza J.G. 1990. Effect of leaf position and anatomy on gas exchange of *Coffea arabica* L. growing in a hedge-row system under Hawaiian conditions. Proc. Of the plant growth regulator society of America. 17th Annual Meeting, 5-9 aug. 1990. It paul, Minnesota, USA. Pp.: 173-178.

Friend D.J.C. 1984. Shade adaptation of photosynthesis in *Coffea arabica*. Phtosynthesis Research Vol.**5** No.4 pp.: 325-334

Gathaara M.P.H. and Kiara J.M. 1984. Factors that influence yield in close-spaced coffee. I. Light, dry matter production and plant water status. Kenya coffee vol. **49** No. 578 pp: 159-167.

Hawkins C.T. 1982. Light interception in cocoa production. O*ut look* Vol. **21** (2): 137-145.

Jackson J.E. 1980. Light interception and utilization by orchard systems. Horticultural Reviews, **2**: 208-267.

Levitt J. 1980b. Responses of plants to Environmental stress. Vol. 2. Water, Radiation, Salt, and other stresses. Academic press, Inc. New York. PP. 25-364.

Tesfaye Shimber. 1995. Influence of seed bed watering frequency, mulching and shading on seedling growth of Arabica coffee. M. Sc. Thesis. Alemaya University of Agriculture, Ethiopia.

Tesfaye Shimber; Yacob Edjamo; Alemseged Yilma and Taye Kufa. 1998. Research Achievements and Transferable Technologies in coffee Agronomy. In Beyene, S. and Aberra, D. (eds.). Proc. Of the Third Technology Generation, Transfer and Gap Analysis Workshop. 12-14 Nov. 1996, Nekemt, Ethiopia. Pp. 70-79.

Thompson L.K. 1987. Irradiation stress. In M.G. Hale and D.M. Orcutt (Eds.). The physiology of plants under stress. John Wiley and sons, Inc. New York. Pp. 103-115.

Yacob E., Tesfaye S., Alemseged Y., Taye K., Anteneh N., Takele N., Mohammednur A.C. and Bekele B. 1996. Advances in Coffee Agronomy Research in Ethiopia. Improving coffee Management systems in Africa. Proc. Of IACO Workshop. Kampala, Uganda, 4-6 Sept. 1995. African Crop Science Society 1996. Pp. 40-55.

Managed Selection Environment: A New Concept in Breeding for High Yields and Superior Quality in Arabica Coffee

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INTRODUCTION

Genotype by environment interaction (GE) is important in crop plants since it results into lack of correspondence between the observed phenotype and the underlying genotype. In Arabica coffee, GE interaction has been reported for yield and yield related traits (Srinivasan et al., 1979; Walyaro, 1983; Bittenbeder et al., 1991). GE interaction reduces selection efficiency and makes variety trial experiments expensive. This paper demonstrates how the effects could be accommodated in variety trial experiments through the use of managed environment techniques.

CHOICE OF SELECTION ENVIRONMENTS IN ARABICA COFFEE

An ideal site for selection and testing of new varieties is one which would allow for the discrimination between genotypes and which adequately represent the various environmental conditions which the variety under improvement is expected to encounter (Figure 1 for the Kenyan situation). Comprehensive coverage of the full spectrum of the environment is however logistically difficult and expensive.

To overcome the constraints associated with comprehensive sampling, the use of managed environments of selection has been proposed for a number of crops (Seed and Francis, 1984; Cooper et al., 1995; Van Oosterom et al., 1996). To effectively design and use such environments however, one need to understand the environmental factors with significant contribution to the GE interaction components and the developmental stages through which they affect performance. In Arabica coffee, both yield and quality develop systematically over a period of nine months (Figure 2). Depending on the period during which environmental stress occurs, yield, bean size bean weight or liquor quality may be affected.

If one considers moisture stress, for example, stress occurring during the pin head stage would have minimal effect on any of the yield or quality traits. Stress during 6-16 weeks would limit the final bean sizes whereas moisture stress 17-24 weeks would affect bean weight and liquor quality. This knowledge could be used to plan managed environments in which the various stress factors are synchronised with the developmental stages most susceptible to their effects (Table 1).

Caution should however be exercised when interpreting the results from managed environment trials since environmental factors such as temperature and humidity would still remain largely uncontrolled in such trials.

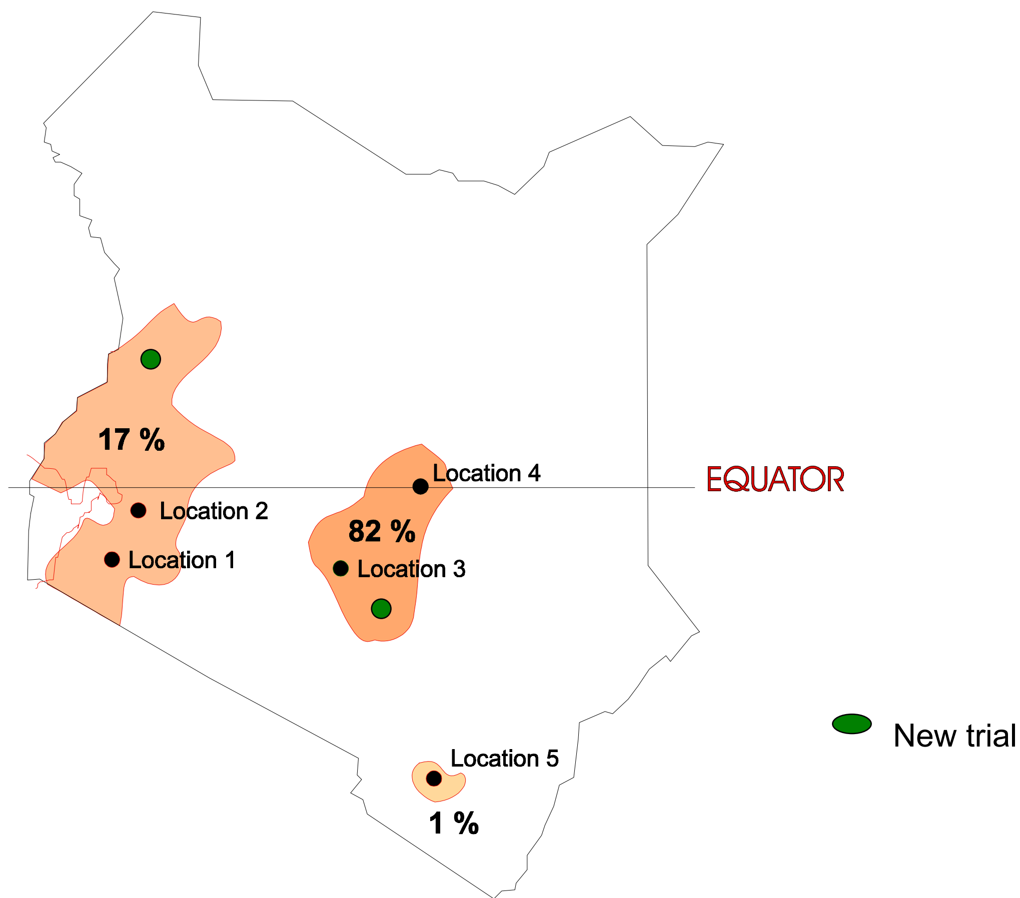

Figure 1. Distribution of test environments in relation to the various coffee growing zones in Kenya

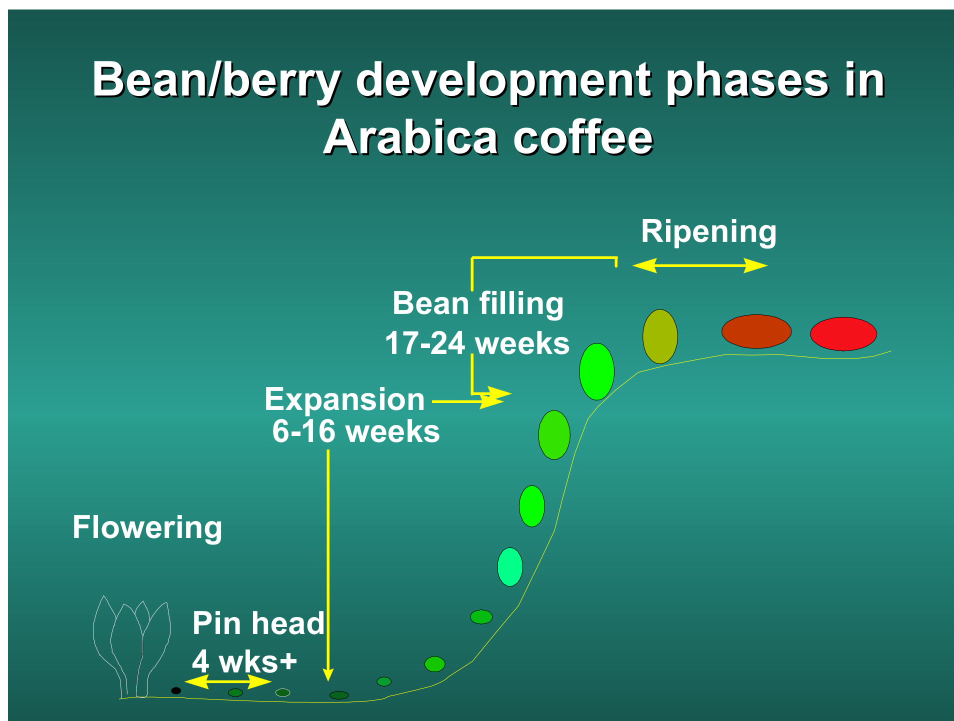

Figure 2. Pictorial representation of the main phases of coffee berry development

Table 1. Synchronise moisture stress with susceptible fruit development stages: in a managed environment selection trial in Arabica coffee

Category	Phases of fruit development						Trial objective
	Pre-flowering	Flowering	Pinhead	Expansion	Bean filling	Ripening	
Trial 1	Stress	Optimum	Optimum	Optimum (Irrigation)	Optimum (Irrigation)		Bean quality & Productivity
Trial 2	Stress	Optimum	Optimum	Sub-optimum (Irrigation)	Mod. stress (? irrigation)		Liquor quality
Trial 3	Stress	Optimum	Optimum	Optimum (Irrigation)	Stress		Stability & Bean weight
Trial 4	Stress	Optimum	Optimum	Stress	Optimum (Irrigation)		Stability & Bean size
Trial 5	Stress	Optimum	Optimum	Stress	Sub-optimum		Adaptation patterns

REFERENCES

Bittenbender H.C., Upreti G., Nagai N.Y. and Cavaletto C.G., 1991. Evaluating performance of coffee cultivars in Hawaii using stability analysis. In: 14th Int. Coll. on coffee, San Francisco, pp. 667-673. ASIC, Paris.

Saeed M. and Francis C.A., 1984. Association of weather variables with genotype X environment interactions on grain sorghum. Crop Sci. **24**: 13-16.

Srinivasan C.S., Vishveshwara S. and Subramanya H., 1979. Genotype-environment interaction and heritability of yield in *Coffea arabica* L. J. Coffee Res. **9** (3): 69-73.

Van Oosterom E.J., Mahalakshmi V., Bidinger F.R. and Rao K.P., 1996b. effect of water availability and temperature on the genotype-by-environment interaction of pearl millet in semi-arid tropical environments. Euphytica **89**: 175-183.

Van Oosterom E.J., Bidinger F.R., Mahalakshmi V. and Rao K.P., 1996a. Effect of water availability pattern on yield of pearl millet in semi-arid tropical environments. Euphytica **89**: 165-173

Walyaro D.J.A., 1983. Considerations in breeding for improved yield and quality in arabica coffee (*Coffea arabica* L.) Doctoral Thesis. Wageningen Agricultural University, the Netherlands. 119 p.

Weed Management Practices in Small Scale Coffee Farms in Kenya

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SUMMARY

Surveys of weed population dynamics and observations on cultural practices and their impact on weed management were carried out in coffee farms in the highlands of East and Central Kenya between 1997 and 1999. About 50 weed species belonging to 19 families were identified. Intercropping suppressed weed growth while chemical weeding controlled annuals and shortened the weeding period. Hand cultivation controlled the difficult weeds but was labour intensive. A combination of both chemical and cultural methods controlled difficult weeds effectively and is recommended as an IWM strategy.

INTRODUCTION

The severity and duration of weed infestations determines magnitude and extent of operations required for weed control (Sagar, 1974). An infestation in one season, unless eradicated, leads to the same considerations in the next. Comprehension of natural (e.g. climatic) and man managed (e.g. crop husbandry/ cropping systems)factors that regulate the size of a weed population become crucial if weed control procedures are to be critically evaluated economically (Mortimer, 1983). This can only be achieved through the study of weed population dynamics (Sagar and Mortimer, 1976), which can then form the basis of strategic planning for weed control (Cussans, 1980). Weeds can reduce coffee yields by up to 50% (Njoroge and Kimemia, 1990). Herbicides are not popular with small scale farmers due to their high costs. Most farmers therefore, use cultural practices of weed control which appear cheaper since they involve the use of family labour which is not costed. Some farmers however resort to herbicides as the cheaper option when family labour is unavailable or inadequate. Nevertheless, an integrated approach is required as a cheaper and sustainable method of weed management. This study focused on weed management practices in small scale farms under different cropping systems in different ecozones. The aim was to identify possible windows for integrated weed management (IWM) intervention, the impact of cultural practices and agroecozone on weed control.

METHODOLOGY

The study was carried out in two phases over a period of two years, where surveys of weed population dynamics were conducted. In the first phase the survey was conducted between November 1997 and May 1998 on eleven farms chosen at random from three coffee growing agroecological zones namely upper, main and lower zones. Four farms were chosen from each zone except for the upper zone where only three farms were chosen. The altitude range for the upper, middle and lower zones were 1600-1800, 1400-1600 and 1400-1200 metres above sea level respectively. Within each farm the experimental unit was a plot of three rows of four coffee trees each replicated three times. Sampling was carried out monthly.

The second phase was conducted between October 1998 to September 1999. Certain coffee management systems identified during phase one led to the targeting of the four most common practices for sampling of farms in phase two. Four farms and four blocks at the Coffee Research Station (CRS), Ruiru were chosen in the main coffee growing agro-ecological zone.

There were four agronomic treatments with each farm and similarly each block in CRS representing a treatment (Table 3). Data on weed cover and species occurrence was collected monthly from plots marked out on the sites and the weed control practices noted. The overall weed cover was visually assessed on the whole plot/experimental unit and scored on a scale of 1-9 representing total control to no control respectively.

RESULTS AND DISCUSSION

A total of 50 weed species belonging to 19 families were identified through the survey period. These were randomly distributed spatially and temporally. The most dominant species however, were *Galinsoga parviflora*, *Digitaria velutina*, *Bidens pilosa* and *Amaranthus spp*. The most common difficult weed species were *Cyperus spp*. and C*ommelina benghalensis* (as described by the farmers) which occurred in patches on farms where they were found.There were no significant differences in weed cover and weed species distribution between the three ecozones (Table 1).

Table 1. Effect of agro- ecozone on mean weed scores (weed population)

Agro-Ecozone	Weed Scores						
	Nov 97	Dec 97	Jan 98	Feb 98	Mar 98	Apr 98	May 98
Um1	4.47a	4.50a	2.83b	2.03a	2.10b	2.07b	2.30a
Um2	5.43a	6.23a	3.83ab	3.27a	3.47ab	4.27ab	4.67a
Um3	5.73a	6.10a	6.10a	1.87a	4.27a	4.83a	3.97a

Means followed by the same letter down the column are not significantly different according to Tukey's Honestly significant test (P=0.05)

In phase one the major weed control method was hand cultivation, carried out between January and May (Table 1). Other weed control practices were intercropping (with *Phaseolus vulgaris/amaranthus/Solanum tuberosum/Ipomea batatus*), mulching, and shading from dense coffee canopy. Weeding was on average done monthly except where other cultural practices were applied (Table 2).

In phase two Weed infestation levels were not affected by the treatments but were affected by the management of the farm/plot. The most common weed species at CRS were *Digitaria velutina, Richardia repens*, *Bidens pilosa, Oxalis* sp., *Cyperus* sp. and *Commelina benghalensis*. On farmers fields *Gnaphalium* spp., *Galinsoga* spp., *Conyza* sp., *Cyperus* sp., *Eleusine indica* and *Bidens pilosa* were prevalent.

Good weed control was observed in all treatments (Table 3). Weeding was done between Oct-Nov and Feb-Aug Both hand cultivation and chemical weeding methods were used. At CRS, weeds were observed to have been controlled using glyphosate at the rate of 1L/ha applied at an average of 2 times in a year and followed by cultivation. On some farmers' fields it was recorded that either paraquat or glyphosate was used at the rate of 0.5-1L/ha twice a year (once each at the beginning of the two rain seasons) followed by hand cultivation on the regrowth.

The difficult weeds like *Commelina, Cyperus* and *Oxalis* occurred only on patches in all the treatments.

Table 2. Agronomic practices and their effect on weed cover on different farms, phase one

Farm	Weed Scores						
	Nov 97	**Dec 97**	**Jan 98**	**Feb 98**	**Mar 98**	**Apr 98**	**May 98**
1	2.00d#	3.67de#	4.67abc#	4.00ab#	1.00d#X	1.33c#	2.67ab#
2	6.33ab	5.67bcd	2.00bcX	3.17ab	4.83abc*	5.50ab	5.17ab
3	6.00ab	5.83abcd	4.83abX	1.67ab	1.67cdX	4.17abc	2.67abX
4	6.67ab	7.33ab+	5.17abX	4.67aX	2.83bcdX	3.33bc#	4.67abX
5	6.00ab+	5.33bcde	7.33a	3.33ab*X	5.17ab	7.00a	4.00abX
6	3.00cd	3.00e	2.67bcX	2.33abX	1.00dO	1.00cX	1.00bX
7	4.50bcdO	4.50cde	4.33abc	1.00bX	1.00dO	2.00cX	1.67bXO
8	8.17a	8.17a	6.50a	1.33abX	6.33a	6.50ab	6.83a*
9	4.33bcd	4.67cde	1.00cX	2.17ab	3.67abcdX	1.00cX	3.33abX
10	5.17bc	6.83abc	2.00bc	4.17ab	6.50a	7.50aX	7.67a
11	3.00cd	4.83cdX+	4.50abc	1.00bX=	1.33dX	1.00cO	1.00bX+

Means followed by the same letter (abcde) down the column are not significantly different according to Tukey's Honestly significant test (P=0.05).
*Key : + - Intercropping = - Forking; X - Hand cultivation; O - mulch, # - Canopy cover; * - Slashing*

Observations showed that coffee prunings were effective in controlling weeds. On these plots coffee prunings arose from the change of cycle on the coffee. It was also noted at CRS on coffee/banana intercrop that trash was used as mulch as opposed to the farmers fields where the trash was mostly removed to feed cattle. Where it was used as mulch, it was spread sparsely rendering them not very effective. Shading by bananas was more at CRS than on farmers fields which effectively suppressed weeds (Table 3).

CONCLUSIONS

The main method of weed control in coffee grown on small scale farms East of the Rift valley is hand cultivation which effectively controlled the difficult weeds. Herbicides were mainly effective on annuals. Intercropping, mulching and shade from dense coffee canopy suppressed weed growth. A combination of hand cultivation and herbicides was effective in controlling all weeds and is recommended as an IWM strategy where herbicides could be used during critical weeding periods (e.g. during the rainy season) followed by hand cultivation (after the rains).

Table 3. Agronomic practices and their effect on weed cover on different farms, phase two

Farm	Weed Scores											
	Oct 98	Nov 98	Dec 98	Jan 99	Feb 99	Mar 99	Apr 99	May 99	Jun 99	Jul 99	Aug 99	Sep 99
1	3.33bc	7.37a	7.10ab	2.03bc X	1.20b	1.20b X	7.50a O	6.23abcd	3.77bc @	1.10c X	1.20c X	1.10b
2	1.13c	8.10a	7.50ab	1.17c X	1.10b X	2.67b	7.80a @	4.10bcde @	1.77c	1.13c	1.13c	1.17b
3	8.30a	8.13a	8.17ab	8.20a	1.20b @	3.67ab X	8.90a X	2.63de X	1.43c XO	3.30bc =	1.73bc X	5.37ab
4	1.17c X	2.33bc	3.67bcd	8.27a	1.13b X	2.67b X	6.61a O	4.83abcde	2.73bc =	1.93c X	2.67bc	6.77ab
5	1.03c	1.13c @	1.17d	1.40c	3.83ab O	1.37b O	6.07ab	6.97abc @	3.17bc	2.70bc O	2.43bc O	3.03ab
6	5.33ab X	5.67ab	4.67abc d	5.40ab	5.27ab @	2.33b	5.50ab O	6.67abc @	5.50ab X	4.83b X	5.00ab X	5.67ab
7	1.07c X#	1.20c #	1.67d #	1.10c #	1.10b #O	1.20b #O	5.07ab #O	3.57cde #O	1.43c #O	1.13c #O	2.13bc #O	1.13b #O
8	1.10c #	1.10c #	2.37d #	1.20c #	1.73ab #O	1.37b #O	2.33b #O	2.10e #O	2.70bc #O	1.17c #O	1.10c #O	1.67ab #O

Means followed by the same letter down the column are not significantly different according to Tukey's Honestly significant test (P=0.05)

Key

Treatments

1 - Ruiru 11 monocrop - on farm (Gatundu)
2 - SL 28 monocrop - on farm (Gatundu)
3 - Ruiru 11/banana intercrop - on farm (Gatundu)
4 - SL 28/banana intercrop - on farm (Gatundu)
5 - Ruiru 11 monocrop - CRS
6 SL 28 monocrop - CRS
7 Ruiru 11/banana intercrop - CRS
8- SL 28/banana intercrop - CRS

Weed control practices

= *- Forking*
X *- Hand cultivation*
@ - Herbicide
O *- mulch*
 # - Banana shade

REFERENCES

Cussans G.W. 1980. Strategic planning for weed control - a researcher's view. Proceedings, 15th British weed control conference **3**, 823 - 832.

Mortimer A.M. 1983. On weed demography. In “Recent advances in weed Research” eds. W.W Fletcher. Commonwealth Agricultural Bureaux. 1983. Greshaan Press. pp. 3-40

Njoroge J.M and Kimemia J.K., 1990. A comparison of different weed control methods in Kenya. Kenya Coffee **55**. 644 pp 863-70.

Sagar G.R. 1974. On the ecology of weed control . In “Biology in disease and Pest control.” British Ecology Society Symposium. Eds. P. Jones and S.E., Solomon **13**, 42-58 Blackwell Scientific publications.

Sagar G.R. and Mortimer A.M. 1976. An approach to the study of population dynamics of plants with special references to weeds. In “Applied Biology”. eds T.H Coker PP. 1-47. Academic Press London UK.

New Concepts on Fertilizer Recommendation for Coffee – Nigerian Case Study

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SUMMARY

Coffee production in Nigeria is done over a variety of agroclimatic zones ranging from the typical rainfall region in the South to the Guinea Savanna in the middle belt to montain savanna in the North. The variety of agroclimatic zones also connotes variety of soil types. Coffee is therefore produced in Nigeria on Alfisol, oxisols and ultisols. These soils have different nutrient supply powers responses of coffee to fertilizer also vary considerably. In Nigeria, and in must coffee producing countries, single fertilizer recommendations are being used irrespective of differences in soil type and climatic conditions.

Recommendations in Nigeria for coffee are 65 kg N, 15 kg P, and 70 kg K across the different climatic zones.

This blanket fertilizer recommendation has not achieved the desired increase yield across the coffee producing areas. Consequently yield levels in Nigeria have remained exceedingly low due to wrongful fertilizer wage. The new concept of fertilizer recommendation sought to take into considerations soils, agroclimatic conditions of the producing area and the expected yield level. Similar works on cocoa in Nigeria have also produced encouraging results. Observations so far indicate that the new soil series based fertilizer recommendations will seriously reduce wastage and significantly increase yield.

INTRODUCTION

Coffee production world wide has been found to require the external input of additional nutrients in form of fertilizers. This is primarily because the crop exerts a lot of demand on the soil in terms of nutrient uptake. This is further aggravated if the variety planted is expected to produce high yield.

In Nigeria, and in most coffee producing countries, fertilizer requirements of coffee have been met based on field trials of different rates of fertilizers. In some cases, these trials have been at only a single location in some other cases, they have been multilocational. One important aspect missing in these trials have been that none of these trials have adequately taken into cognizance the demand of the harvested berries and consequently the yield of the plant.

Obatolu (2000) in a similar work on cocoa had proposed that due cognizance be taken of exports of nutrients from the plantations as a result of harvest in fertilizer recommendations.

Fertilizer recommendations that are soil series specific are more likely to be more economical and beneficial than blanket recommendations. It is in this light that this study was initiated and carried out. This study was therefore designed to evaluate fertilizer recommendations based on the different soil series on which coffee is grown in Nigeria. This report is the first

on the trials and is expected to steer in a new direction in fertilizer recommendation for coffee.

MATERIALS AND METHODS

Soil samples were collected from the following coffee growing areas in Nigeria: Ibadan Oyo State (slong steep oxic Tropudalf), Owena, Ondo State (gently undulating oxic Tropudalf), Uhonmora, Edo State (oxic Palenstalf) Ibeku, Abi State, Kabba, Kogi State (Alfisol) and Ikom,Cross River State (Alfisol) Obatolu and Owaiye, 1996.

The soil samples collected from each of these locations were collected from several points and bulked. About 10kg of each of these bulked soil from the locations were put in a 15kg capacity plastic pot which had five holes drilled at the bottom to allow for proper aeration and drainage. Soils from each location formed a treatment and there were six locations in all.

Coffee berries were also collected from each of the locations and analyzed for their nutrient contents. The berries were separated into the pulp + parchment and the clean coffee beans for the purpose of these analysis.

In each pot one year old coffee robusta seedling of quillou variety were planted and allowed to grow for two years without fertilizer application. At the end of two years, each plant was destructively sampled and analyzed for their dry matter yield and nutrient contents. The following nutrient elements were determined in both soil and plant samples:

In the soil

Total nitrogen according to the macro-kjeldahl procedure as described by Jackson, (1958); pH was determined in water (1:2 soil: water ratio) using pH-meter with glass electrode.

Phosphorus was determined using the Ray no. I method as described by Bray and Kurth (1945);

Exchangeable K, Ca, Mg were determined by extraction with IN ammonium acetate and the amount of K, Ca, in the filtrate were determined using a coming flame photometer with appropriate filter while Mg was determined using a Perkin-Elmer Atomic Absorption Spectro-photometer.

In the plant samples

N was determined using a Technicon Autoanalyser;
P with the Vanada – Molybdate method colorimetrically;
K from the digest using a Corning Flame photometer.

Nutrient uptake was calculated from the nutrient content X dry matter yield. This was then used to calculate the fertilizer requirement using the formulae of Enzmann, (1977) as quoted by Obatolu (2000). The nutrient content of the clean coffee beans was regarded as the export from the farm since this is usually taken away from the farm. This was taken care of in the fertilizer requirement.

RESULTS AND DISCUSSIONS

The results of the analysis of the different coffee growing soils are presented in Table 1 below:

Table 1. Analytical results of different nigerian coffee growing soils

Location	Vegetation	pH (H20)	Total N (%)	Available P (ppm)	Exchangeable cations CMol/Kg Soil K	Ca	Mg	Fertility Classification
Ibadan	Rainforest	6.30	0.12	7.20	0.43	1.65	0.40	Medium
Owena	Rainforest	6.10	0.15	13.00	0.24	4.20	1.25	Medium
Uhonmora	Guinea Savanna	6.20	0.16	20.00	0.41	0.82	0.38	Low
Ibeku	Rainforest	4.60	0.10	48.00	0.10	1.28	0.45	High
Kabba	Guinea Savann	5.80	0.21	12.06	0.38	1.04	0.40	Medium

From Table 1 the diversity in the different soils on which coffee is grown both in soil reaction (pH) and the nutrient content and fertility status, even when they belong to the same group. This then implies that fertilizer recommendation based on one location may not be necessarily valued for another location. Table 2 shows the dry matter yield of coffee plants grown on different soils.

Table 2. Dry matter yield of coffee grown as different soils

	Dry Matter Yield g/Pot			
Location	**Leaves**	**Stem**	**Root**	**Total**
Ibadan	2.15	13.25	8.70	24.10
Owena	5.10	6.59	46.00	57.69
Uhonmora	2.60	3.65	7.90	14.15
Ibeku	4.60	6.40	14.75	25.75
Kabba	2.60	5.10	9.50	17.20
Ikom	1.60	4.80	2.90	9.30
LSD (5%)	1.35	3.28	15.02	16.57

From Table 2 above, it is observed that the dry matter yield of coffee varied significantly from soil to soil and further goes to affirm the need to consider each soil on its fertility status for the purpose of fertilizer recommendations.

Table 3. Nutrient contents (%) and uptake (g/pot) of coffee from different soils

Location	Leaf Content N	P	K
	Percent Dry Matter		
Ibadan	3.00(72.30)	0.22(5.30)	2.72(65.95)
Owena	2.80(61.53)	0.16(9.23)	0.87(50.19)
Uhonmora	1.80(25.47)	0.29(4.10)	2.84(40.19)
Ibeku	3.20(82.40)	0.29(7.47)	2.58(66.44)
Kabba	3.10(53.32)	0.25(4.30)	2.99(51.43)
Ikom	2.50(23.25)	0.22(2.05)	1.05(9.77)
LSD (%)	0.49.(48.83)	0.05(2.47)	0.91(20.03)

In Parenthesis () nutrient uptake in g/pot

The values in Table 3 showing nutrient contents and in parenthesis nutrient uptake show very clearly that there are significantly different uptake for the nutrient elements by the coffee plants grown on different soils. This agrees with values obtained in Tables 1 and 2 and emphasis the need for soil series based fertilizer recommendations.

Enzman (1977) and Obatolu (2000) calculated optimum nutrient requirements based on nutrient uptake as follows: on poor or medium soils, P requirement will be uptake +100% or 50%, whereas for K it will be the uptake +1.5% or 0.8%. Due considerations have to be given to nutrient exports through harvest. In this study, 0.8% N, 0.4% P and 5.11% K were found in the coffee parchments whereas 1.2% N, 0.4% P and 3.41% K were found in the beans. To compute the fertilizer requirements based on the nutrient uptake presented in Table 3 above and the formulae of Enzman, 1977 the following requirements in Table 4 are obtained.

Table 4. NPK Fertilizer requirements of coffee on different soils in Nigeria

Location	*Fertility Status*	*Fertilizer Requirement*		
		N	**P**	**K**
		Kg per hectare		
Ibadan	Medium	75.19	8.27	54.54
Owena	Medium	167.99	14.40	41.76
Uhonmora	Low	52.98	6.40	33.44
Ibeku	High	85.69	7.77	55.28
Kabba	Medium	55.45	6.76	42.78
Ikom		24.18	3.19	8.13
Current blanket fertilizer rate	Medium	65.00	15.00	70.00

Depending on the yield level desired the above fertilizer rates will have to be adjusted using the total amount of nutrient "export" through the clean beans.

CONCLUSION

From the results of these trials which show significant different in the nutrient requirements of coffee from soil to soil, the soil series based fertilizer recommendation will be more appropriate than the blanket fertilizer rate across the different soils which have not yielded the desired high productivity.

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REFERENCES

Bray R.H and Kurth L.T. (1945):Determination of total, organic and available forms of phosphorus in soil. Soil Science Journal **50** P 39-45.

Enzman Josef (1977): Plant Nutrition and Soil Fertility – Karl Max University Leipzig, Germany – Lecture Notes for Postgraduate students 1977.

Jackson M.L. (1958): Soils Chemical Analysis. Constable and Co. Ltd. London.

Obatolu C. R. and Owaiye A.R. (1996): Coffee Soils of Nigeria. Nigerian Journal of Tree Crop Research Vol. **2** No. 1 1996 P 114-127.

Obatolu C.R. (2000): Development of Soil Series Based Fertilizer. Recommendation for New Generation Cocoa Materials in Nigeria. Paper presented at the 13th International Cocoa Conference – Malaysia 4 pp.

The Effect of Shading Stock Plant on Rooting in Coffee

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SUMMARY

Studies were carried out in the wet and dry seasons to investigate the effect of stock plant shading on rooting in two clones and two wood types. Two shade regimes; Light (37% full day light)and Heavy Shade (17% full day light) were compared with No Shade treatment (control; where all the plants were fully exposed to sunlight). The experiment was a split plot in a randomised complete block design with four replicates. Rooting was done in rice husk in concrete propagation pits. Cuttings were assessed for callusing, sprouting and rooting at two weekly intervals for 12 weeks. There was no significant effect of shade on rooting percentage, however significantly longer roots were produced by cuttings from the Light Shade treatment. In general semi-hard wood cuttings gave significantly (P=0.05) better rooting than terminal cuttings.

INTRODUCTION

Coffee in its natural habitat grows under other trees and shows a tendency to be moderately sun-loving with peak photosynthetic activity resulting from the first morning and late evening rays of the sun as well as the light through cloudy days. Thus strong sun light at mid-day arrests and slows down photosynthetic activity, (Snoeck, 1988). Hence the use of shade on coffee vary considerably according to ecological conditions, local tradition and the level of management practices (Amoah et al., 1997; Mitchell, 1988). Similar to other crop species, shading affects both anatomical and physiological processes in coffee plants (Beakbane 1961; Thompson, 1951). There is some increase in individual leaf area under shade and in some species there is a reduction in lamina thickness and a decrease in the proportion of palisade tissue (Turell, 1936; Wylie, 1951). Shading of stock plants also leads to etiolating of suckers and promotes the formation of adventitious roots (Howard and Schmidt, 1979). Shading is also reported to reduce the amount of mechanical tissue which would have otherwise restricted root emergence and increased the juvenile phase of plant by delaying tissue maturation (Beakbane, 1961). Modification in the micro-environment of stock plants by shade may therefore influence aspects of cutting morphology such as stem length as well as biochemical and physiological factors which affect rooting (Leaky, 1983). Different plant species and even genotypes of the same species may respond differently to various levels of shading in their rooting performance (Moe, 1988).

Optimal rooting is obtained from juvenile trees and this increases in general with increasing age of the cutting material. This can however be reversed by perpetuating the juvenile character by shading of stock plants which could induce etiolating that is reputed to enhance rooting in cuttings (Harrison-Murray and Howard, 1981). The main objective of this study was to investigate the effect of artificial stock plant shading with Tildenet on the rooting success in different types of coffee cuttings.

MATERIALS AND METHODS

The study was carried out at the Bunso cocoa Station at Bunso (00'22'1'/, 12.8 N, 198~? a.s.l) fin the Eastern Region of Ghana from September 1996 to September 1997. Two robusta coffee cultivars 181 and 197 were used for the study. The stock plants were about six years old and were coppiced in October 1996 for new sucker production. Two shade regimes; 'Light shade' (37% full day light) and 'Heavy shade' (17% full day light) were investigated in this study. The light and heavy shade treatments were respectively achieved by erecting sheds with a single arid double layer of Tildenet 63% LSD over the coppiced stumps. Each shed measured 7.5 m long by 5m wide and 2.5 m high. These were compared with a control where all the plants were fully exposed to sunlight. The experiment was a split plot in a randomised complete block design with four replicates. The factors studied involved shade, cultivar and two physiological ages of cuttings (soft wood and semi hard wood cuttings). Soft- and semi-hard wood single node cuttings (10-15 cm long) were taken from 12 weeks old plagiotropic suckers and the leaves trimmed to 50%.

Rooting was done in propagation pits using at least 30cm deep cured rice husk as the rooting medium. The pits were covered with white transparent polythene sheet (150!l).and frequently watered to maintain a high relative humidity for the cuttings. Cuttings were treated with a 3% solution of Dithane M45 fungicide at weekly intervals to reduce rotting. Cuttings were assessed for callusing, sprouting and rooting at two weekly intervals. Five rooted cuttings were potted from each treatment for weaning studies. The experiment was carried out in both the wet and dry seasons.

RESULTS

Significantly longer roots (p= 0.05) were produced from light shaded suckers (37% full day light) at 10 and 12 weeks in the propagation with the unshaded suckers producing the shortest roots. In both seasons, semihard cuttings attained higher rooting than terminal cuttings (Table 1). Semihard wood cuttings also had significantly better rooting at 4 weeks than the terminal cuttings especially in the dry season.

Table 1. Effect of Physiological age on rooting percentage of stem cuttings of shaded plants

Weeks	Wet season		Dry season	
	Terminal	Semi hard wood	Terminal	Semi hard wood
2	0	0	0	0
4	0	5,81	2,21	6,18
6	31,73	44,52	19,01	39,83
8	57,74	66,44	39,87	66,29
10	71,91	74,83	53,96	84,33
12	83,01	86,41	66,32	92,41
Means	40,73	46,5	30,23	48,17
LSD (P=0,05)	4,131		3,82	

In the wet season, semihard wood cuttings produced significantly (P=0.05) longer roots than the terminal cuttings (Table 2). No differences in root length were noticed between type of cuttings (Table 2). Cultivar 181 produced significantly higher rooting percentage in the wet season at 10 and 12 weeks than cultivar 197 (Table 3).

In the dry season however, rooting was significantly better (P=0.05) in cultivar 197 than cultivar 181. Whilst cultivar 197 achieved 50% rooting at the 7th week, cultivar 181 achieved 50% rooting at the 9th week. However cultivar 181 produced significantly higher mean root length than cultivar 197 in the wet season at 10 and 12 weeks (Table 4).

Table 2. Effect of physiological age on mean root length (cm) of stem cuttings of shaded plants

Weeks	Wet season		Dry season	
	Terminal	Semi hard wood	Terminal	Semi hard wood
2	0	0	0	0
4	0	3,83	0	0
6	1,72	2,95	0,8	1,25
8	3,28	3,81	1,5	1,8
10	5,13	5,23	1,9	2,1
12	7,24	6,93	2,1	2,2
Means	2,89	3,79	1,55	1,86
LSD (P=0,05)	1,034			

Table 3. Effect of cultivar on rooting percentage of stem cuttings of shaded plants

Weeks	Wet season		Dry season	
	Terminal	Semi hard wood	Terminal	Semi hard wood
2	3,81	3,9	0	0
4	4,01	9,23	2,1	6,13
6	38,24	47,53	22,32	38,01
8	69,85	68,12	43,91	62,11
10	84,24	72,51	63,82	75,89
12	90,12	78,13	75,9	80,12
Means	48,38	46,37	34,68	43,71
LSD (P=0,05)	4,131		6,213	

Table 4. Effect of cultivar on mean root length of stem cuttings of shaded plants in the wet season

Weeks	Cultivar 181	Cultivar 197
2	0	0
4	0	0,4
6	1,59	2,61
8	3,49	3,5
10	6,68	4,4
12	8,41	4,61
Means	3,36	2,59
LSD (P=0,05)	2,11	

DISCUSSION

The overall performance of the shade treatments indicates that moderate shade enabled cuttings to achieve superior rooting than the other shade regimes. This observation confirms the explanation given by Wrigley (1988) and Nutman (1975a, b) that moderate shade gave maximum photosynthesis and assimilates for rooting The poor rooting in the 'no shade' treatment could therefore be attributed to the decreased photosynthesis and possible low carbohydrate content in the unshaded coffee cuttings (Leaky, 1983; Moe, 1988)

The effect of physiological age of cuttings on their rooting ability is well documented in the literature. Hartman and Kester (1983) observed in other crop species that the ability of cuttings to fonn adventitious roots decreases with increasing age. Cuttings from juvenile materials often fonn new roots much more easily than those taken from mature plants However semi-hard wood cuttings gave higher rooting percentage and longer roots in the dry season than the tenninal cuttings probably because of their higher nutrient and water contents than the tenninal cuttings which were wilting. Evans (1952) working on cacao and Rajagopal and Anderson (1980) working on pea cuttings showed reduced rooting when the cuttings were taken from stock plants having a water deficit Hartman and Kester (1983) observed that for good rooting of leafy cuttings it is essential that they maintain a high leaf water potential. Environmental conditions were favourable in the wet season with high relative humidity and relatively stable temperatures thus improving the perfonnance of the tenninal cuttings in the wet season. The perfonnance of the two cultivars in the dry season showed that cultivar 197 gave higher rooting percentage than cultivar 181. It is possible that genetically, cultivar 197 roots more readily than cultivar 181 however cultivar 181 perfonned slightly better than cultivar 197, under the optimal environmental conditions prevailing at the time. It is also possible that the nutrient status of cultivar 181 improved in the wet season with high photosynthetic activity as well as protein synthesis hence its improved rooting perfonnance. Visual observation of the stock plants also indicated that cultivar 181 was more stressed with yellowish drooping leaves than cultivar 197.in the dry season This suggest that cultivar 197 may be more drought tolerant than cultivar 181 and hence the better perfonnance ofcultivar 197 in the dry season than cultivar 181.

CONCLUSION

Semi hard wood perfonned better than tenninal cuttings and thus preferred as cutting material. Setting cuttings in the wet season is preferable to setting in dry season. Pre-shading of the parent coffee plants before cutting materials are prepared is not really necessary hence material can be obtained from shaded or no-shaded plots. Cultivar 181 has better rooting than cultivar 197 thus indicating varietal effects on rooting.

REFERENCES

Amoah F. M., Osei-Bonsu K. and Oppong F. K. (1997). Response of improved robusta coffee to location and management practices in Ghana. Experimental Agriculture, Volume **33**, pp 103-111.

Beakbane A. B. (1961). Structure of the plant stem in relation to adventitious rooting. Nature, **192**: 954-955.

Evans H. (1952). Physiological aspects of the propagation of cacao from cuttings. Proc.13/hlnt. Hort. Congress, **2**: 1179-1190.

Harrison-Murray R. S. and Howard B. H. (1981). Preconditioning shoots for rooting, etiolating and Blanching ofM9 an other apples. East MaIling Research Station Report, pp 57-60.

Hartman H. T. and Kester D. E. (1983). Plant propagation principles and practices (4th Ed.)

Prentice-Hall Inc. En.elewood Cliff PP. 250-264.

Howard. B. H. and Schmidt, G. L. (1979). Propagation by leafy soft wood cuttings under mist Report East Mailing Research Station. Pp 73- 74.

Leaky R. R. B. (1983). Stock plant factors affecting root initiation in cuttings of Triplochiton sceroxylon K. Schum an indigenous hard wood of West Africa. J. Hort. Sci. **58**: pp 277-290.

Mitchell H. W. (1988). Cultivation and harvesting of the arabica coffee ree. In coffee Vo1 **4**. R.J. Clarke and R. Macrae (Eds.) London. Elsevier Applied Science pp 334.

Moe. R. (1988). Effect of stock plant environment on lateral branching and rooting, Acta Hort. **226** (2) pp 431-444.

Nutman, F. J. (1937a). Studies on the physiology of Coffea arabica L (i) Photosynthesis of coffee leaves under natural conditions. Ann. Bot., **1** (3): pp 353-362.

Nutman, F. J. (1937b). Studies on the physiology of Coffea arabica L (ii) stomatal movement in Relation to photosynthesis under natural conditions. Ann. Bot., **1** (4): pp 687-693.

Rajagopal, V. and Anderson, A.S. (1980). Water stress and root formation in pea cuttings. Physiol.Plant., **48**: 144-149.

Snoeck, J. (1988). Cultivation and harvesting of robusta coffee. Coffee Agronomy vol. **4**, R. J. Clarke and R. Macrae. (Ed. London: Elsevier Applied Science. England. Pp 334.

Thompson, B. F. (1951). The relationship between age at the time of exposure and response of parts of the Avena seedling to light Amer. Jour. Bot. **38**: pp 635-638.

Turell, F. M. (1936). The area of the internal exposed surface of dicotyledon leaves. Amer. Jour. Bot. **23**: pp 255.

Wrigley, G. (1988). Coffee. Tropical Agriculture series. Singapore: Longman.

Wylie, R. B. (1951). Principles of foliar organisation shown by sunshade leaves from ten species of deciduous dicotyledon trees. Amer. Jour. Bot., **38**: 355.

The Effect of Different Media on the Success of Rooting Ramets of Coffee Clones Directly in Polybags

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SUMMARY

In view of the high overhead cost of rooting coffee cuttings in propagators, it became necessary to find other ways of rooting coffee cuttings. To achieve this objective, an experiment was set up at the Cocoa Research Institute of Ghana (CRIG) to investigate the effect of using polybags containing different media in a simplified technique for raising coffee ramets for planting. Single node cuttings (ramets) of four different coffee clones were rooted in holes dibbered in black soil contained in black polyethylene bags measuring 10cm x 20cm. The holes were filled with different rooting media including rice husk, sawdust, river sand, rice husk plus river sand (1:1), or sawdust plus sand (1:1) and a control using black soil alone. Half the number of polybags containing each medium received 5g of triple superphosphate (TSP) per bag whilst the other half did not receive any fertilizer. Significantly ($P \leq 0.01$) higher flushing, rooting and root lengths were recorded from two of the clones (Nos. 96 and 149) but not from the other two (Nos. 138 and 181) within 4 months. The river sand and the topsoil media produced taller suckers probably owing to faster rooting than in the other media. Clones 96 and 149 continued to produce taller suckers and retained more leaves with larger leaf area than clones 138 and 181 after 8 months. It is concluded that coffee cuttings can be successfully rooted directly in polybags with topsoil in the nursery to reduce infrastructural costs.

INTRODUCTION

Coffee cultivation has not received much attention in the past in Ghana. Many coffee farms have been planted from seed materials with uncertified genetic background thereby giving poor yields (100-200 kg/ha) to farmers (Ampofo and Osei-Bonsu, 1988; Anon, 1996). A World Bank funded programme, the Agricultural Diversification Project (ADP) aimed at revitalizing coffee cultivation through research and extension was initiated in 1990 (Anon, 1996). A working group of the ADP proposed that to achieve the above objective, there was the need to encourage the planting of high yielding and disease resistant clonal coffee material (Anon, 1997).

The group therefore suggested the establishment of wood gardens of high yielding clones at selected sites to produce rooted cuttings for farmers. Very heavy investment was undertaken to provide propagating structures (namely bins and sheds) for the nurseries (Ampofo and Osei-Bonsu, 1988). Cocoa Research Institute of Ghana (CRIG) was assigned to develop and propagate the initial planting stock for the wood gardens which were to be operated by the Cocoa Services Division. During a working visit to the Uganda Coffee Industry (Anon, 1998) it was noticed that coffee cuttings could be rooted successfully in polybags under raised sheds which did not require the heavy capital investments incurred in Ghana. The additional advantage of the system was that it could be located anywhere with ease.

An experiment was therefore set up to investigate the use of polybags and different rooting media in a simplified technique for rooting coffee cuttings in Ghana. It was hoped that the success of this method would help reduce infrastructural costs for raising coffee ramets for planting and make the technique adoptable by small- scale nurseries.

MATERIALS AND METHODS

The trial involved the use of six rooting media, with or without fertilizer and combined factorially with four robusta clones in a randomised block design with four replicates.

The rooting media tested were:

1. Decomposed rice husk
2. Decomposed sawdust
3. River sand
4. Decomposed rice husk plus river sand (1:1 ratio)
5. Decomposed sawdust plus river sand (1:1 ratio)
6. Control with no rooting media

Small size black polyethylene bags measuring 10 cm x 20 cm were filled with topsoil. A hole about 5cm diameter by 10 cm deep was made in the centre of the soil in each bag with a dibber. Half of the bags received 5 g of triple superphosphate (TSP) in the hole whilst the remaining half did not receive any fertilizer. Single node cuttings with the leaves reduced by half were prepared from robusta coffee clones 149, 96, 138 and 181. Each sub-plot treatment contained 25 cuttings giving a total of 4800 cuttings for the trial. The bags were arranged in columns of 10 x 50 and leaving space for movement between packs. The columns were covered with a translucent polythene sheet held on arched bamboo sticks and tucked under with plants. The experiment was conducted under about 50% shade provided by *Gliricida sepium* and palm fronds. The bags were watered regularly to maintain a high relative humidity for the cuttings. The trial was inspected periodically to remove dead leaves and cuttings.

At four months after insertion, the percentage flushing, number of leaves, height of suckers, percentage rooting, number and length of primary roots as well as number of secondary roots produced were assessed on 5 samples per treatment. At eight months after insertion, 5 samples per treatment were scored for the number of leaves, leaf area and sucker height to determine post-rooting performance.

RESULTS AND DISCUSSIONS

There was a highly significant ($P \leq 0.01$) effect of the type of clone used in all the parameters evaluated (Table 1).

The propagation medium affected only the height of the suckers. From the table of means (Table 2), it was apparent that clones 96 and 146 were superior to clones 138 and 181 in terms of the ease of propagation and some vegetative characters at four months. Both media and fertilizer application did not have any effect on the propagation the coffee ramets.

Eight months after insertion, clone 96 followed by clone 146 continued to give the highest leaf area and the tallest suckers (Table 3), thus confirming the observations made at 4 months. The number of leaves was not affected by the treatments applied to the clones at eight months. These confirm the observations by the Plant Breeder (Personal communication) that clones show different propagation abilities.

Table 1. Analysis of variance for measurements on coffee ramets at 4 months

Factors/Interactions	df	Percent Flushing	No. of leaves	Percent rooting	Height of suckers	No. of Primary roots	Length of Primary root	No. of Secondary roots
Clone	3	7.07***	10.70***	17.31***	57.30***	6.17***	5.85***	5.82**
Medium	5	0.47	0.65	1.28	4.52**	1.77	1.37	1.01
Fert	1	0.16	0.64	0.00	0.01	2.41	ns	0.20
Clone x Medium	15	0.10	0.82	0.51	ns	ns	0.91	0.39
Clone x Fert.	3	ns	0.88	1.11	ns	ns	2.56	0.05
Medium x Fert.	5	0.24	1.19	0.54	ns	ns	0.64	0.35
Clone x Med. X Fert.	15	0.31	1.01	0.64	ns	0.55	0.55	0.01

***Significant at P≤0.01; ***Significant at P≤0.001*

Table 2. Table of means of measurements on coffee ramets at 4 months after insertion

CLONE	Percent* Flushing	No. of** leaves	Height of suckers (cm)	% Rooting*	No. of Primary Roots**	Length of Primary roots (cm)	No. of Secondary** roots
96	58.7	2.0	6.9	45.2	2.6	6.2	4.2
149	57.7	8.7	5.4	41.4	2.7	5.1	3.1
138	49.3	2.1	4.3	31.2	2.3	4.7	3.0
181	52.6	2.0	2.9	29.4	2.1	4.2	2.1
SE of Means	2.35	0.05	0.32	2.58	0.07	0.50	0.49

**Angular transformed data; **Square root transformed*

Table 3a. Analysis of variance for measurements on coffee ramets at 8 months after insertion

Factors	df	No. of leaves	Leaf area	Sucker height
Clone	3	6.428	12.58**	57.21***
Medium	5	0.64	0.69	5.55*
Fertilizer	1	0	0.32	0.91
Clone x medium	15	0.91	0.36	0.76
Clone x fertilizer	3	1.56	1.48	0.23
Medium x fertilizer	5	0.04	0.31	0.25
Clone x medium x fertilizer	15	0.69	0.37	0.53

**Significant at P ≤ 0.05; **Significant at P ≤ 0.01; ***Significant at P ≤ 0.001*

The percentage flushing was generally low compared to what could have been obtained from the conventional propagators. This was attributed to the lack of uniform shade for the trial which was set up under *Gliricidia sepium* nursery shade. Owing to the canopy arrangement of *G. sepium* and shedding of their leaves during the dry season for flowering, direct sunshine penetration caused overheating and scorching of some of the cuttings. Ideally the trial should have been conducted under a properly raised shade of bamboo slats or synthetic polypropylene netting (Ampofo and Osei-Bonsu, 1988) to provide uniform shade. Thus any small-scale nursery operators wishing to adopt this direct insertion technique where the cuttings stay in the bags for up to 9 months must take precautions to provide proper shade to enhance the success of the propagation. Significantly taller ($P \leq 0.05$) suckers were produced from the river sand and control (top soil only) media at both 4 and 8 months (Tables 1 and 3). Rooting of cuttings has been noted to be faster in river sand (E. Anim-Kwapong, Personal

communication). This could have hastened the development of the suckers. A similar situation could have occurred in the topsoil resulting in better-developed vegetative growth. This situation could have accounted for the production of taller suckers from these media. Further investigations need to be conducted to explain these observations.

Table 3b. Table of Means

Clone	No. of Leaves*	Leaf area (m^2)	Sucker height (m)	Medium	Plant Height (cm)
96	2.9	7.9	14.1	Rice husk	9.3
149	2.8	6.5	10.1	Sawdust River	8.3
138	2.4	5.6	7.5	sand	10.4
Sed (df 141)	2.6	6.1	5.8	R. husk; R. sand	7.8
	0.34	0.33	0.67	Sawdust; R. sand	9.1
				Control	11.5
				Sed (df.141)	0.82

**Square root transformed*

CONCLUSION

Coffee ramets can be rooted using a simplified technique of planting directly in polybags. The type of rooting medium may not be important in determining the success of the propagation. It has been demonstrated from this trial that cheap and readily available media like topsoil could also be used. However care should be taken to provide adequate shade and high relative humidity for the system to enhance the rate of success.

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REFERENCES

Ampofo S.T. and Osei-Bonsu K. (1988). A practical guide for coffee extension workers. Tech. Bulletin, Cocoa Research Institute of Ghana (CRIG), Tafo.

Anon (1996). Ghana coffee study. Cargill Technical Services Ltd., Knowle Hill Park, Surrey, U.K. Consultant Report for the Government of Ghana.

Anon (1997). Revamping the coffee Industry: Short term programme to increase the production of coffee in Ghana. Ghana Agricultural Diversification Programme, Coffee component. Coffee Working Group, Accra, Ghana, May 1997.

Anon (1998). Report on the visit to the Uganda Coffee Industry – Uganda Coffee Development Authority, UCDA, January 1998.

GFAR and International Cooperation on Commodity Chains

Hubert OMONT

GFAR

GLOBAL FORUM ON AGRICULTURAL RESEARCH
FORUM MONDIAL DE LA RECHERCHE AGRICOLE
FORO GLOBAL DE INVESTIGACION AGROPECUARIA

GFAR and International Cooperation on Commodity Chains

GFAR

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What is GFAR?

The Global Forum on Agricultural Research (GFAR) was established in 1996 as a neutral and transparent platform involving all the stakeholders of the Agricultural Research for Development.

The **mission of GFAR** is to mobilise the scientific community and all stakeholders in agricultural research for development in their efforts to alleviate poverty, increase food security, and promote the sustainable use of natural resources.

Stakeholders

- Private Sector
- NARS
- Donors
- ARIs
- CGIAR
- NGOs
- Farmers' Organisations

The GFAR current Programme of Work is to be developed along four lines of action:

- Development of a shared vision and of a strategic agenda for ARD
- Promotion of innovative research partnerships
- Development of a global knowledge system for ARD
- Strengthening NARS and their regional and sub-regional Fora.

The GFAR priorities for innovative research partnerships:

- ✔ Genetic Resource Management and Biotechnology
- ✔ Natural Resource Management and Agro-ecology
- ✔ Global Programmes on Commodity Chains
- ✔ Policy Management and Institutional Development.

subsidiarity, complementarity, additionality, involvement of all stakeholders, and partnership

are GFAR guiding principles

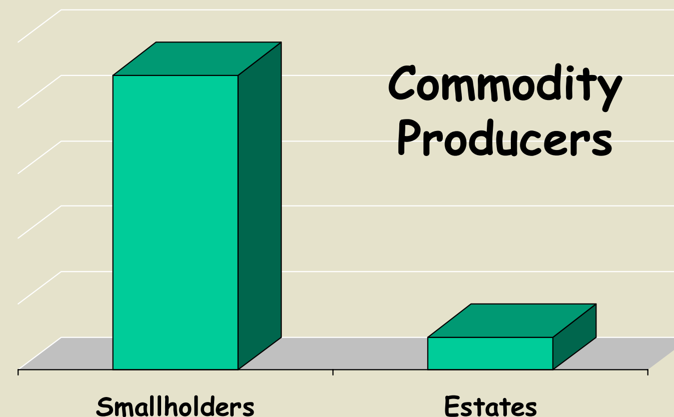
www.egfar.org

Why Commodities?

Crucial economical importance to generate income and to achieve integration in international exchanges

Research carried out by NARS with limited resources

Decreasing support to smallholders due to liberalisation and privatisation



Research Under-funded

- ✔ **Long lasting low prices for most commodities**
- ✔ **Limited Donor support**

Increasing cooperation needs between producers and the industry to provide quality products that suit consumers' demand at a satisfactory price for both.

The private sector's research contribution limited to some competitive sectors

New mechanisms are required to build on and coordinate existing private and public sector activities to produce more "public goods"

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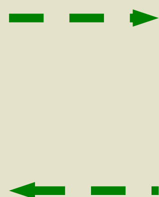
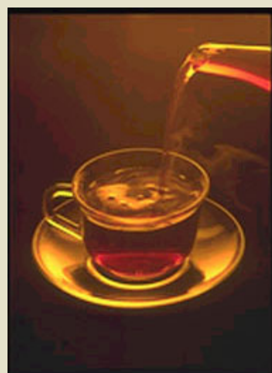
The Concepts

To address problems or challenges that are of global concern,
and can not be effectively tackled at local or regional levels

A Commodity Chain Approach





For a given crop, the approach is not restricted to the conventional agricultural components related to increases in productivity, but rather the crop is considered "as a whole" in all aspects of a chain (or a system), from its production through to its consumption or use by the consumers.

Global Programmes

- ✔ Coordinated set of activities ...
- ✔ Wide range of programme participants ...
- ✔ To solve a specific problem/challenge ...
- ✔ Identified at the global level.

Philosophy

- Build on existing achievements
- Based on on-going activities
- Equity and subsidiarity

Benefits

Global research priorities
Improved access to information and resources
Closer interaction among research teams
Interdependent research projects
Improved funding possibilities

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Action Undertaken

Montpellier Facilitation Unit - IPGRI/Cirad September 1999

Information on stakeholders and their on-going collaboration

Identification of stakeholders,
Existing cooperation mechanisms,
On-going and planned projects.

Contacts, possibilities and interest for global programmes

- **Stakeholders' initiatives**
- **GFAR initiatives**
- **Montpellier Unit initiatives**

Assistance in the development of global programmes

Existing

Banana

In preparation

Coconut **PROCOCOS**

Cocoa

PROCACAO

GFAR

GLOBAL FORUM ON AGRICULTURAL RESEARCH
FORUM MONDIAL DE LA RECHERCHE AGRICOLE
FORO GLOBAL DE INVESTIGACION AGROPECUARIA

Assistance in the setting up of Commodity Global Programmes

Initiative

Initiative

Global Challenge / Problem

Initiative

Identification

Stakeholders' Consultation

Divergent Approaches

Separate Programmes

Common Agenda

Elaboration

Stakeholders' Consultation

Global Programme

Implementation

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Biological Control of the Coffee Berry Borer

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With the announcement of the arrival of the coffee berry borer (CBB, *Hypothenemus hampei* (Ferrari)) in Costa Rica in December 2000, this tiny 2 mm long beetle has finally completed its conquest of the world's major coffee producing countries. It was first noticed in the field in Gabon in 1901 (Chevalier, 1947) and soon became apparent in Java by 1909 and in Brazil by the 1920s (Le Pelley, 1968) and from these introductions, and mostly likely others too, it has gradually spread to more than 50 coffee-producing countries. This geographical spread, together with the economic loss it causes, makes it the most important insect pest of coffee of the 20th century.

The earliest method of control was simply to collect all berries after harvest to leave no food sources for this insect, and remarkably this is still the standard recommendation in many countries. However, with the current historically low prices for coffee this is no longer a very welcome recommendation for many farmers. And with the current interest in organic and sustainable agriculture and increasing concerns for health and safety of operatives who rarely take adequate precautions whilst spraying, the chemical alternative is not attractive. Additionally, for many small farmers who grow coffee on mountainsides, the chemical option is also very expensive and in the present price crisis, very few are spraying their coffee.

The principal preoccupation of our unit at CABI is to help small farmers produce high quality coffee whilst incurring the lowest possible economic, environmental and health costs so that they can maximise their earnings. We believe that biological control should play a central role in helping them control insects and diseases where at all possible because it is non-toxic, environmentally safe, and, when it works well, extremely cost effective. We argue therefore that if sustainable coffee farming is to prosper, problems such as CBB have to be solved in an environmentally compatible fashion, and this requires rigorous research on a range of possible alternatives.

Hence the subject of this paper is biological control which, in its various forms, our institute has championed over the last 70 years.

WHAT IS BIOLOGICAL CONTROL (BIOCONTROL)?

Remarkably few of the millions of insect species are pests, and the principal reason for this is that their numbers are severely restricted by various parasites, parasitoids (a parasite that invariably kills its host), predators and diseases. To date 1424 pests of coffee have been recorded from 101 countries but very few of these are major pests, due mainly to the 705 species of natural enemy also reported (data from Bigger, 1999, and personal communication, 2001). Biological control simply uses these natural enemies in a variety of ways, but the central principle is always the same, to reduce population levels of a target pest by natural means. And we want to use it whenever possible, because once chemical control becomes common, the chances increase of upsetting the delicate balance of so many potential pests and their natural controllers. Many of the birds and other wild animals found in coffee (which can add value to the harvest) are directly or indirectly dependent on the diverse insect life supported by the crop, making this a further reason to understand and use biocontrol.

In the case of CBB, our initial aim is to introduce the African natural enemies it escaped from when it was introduced to other countries. This is known as 'classical' biological control and once the organism is released and established, no further intervention is required. Sometimes, classical biocontrol can be a complete solution to a pest problem, because the agent concerned carries out the control so efficiently that the farmer may eventually become completely unaware of the pest and the natural enemy at work in his fields.

Unfortunately, in many cases the classical option does not produce complete control, which leads us either to try augmentation of the agent, by culturing and regular release, or to adopt other control measures that are compatible with biological control. But whatever methods are used, we always try to encourage biocontrol because it is the only method that is 'density-dependent',- this means that as pest numbers rise, control tends to become more effective, whilst as numbers fall, control declines so that a fluctuating equilibrium is reached. Hence biological control is concerned with ecological balance, which is a centrally important concept in sustainable coffee growing.

IS BIOLOGICAL CONTROL RISKY?

In recent years biological control has come under increasing attack, mainly because of the fear that the introduced agent may attack other insects, some of which could be beneficial. However, if prior testing is carried out to measure specificity of the control agent, problems of this sort are very unlikely to emerge. And even in the few cases where non-specific agents have been released, to date there are no serious biological problems reported for insect control campaigns. Of the nearly 5,000 biocontrol introductions during the 20th century (Biocat biocontrol database, Greathead and Greathead, 1992; personal communication, 2001) there is no insect biocontrol mistake that has caused human suffering. Insect biocontrol has no Bhopal or 'Silent Spring', and unlike pesticides, it is not responsible for a regular toll of lives impaired or shortened by their routine use. Insect biocontrol is a farmer-friendly technology.

AN HISTORICAL PERSPECTIVE OF INSECT BIOLOGICAL CONTROL

Before we look at the specific case of CBB, it is worth studying what we know of biocontrol from previous programmes. As mentioned above, there have been a large number of introductions, and many of them have failed. Regrettably the reasons for failure are frequently hard to determine because insufficient data exists, indeed good follow-up studies are very rare. However, we know that many projects fail because of insufficient time and resources to introduce sufficient individuals, often far from ideal circumstances, to form a critical mass to

establish viable colonies. But if we set aside these financial and logistical problems, certain patterns in the data become apparent. Hawkins (1994) looked at these in detail and came to some interesting conclusions.

First, he found that rather few boring insects have been controlled successfully by biocontrol alone. Instead he found that external feeders (e.g. many lepidopteran larvae) and those near the surface (e.g. leafminers) had higher success rates. Hawkins also found a significant relationship between the maximum parasitism rate detected, and the success of a biocontrol campaign. Anything below 40% parasitism was almost always a failure and he found mean maximum parasitism for borers to be around 20%.

These facts alone however should not discourage us from introducing natural enemies against CBB, there are always exceptions to any rule and even modest control can be very beneficial and cost-effective.

Hawkins (1994) also pointed out another problem; borers tend to have a limited 'guild' of specific natural enemies. This is perhaps not surprising, since an insect hidden away, deep inside the tissues of a plant, is not easy to discover and hence few predators or parasitoids are likely to have evolved to find ways to routinely live off them.

If we look now at the known parasitoids of the CBB (Murphy and Moore, 1990) we see that there are only five: *Cephalonomia stephanoderis*, *Prorops nasuta*, *Sclerodermus cadavericus* (all Bethylidae) *Heterospilus coffeicola* (Braconidae) and *Phymastichus coffea* (Eulophidae). Interestingly, five is just about what Hawkins (1994) predicts from the Biocat database. Hawkins et al. (1997) also found that internal feeders were especially resistant to non-specific predators (e.g. ants) and pathogens, and that history clearly indicated that parasitoids tend to be considerably more important as agents of mortality to this group.

What emerges then from the last 100 years of biocontrol work, is that although the number of parasitoid natural enemies and their effectiveness is likely to be lower for borers than for several other insect groups, they are still the most important agents of CBB mortality. And so, if we believe in sustainable coffee production, it is our duty to try to make them freely available to farmers who will have few other resources at hand to help control this pest. Accordingly, the few available biocontrol species of CBB should be studied and tried out in as many ways as possible to see how they can be most useful to the farmer.

THE BETHYLID FAMILY OF PARASITOIDS OF CBB

Classical control

These wasps are *Cephalonomia stephanoderis, Prorops nastua* and *Sclerodermus cadavericus,* the last named has not been used because its bite causes a dermatitis which would make it difficult to rear. The other two bethylids have now been released and established in many Latin American countries over the last 15 years, thanks to programmes funded by GTZ, DFID, EU, IDRC and various national programmes. The pattern of parasitism found after release shows sharp declines, some of the most detailed work on this, (Barrera 1994) clearly shows that parasitism levels decline to less that 5% after the first year of release. Quintero et al. (1997) carried out similar studies in S. Colombia two years after releases of *C. stephanoderis* and *P. nasuta* was last released. They found *C. stephanoderis* in only 27% of the release sites whereas for *P. nasuta* it was found in 73% of sites. Mean parasitism rates were less than 5% for both species. A new and indigenous bethylid, *Cephalonomia hyalinipennis* has recently been found parasitising CBB in Mexico (Perez-

Lachaud, 1998) but we believe that parasitism rates for this species will also turn out to be very low.

Figure 1. ***C. stephanoderis***

Hence classical biocontrol of CBB by bethylids is slight. Nevertheless, even if the parasitoid saves only 1% of berries from becoming infested, when summed over the whole of Latin America, the cost of introducing and establishing the agents is easily paid for.

Augmentative control

Could bethylids be reared and released cheaply enough, say three or four times a year, to bring about control?

Results from our Colombia project (Baker, 1999, a CABI Commodities and Cenicafé joint project funded by DFID (UK) and the Colombian Coffee Federation) suggest that *C. stephanoderis* can control field populations of CBB when released massively, but that the numbers required, and hence the cost, make this method impractical.

Salazar (see Baker, 1999), carried out releases of quantified numbers of *C. stephanoderis* in coffee plots where, through intensive sampling he had calculated the absolute number of CBB present. In each of two replicates of three plots he variously released three ratios of parasitoid to infested berry; 10:1, 50:1 and 100:1 four times during 12 months of study and he included two control plots. No other control method was used except in the control plot where hand picking was required to keep the numbers from getting excessively high. Only at the 100:1 release rate was the damage to harvested parchment coffee kept under complete control. For all other treatments, the level of damage increased, albeit gradually, during the year. A further experiment with monthly or bi-monthly (6 times/yr) releases of 20 parasitoids per infested berry (Figure 2), showed that the monthly releases managed to keep parchment levels below 2% infestation. Perhaps the most disappointing aspect of the work is that we would hope to see at least some evidence of a second wave of parasitism occurring around five to six weeks after the original release, as F1 offspring emerge and attack new CBB-infested berries. But despite intensive sampling, no such pulse was seen.

Since stabilisation of CBB damage was recorded, augmentation is therefore a possibility but a very cheap method of producing them would be required, and the current methods are too expensive by at least an order of magnitude. Extensive laboratory studies by Portilla (see Baker, 1999) found that *C. stephanoderis* requires a large number of CBB stages to produce

offspring, roughly three CBB stages per new live female birth. Demographic parameters calculated from these laboratory studies show that with a mean generation time of 36 days and a sluggish fecundity, it would be too expensive to rear in the laboratory (finite rate of increase is approximately 1.1). This also implies that it is too inefficient in the field to breed fast enough before harvest to cope with the often mass attack of CBB emerging from fallen berries after the first rains.

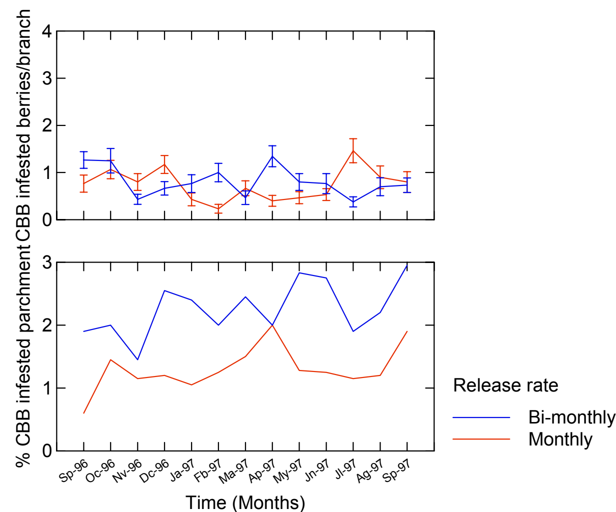

Figure 2. Top: infestation of CBB/branch, Bottom: % CBB damage to parchment beans from plots with 20:1 wasp releases every month or every other month

Indeed, studies on dispersal of the wasp by Aristizábal (see Baker, 1999) show that even though percentage parasitism around the release site is promisingly high (>40%) this level declines rapidly with distance from release site, and, surprisingly, with time. Numbers of adult wasps inside infested berries declined significantly between the first and second observations (at 7 and 15 days after release), suggesting that many wasps that originally entered berries were either departing again or being predated by unknown agents. Since predation of CBB-infested berries is generally very low, the most likely explanation is that the wasps chose to leave the berries, perhaps because conditions there were not favourable to them. Dufour however (cited in Dufour et al., 1999) found very high levels of parasitism (64%) with a 1:1 release rate, though there was no follow-through to measure the rate of CBB attack in parchment coffee harvested from the experimental plots to see whether there was a protective effect. In Mexico on the other hand, Damon (1999) found that the field impact of *C. stephanoderis* was very low, and suggested that this was simply because so few released wasps manage to find an infested berry.

On balance, the low natural level of parasitism found in the field, the low fecundity of the wasp and its apparent inability to protect against parchment damage, all militate against the use of this wasp as an augmentation agent. Since *Prorops nasuta* is a close relative of *C. stephanoderis* we infer that the situation is broadly similar for this wasp also. And since initial trials with *Phymastichus coffea* are more promising, it seems sensible now to concentrate limited research funds on this wasp.

THE THIRD PARASITOID

The recently discovered *Phymastichus coffea* (Figure 3) is an unusual parasitoid because it attacks the adult insect. Very few parasitoids attack adult beetles, and possibly CBB is an exception because it is exposed and immobile in the entry tunnel to the coffee bean during the

initial stages of its attack on the berry. The female normally injects two eggs into the body of the adult female CBB where they develop, one in the abdomen, the other in the thorax. Unlike the bethylids, one wasp can parasitise more than one infested berry, which makes it inherently more interesting as a biocontrol agent than a bethylid which should normally stay in the berry that it attacks to guard its brood.

Figure 3. ***Phymastichus coffea***

The initial experiments on *P. coffea* are quite encouraging. In Guatemala for instance, experiments by Anacafe (Garcia et al., 2000) show that releases of the wasp quickly disperse over a period of 90 days to cover at 10 hectare area of coffee. Since the adult is short-lived, this is good evidence that the wasp is breeding freely and first and second generation descendants are present at detectable levels. In several arabica and robusta plots in Guatemala, parasitism rates of 15, 21, 23, 33 and 46% were recorded in plantations. Furthermore, an initial study by Vergara (1998, and cited in Baker, 1999) released 30,000 wasps into a plot with a 13% natural infestation and where he had artificially infested 26,700 CBB onto marked branches, achieved a mean parasitism of 46%.

These studies, if confirmed by others, therefore suggest that quite high levels of parasitism can be achieved from a single release and that two or more measurable generations may arise from a release. This is encouraging because the residual effect would give longer control for each release for no extra cost.

THE FOURTH PARASITOID

The fourth and possibly the last parasitoid of CBB, the braconid *Heterospilus coffeicola*, has yet to be reared successfully and hence we know less about its potential than the others. The female can visit more than one infested berry where she lays only one egg per berry, which hatches out and consumes the younger stages of the CBB. Recent work in Uganda by Murphy et al. (2001) suggests that the parasitoid lays eggs predominantly in berries with young CBB broods, peaking at around one week after CBB perforation of the berry. Other preliminary data gathered so far suggest this species prefers shade and is most active early in the morning. It was attracted to trays of infested berries placed in the field and tended to preferentially attack higher concentrations of CBB infested berries. Dissections of females reared from field-collected berries and fed on different combinations of honey, sugar, water, pollen etc., revealed very few eggs however, suggesting that an important element of its diet is still missing to ensure adequate fecundity.

CBB PREDATORS

A number of CBB predators have been reported, though numbers are usually very low and seem to be mostly generalist predators including ants such as *Crematogaster* spp. Biocontrol practitioners often try to avoid perdators precisely because they are more likely to attack non-target insects. But an exception to this may be the recent discovery of *Leptophloeus* sp. near *punctatus* (Vega et al., 1999) which may be more specific, though this requires further research. If it were specific, it could be of interest for augmentation work since it might be easier to mass rear than parasitoids.

BIOPESTICIDES

There has been considerable research on *Beauveria bassiana* (Bb) for control of CBB over the last decade of the 20th century and there is insufficient room here to deal comprehensively with this work. But in summary, the authors know of no convincing published evidence that this technology is cost-effective. There is no doubt that a spray of Bb will kill CBB, but the amount required to reliably kill the same amounts as a chemical insecticide, can be prohibitively expensive. A plot of all available field data from several experiments in Colombia (Figure 4), suggests that with current technology, 10^{10} spores per tree are required to achieve 80% mortality of recently infested berries (equivalent to the kill seen after a chemical application). This is too expensive by roughly an order of magnitude. A serious problem is the difficulty of ensuring that most CBB-bored berries are adequately covered by spore-containing droplets. The simple knapsack sprayers used by farmers tend to give very poor coverage.

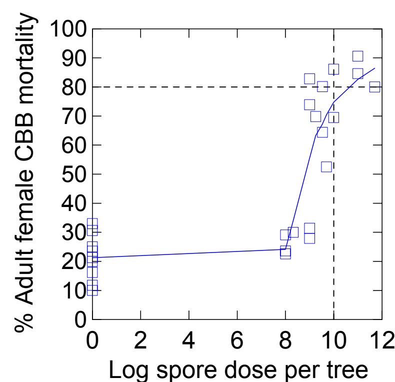

Figure 4. Composite figure of all available field tests using various dosages of Bb. Line fitted by smoothing function, dotted lines indicate approximately 10^{10} spores/tree needed to get 80% mortality (equivalent to an insecticide spray)

We suspect that control will always be difficult using this technology because:

1) The fungus deactivates fast, especially where shade is light and temperatures elevated.
2) Good coverage is difficult with inexpensive traditional spray machinery.
3) The fungus does not penetrate well into the bored berry so will be most effective when applied on berries of a major flowering where large numbers of CBB are in the early peripheral attack phase.
4) Commercialising the product and ensuring viability whilst it reaches remote mountainous areas means it is unlikely to be a cheap alternative for many farmers.
5) Several chemical insecticides are often reasonably effective against CBB so there might be no incentive for farmers to change.

6) Since many farmers can not afford to spray anyway, (it can take up to 5 days to spray one ha of mountain coffee) it does not match the criterion of a sustainable control method.

This is not to say that a commercially viable product is impossible, only that it will be likely to work sustainably if several conditions are in place:

1) A reliable source of Bb with independent quality control enforcement (when CBB became widespread in Colombia, several firms started producing Bb but quality was very variable). This would most likely occur on the heels of a proven local success, e.g. Bb used on high-value horticulture/cut flower industries which would afford economies of scale and freshness for suppliers.
2) Shade coffee (most trials in Colombia were on sun-coffee, which is a harsher environment for Bb).
3) A medium/large farmer with ULV spraying machinery and the know-how to use it.
4) A distinct dry season, so that most berries become susceptible to attack at the same time where a well-timed spray will have the maximum chance of causing high mortality.
5) A compelling reason for farmers to use Bb rather than a chemical.

This would therefore suggest that, say, a medium or large Central American organic shade coffee farmer, either with his own production or fairly close to a Bb facility, would be the most likely producer to make this technology work cost-effectively.

OTHER BIOCONTROL AGENTS

Apart from parasitoids, predators and fungi, there are a few other possibilities. Entomopathogenic nematodes (EPNs) are an interesting prospect because theoretically at least they should find the under-tree habitat of fallen infested berries favourable and we know that fallen berries are a very significant reservoir of infestation that is rarely controlled. Experiments at Cenicafé by López (López and Briscoe, 1999 and cited in Baker, 1999) show that the EPNs *Steinernema glaseri* and *Heterorhabditis bacteriophora* are capable of finding and attacking CBB-infested berries, though it remains to be seen whether they could become a either a classical or augmentative agent. On the face of it, it seems unlikely that small farmers would regularly buy nematodes to release, but if an occasional inoculation were to be reasonably self-sustaining, then this could have a beneficial effect on fallen berries where CBB mortality rates are frequently very low.

BIOLOGICAL CONTROL OF THE BORER, HOW FAR CAN IT GO?

The released agents up to now have shown no major reduction in damage by the CBB, though we argue that since at least two and probably three parasitoids have now established in several countries, that they must exert some level of control. Furthermore, though evidence is only anecdotal, the biocontrol agents must exert a much greater effect in abandoned farms, so biocontrol in these cases will reduce these reservoirs of CBB that disperse outwards to operational farms. Hence biological control acts in the reverse sense to the effects of coffee price: when prices are high, farmers apply more control methods and make more regular collections of berries, all of which will keep CBB under control. When prices fall, farmers stop controlling, CBB populations build up and biocontrol becomes more significant. Indeed when prices are at the current historical lows, biological control, essentially free to the farmer, may well be his only form of control. For this reason alone, we believe that the investment in CBB biocontrol to date has been well worth it.

Can we build on this situation to gradually improve the degree of control? If a combination of one, two or possibly three parasitoids does not materially affect CBB populations, we might then start to look at trying to change the coffee habitat in order to promote greater numbers of mostly indigenous natural enemies, many of them non-specific. This could include denser shade to promote better conditions for natural epizootics of Bb and inclusion of shade tree or weed species that are attacked by similar pests to CBB, which would thus provide reservoirs of natural enemies (including pathogens). These modifications, combined with changed agronomic practices, e.g. to leave, say, one in 20 trees un-picked as a reservoir of natural enemies or as foci to inoculate small amounts of natural enemies might start to have a cumulative effect.

However, there are problems with developing this sort of work: such experiments take considerable effort to set up and evaluate and they have to be rigorously quantified over several seasons; many funders would lose patience before progress could become apparent. Prior to this therefore, modelling of the economic and biotic effects of different practices could save a lot of trial-and-error tests in the field. A prototype model already exists, developed by Leach et al. (1999) which could serve as the starting point for this type of approach. And wider surveys, in Africa and local ecotopes in Latin America could reveal circumstances where CBB is rather scarcer than might be expected. If studied thoroughly, these situations could help us to gradually alter the balance of control in favour of the farmer.

On a more interventionist note, other methods suggested have included ways to return some of the harvested CBB natural enemies to the coffee plots, and traps to auto-infect CBB and then release them to disperse the pathogen in question. Whatever additional method, the guiding principal must be to find easy and labour-saving ways of helping small farmers, for they are going to find it increasingly difficult to make a living from growing coffee. It is often believed that small farmers have recourse to abundant cheap family labour, e.g. to hand remove infested berries, but studies show that this is frequently a myth (Baker, 1999).

THE FUTURE

Get the four parasitoids

We argue that we must strive to ensure that all four parasitoids are available for use anywhere so that national programmes can then test them in various combinations to see how effective they might be. We believe that there is a good chance that either *P. coffea* or *H. coffeicola*, or a combination of both could exert an appreciable effect in some situations, especially where there are multiple populations of berries to provide a continuous supply of CBB. These tests should be fully quantified to determine if the parasitoids have any future as augmentative agents (see below).

This is the only short to medium term possibility for an outcome that would reduce the cost of CBB control, all the other methods, whether augmentation, spraying or cultural control, will cost the farmer money each year that he can ill afford.

Evaluate the parasitoids' potential for mass release

For augmentation, it should soon be possible to rear large numbers of CBB very economically, thanks to the on-going studies at USDA Mississippi by Portilla. She has now continuously and stably reared CBB for 13 generations on an inexpensive diet and with low levels of contamination (Portilla, 2001, personal communication). If this can then be bulked up to true mass-rearing (at least 1 million times the average female fecundity per generation,

Figure 5) this could supply very large numbers of very cheap CBB on which to rear the parasitoids in probably fairly simple rearing stations close to the coffee fields. If the economics of this prove to be favourable, the next step would be to mount a pilot project to try to control, say, a few hundred hectares of coffee by mass releases alone. At the moment, *P. coffea* is the obvious candidate for such a trial.

If mass release is not economic

If economic evaluation of mass release is unfavourable, and if all the parasitoids have been evaluated fully and prove to be inadequate to control CBB, there are then two very different avenues.

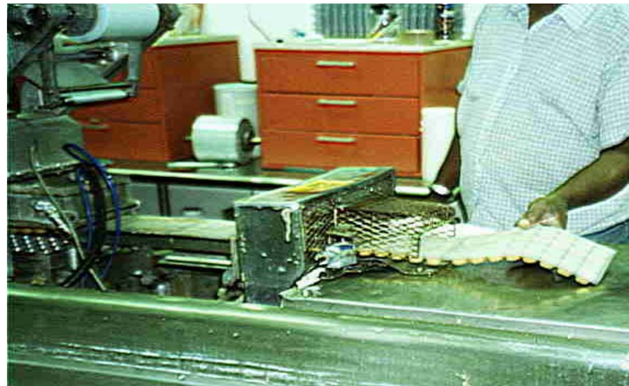

Figure 5. A form-fill-seal machine preparing diet packs; one machine could supply the needs for a whole country's parasitoid production

The first is the ecological approach mentioned earlier, where as many different biotic agents as possible are ranged against CBB, possibly facilitated by very simple trapping devices though rather higher levels of CBB might have to be tolerated. But with the advent of increasingly sophisticated electronic sorting devices, it might eventually be feasible to select out the, say, 10 to 15% of residual CBB-infested beans achievable by natural control and provide the farmer with the top value for the 85 to 90% of perfect beans. Modelling could help to develop scenarios that would be favourable to the farmer.

The second approach is plant resistance. This in itself will not be easy and the most likely line of research, genetic engineering, has added difficulties since most likely a gene coding for a toxin would have to be expressed in the bean itself, which might face consumer resistance.

SYNOPSIS

There can be little argument that progress on biological control of this pest has been slow. There may be several reasons for this, but they are probably all related to two fundamental and unalterable facts, a) the borer lives deep inside the berry making it hard to contact by most control methods and b) picking removes natural enemies, which we make no apologies for stressing is the only self-sustaining, self-regulating and entirely natural method available.

We believe that the current concerns about small farmer livelihoods, sustainability, environmental pollution, food safety, etc. (Figure 6) will continue to grow and that the concept of biocontrol should become a universally acknowledged central pillar of modern coffee production.

Figure 6. A coffee farmer with accompanying cloud of endosulfan

REFERENCES

Baker P.S. (1999) The Coffee Berry Borer in Colombia. Final report of the DFID-Cenicafé-CABI Bioscience IPM for coffee project. ISBN 958-96554-1-6

Barrera J.F. 1994. Dynamique des populations du scolyte des fruits du caféier, *Hypothenemus hampei* (Coleoptera: Scolytidae), et lutte biologique avec le parasitoide *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae), au Chiapas, Mexique. Doctoral thesis, University Paul-Sabatier Toulouse III.

Bigger M., 1999. Insects on coffee, a world checklist of species recorded in literature up to 1999. Unpublished ms, pp 353.

Chevalier A., 1947. Les caféiers du globe fasc. III: Systématique des caféiers et faux-caféier, maladies et insectes nuisibles, Encyclopédie Biologique no. **28**, 356 pp. Paris, P. Lechevalier.

Damon A.A., 1999. Evaluation of current techniques and new approaches in the use of *Cephalonomia stephanoderis* (Hymeoptera: Bethylidaea) as a biological control agent of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae), in Chiapas, Mexico. PhD thesis, University of London.

Dufour B., Barrera J.F., Decazy B., 1999. La broca de los frutos del cafeto: La lucha biológica como solución? In: 'Desafios de la caficultura en Centroamerica. IICA Promecafe Costa Rica, pp 293-325. ISBN 92-9039-391 2

Garcia A., Barrera F., Muñoz R., Canet G., 2000. Proyecto MIB-CFC-OIC-CABI-PROMECAFE, Informe annual año tres. Unpublished report Promecafe Guatemala.

Greathead D.J., Greathead A.H., 1992 Biological control of insect pests by insect parasitoids and predators: the BIOCAT database. Biocontrol News and Information, **13** (4): 61N-68N.

Hawkins B.A., 1994. Pattern and process in host-parasitoid interactions. Cambridge University Press, Cambridge, UK.

Hawkins B.A., Cornell H.V., Hochberg M.E., 1997. Predators, parasitoids and pathogens as mortality agents in phytophagous insect populations. Ecology, **78**: 2145-2152.

Leach A.W., Stonehouse J.M., Baker P.S., 1999. A population and management model of the coffee berry borer, Hypothenemus hampei, to evaluate the returns of integrated management strategies in Colombian coffee. Aspects of Applied Biology **53**, 173-180.

Le Pelley R.H., 1968. The pests of coffee. Longmans, London.

López J.C., Briscoe B., 1999. Efecto de entomonematodos en el desarrollo de la broca del café en condiciones de invernadero. In: Congreso de la sociedad Colombiana de Entomología, 26. Bogotá, 1999 p. 162.

Murphy S.T., Day R., Orozco J., Kucel P., 2001. Investigations into the use of *Heterospilus coffeicola* for the biological control of the coffee berry borer in Latin America and Africa. CABI Commodities unpublished report for the period Nov 2000-July 2001.

Perez-Lachaud G., 1998. A new bethylid attacking the coffee berry borer in Chiapas (Mexico) and some notes on its biology. Southwestern Entomologist **23**, 287-288.

Quintero C.V., Bustillo A.E., Benavides P., Chaves B., 1997. Establecimiento y dispersión de *Cephalonomia stephanoderis* y *Prorops nasuta*, parasitoides de la broca del café, en el departamento de Nariño. In: Congreso de la sociedad Colombiana de entomología, Pereira, July 16-18 1997.

Vega F.E., Mercadier G., Damon A., Kirk A., 1999, Natural enemies of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in Togo and Cote d'Ivoire, and other insects associated with coffee beans. African Entomology **7**, 243-248.

Vergara J.D., 1998 Biología y comportamiento de *Phymastichus coffea* La Salle (Hymenoptera: Eulophidae), parasitoide de la broca del café en condiciones de campo. Thesis for degree of Ingeniero agrónomo, Universidad National de Medellín, pp 67.

Recent Investigation on Coffee Tracheomycosis, *Gibberella xylarioides* (*Fusarium xylarioides*) in Ethiopia

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SUMMARY

Tracheomycosis is a typical vascular wilt disease of coffee incited by *Gibberella xylarioides* (*Fusarium xylarioides*). The fungus was earlier reported to be a well-known pathogen of *Coffea excelsa* and *C. canephora* in West and Central Africa (Central African Republic, Cameroon, Ivory Coast, Zaire and Guinea) in the 1950s. The disease was observed again in Zaire in the early 1980s and noticed for the first time in Uganda in 1993, it is now causing economic losses to Robusta coffee in both countries.

In Ethiopia, the occurrence of the pathogen on Arabica coffee was established in the 1970s although its presence was speculated earlier. It has been considered as a minor coffee disease, but so far documented surveys emphasize that tracheomycosis develops to an important disease on Arabica coffee, too. The recent investigations indicated the existence of variations both in resistance levels of the host genotypes and in agressiveness of the fungal isolates in the country.

INTRODUCTION

Tracheomycosis, induced by *Gibberella xylarioides* Heim & Saccas (*Fusarium xylarioides* Steyaert), is a typical vascular disease syndrome caused by extensive necrosis of the vascular tissue leading to wilting and death of the infected plant. The early symptom of infection on mature and young Arabica coffee trees are epinasty of leaves on some branches in the lower tree canopy that turn brownish or dark brownish within two or more weeks, and finally drop-off from the branches. These external symptoms most frequently begin on one side of a coffee tree, and then gradually progress throughout the plant. Later in the season, completely wilted trees become dried and stand barely with leafless branches, and these trees cannot be easily pushed and uprooted as opposed to coffee trees died of root rot disease caused by *Armillaria mellea*. Brown or dark reddish discoloration on the exposed wood of the stem is a typical internal symptom of tracheomycosis. Brownish black fruiting bodies of the pathogen can be observed in barks of stems and branches of dead coffee trees (Van der Graaff and Pieters, 1978; Girma et al., 2001).

Tracheomycosis had caused severe damage to Excelsa, Liberica, and Robusta coffees in the Central African Republic, Cameroon, Ivory Coast, Zaire and Guinea (Kranz, 1962; Booth, 1971; Coste, 1992). In these countries, the disease could be controlled by the use of resistant cultivars (Kranz, 1962; Coste, 1992; Flood, 1996). However, it has reappeared on Robusta coffee in Zaire in the early 1980s (Mfwidi-Nitu, 1994), and also noticed for the first time in 1993 in two Robusta growing districts of Uganda that border Zaire (Flood, 1996, 1997; Lukwago and Birikunzira, 1997). These authors have reported that the disease is causing economic losses to

Robusta coffee in both countries, but they did not observe the disease on Arabica coffee and improved clonal Robusta cultivars both in Zaire and in Uganda.

The first record of tracheomycosis on Arabica coffee from Ethiopia was by Stewart (1957). He described the wilting symptom and also identified the causal organism to be *Fusarium oxysporum* f.sp. *coffeae*, but later on, Kranz and Mogk (1973) authentically confirmed that the fungus inciting the disease is *Gibberella xylarioides*, of which *Fusarium xylarioides* is the teleomorphic state. During their survey in 1971, they observed the disease on a few single trees scattered in some plantations near Agaro, Jimma, and Bonga areas of Ethiopia. Since then tracheomycosis was recorded in major coffee producing regions of the country (Robinson, 1974; Van der Graaff, 1979; Merdassa, 1986). Although its damage occurred in restricted areas and considered as a minor disease, as compared to CBD, so far documented surveys emphasize that tracheomycosis develops to an important disease on Arabica coffee, too (Eshetu et al., 2000; Girma et al., 2001).

In this paper, some recent investigations on coffee tracheomycosis, *Gibberella xylarioides* (*F. xylarioides*) in Ethiopia are discussed.

MATERIALS AND METHODS

Disease assessment and sample collection

The incidence of tracheomycosis was assessed in different coffee fields, each field consisting of a large number of selections with known history of coffee tree death. All coffee trees in each sample field were diagnosed, and based on the characteristic external and internal symptoms, the number of healthy and infected trees were counted and recorded. The disease incidence was computed and transformed to logits before statistical analysis. At the same time specimens of roots, stems, branches and berries were collected from sample trees with various symptom groups including asymptomatic ones (Girma et al., 2001).

Isolation and identification of the fungus

The fungus was isolated and identified following the standard procedures and taxonomic descriptions of Booth (1971); and our provisional identification was further verified by *Fusarium* specialists at the International Mycological Institute (IMI), CAB International, UK (Girma and Mengistu, 2000).

Pathogenic variability study

The pathogenic variability in the fungus population was studied by inoculating 4 representative *G. xylarioides* isolates collected from different localities (Bebeka, Teppi, Jimma and Gera) on seedlings of 9 Arabica coffee cultivars observed to possess various levels of resistance to tracheomycosis under field conditions. Seedlings (30 per box) were raised and inoculated with viable conidia of each isolate by stem nicking procedures (Pieters and Van der Graaff, 1980; Girma and Mengistu, 2000). The experiment was conducted in a split-plot design with 9 x 4 factorial combinations of the cultivars as main plot and the isolates as sub-plot treatments with 2 replications in the greenhouse at Jimma Agriculture Research Center. The number of healthy and wilting seedlings were identified and recorded fortnightly for 210 days. Incubation periods, and the percentage of dead seedlings were computed and transformed to angular values for analysis.

RESULTS AND DISCUSSION

Incidence of tracheomycosis on different coffee selections

The disease incidence varied among the coffee selections in the field. At Gera in a field consisting of 1981 CBD resistant selections (n = 30) and including both wilt susceptible and resistant checks, significant differences were obtained in the percentage of dead coffee trees. Mean percentage of tree losses in this field ranged from 95.6 for the susceptible check 74304 to 12.2 for selection 8150. The coffee selection 74141, included as a resistant check, showed insignificantly different percentage of dead trees from the susceptible variety 74304 (Table 1). At the same locality, coffee selections (n = 20) planted in another field also revealed significantly different percentage of dead trees caused by the wilt disease. Some selections such as 8211 and 827 showed low incidence of 4.2 and 13.2%, respectively, whereas selections 8214 and 823 had the highest infection levels of 83.4 and 88.9%, respectively (Table 2). The released CBD resistant selection 741 had consistent performance in both fields with relatively low tree death rate of about 35.2% (Tables 1 and 2). Similar results that indicated the existence of varietal differences under field conditions were previously reported (Van der Graaff and Pieters, 1978; Girma et al., 2001).

Table 1. Incidence of tracheomycosis on 1981 CBD resistant selections in the field at Gera, 1997

Coffee selection	Mean Percentages of dead trees	Coffee selection	Mean Percentages of dead trees
741	35.2 c - e	8138	82.5 a – c
754	56.3 a - e	8140	35.9 c - e
813	94.1 ab	8142	38.4 c – e
814	26.3 de	8143	42.4 b - e
815	49.9 a - e	8144	37.0 c - e
816	39.0 c - e	8146	34.6 c - e
817	48.8 a – e	8148	56.2 a - e
7395	30.0 c – e	8149	63.4 a - e
8112	63.1 a - e	8150	12.5 e
8116	56.4 a -e	8151	53.5 a - e
8118	33.2 c - e	74140	43.3 b - e
8121	35.2 c - e	74141 (resistant)	79.5 a – d
8123	38.6 c – e	74262	80.7 a – c
8128	76.5 a - d	74304 (susceptible)	95.6 a
8133	19.2 e	Mean	49.5
8136	29.4 c - e	LSD P = 0.05	43.3
		CV (%)	53.4

Means followed with the same letters are insignificantly ($p < 0.05$) different according to Duncun's Multiple Range Test (DMRT).

In addition to the susceptibility of coffee selections, the incidence of tracheomycosis was observed to be aggravated by some cultural practices, mainly intensive slashing of coffee weeds. In this case it was observed that almost all stems of coffee trees were wounded at the ground level or a few centimeters above. Ninety eight percent of the diseased and 95% of healthy trees were estimated to have 1-3 wounds per stem, on the average. As the fungus is known to penetrate coffee trees through wounds either above or below the ground (Wellman, 1961; Booth,

1971), the slashing activity with bushman knives provides avenues for entry of the pathogen or it may serve as inoculating agent as the tool can easily be contaminated with the fungal conidia either from the soil or from infected trees. Therefore, it is recommended that such agronomic practices as slashing, digging and pruning should be carried out with caution, particularly, in areas where the disease is prevalent.

Table 2. Incidence of tracheomycosis on 1982 CBD resistant selections in the field at Gera, 1997

Coffee Selection	Mean percentages of dead trees	Coffee Selection	Mean percentages of dead trees
741	35.2 c - f	8212	39.7 b - f
821	38.1 b - f	8213	47.2 a - e
822	42.5 b - f	8214	83.4 ab
823	88.9 a	8215	54. 1 a - e
824	44.5 a - f	8219	38.9 b - f
825	33.0 c - f	8221	38.9 b - f
826	31.5 c - f	8228	62.0 a - d
827	13.2 ef	8230	62.1 a - d
828	55.9 a - e	8236	67.9 a - c
829	53.7 a - e	Mean	45.4
8211	4.2 f	LSD P = 0.05	39.1
		CV	52.2

Means followed with the same letters are insignificantly ($p < 0.05$) different according to DMRT.

Isolation and identification of the causal agents

The isolation and identification work confirmed that wilting of coffee trees was predominantly (60%) caused by *G. xylarioides* (Figure 1). The fungus was recovered from all plant parts (except seeds) collected from coffee trees with dead, complete and partially wilting symptoms including apparently healthy looking ones. Both sexual and asexual spores of the fungus are observed in the fruiting bodies produced in the barks of dying and dead trees (Girma et al., 2001). These facts elucidate that any part of infected trees, except seeds, and possibly the adjacent asymptomatic plants and soils serve as survival media and could be potential sources of the pathogen for infection. Further more, the movement and use of infected coffee trees for fire wood and for fencing purposes; and indiscriminate use of the bushman knives could be mechanisms for spread of the fungus in coffee fields, locally or over long distances.

In addition, three other *Fusarium* species, namely *F. solani*, *F. stilboides* and *F. oxysporum*, were isolated and identified in association with the target species (Figure 1). *F. oxysporum* and *F. solani* were isolated from coffee root, husks and soil samples obtained from infected trees with coffee wilt disease (Flood, 1997). Formae speciales of *F. solani* and *F. oxysporum* are cited to induce varying types of wilting on coffee, the former in Kenya (Baker, 1972) and the latter in India, Brazil and Central America (Wellman, 1961). Hence, the importance of these *Fusarium* species in the coffee wilt disease complex may need further observations.

Variation in pathogenicity of *G. xylarioides* isolates

In the pathogenic variability study, highly significant differences among coffee cultivars, and among the fungus isolates; and also a significant cultivar x isolate interaction, were obtained both in percentage of dead seedlings and in length of incubation periods (Tables 1 and 2). Seedlings of cultivars 61/85, 24/85 and F-17 had shown significantly higher disease levels with 62.6, 60.5 and 51.4%, respectively (Table 3).

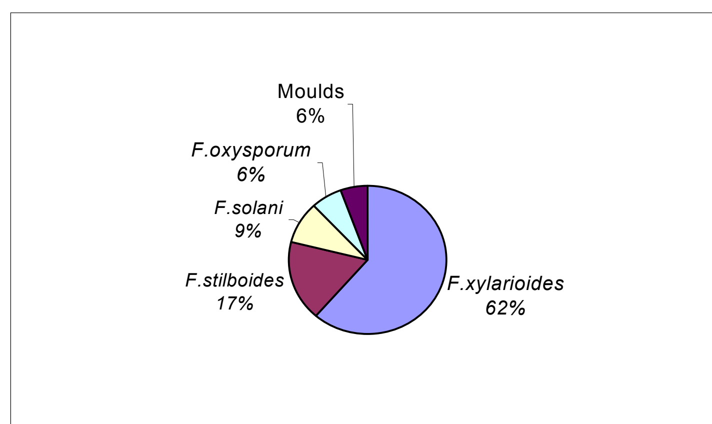

Figure 1. Proportions (%) of Fusarium species isolated from infected coffee trees with tracheomycosis

Table 3. Percentage of dead seedlings (transformed to angular values) of 9 Arabica coffeecultivars inoculated with 4 *G. xylarioides* isolates in the greenhouse at Jimma, 1997

Coffee	Gibberella xylarioides isolates*				
Cultivars	Gx12	Gx26	Gx31	Gx43	Mean
74165	0 j	40.5 e - i	33.9 f- i	22.6 g- j	24.3 E
7440	0 j	17.1 h - j	11.6 ij	19.4 h - j	12.0 F
74304	0 j	64.5 a - f	48.8 b - h	38.0 f - i	37.8 CD
F-17	0 j	77.8 a - c	52.6 a - g	75.0 a - d	51.4 AB
F-61	0 j	54.7 a - g	57.1 a - f	70.8 a - e	45.7 BC
SN-5	0 j	70.8 a - e	62.4 a - f	46.8 c - h	44.9 BC
35/85	0 j	43.8 d - h	35.2 f - i	35.9 f - i	28.8 DE
24/85	0 j	73.8 a - d	82.9a	85.2 a	60.5 A
61/85	0 j	80.4 ab	85.1 a	85.1 a	62.6 A
Mean	0. N	58.2 M	52.2 M	53.4 M	

**Gx12, Gx26, Gx31, and Gx43, were G. xylarioides isolates obtained from Bebeka, Teppi, Jimma and Gera, respectively. Means followed with the same letter(s) are not significantly (P < 0.05) different from each other according to Duncun's Multiple Range Test (DMRT). LSD values for the cultivars, the isolates and the interactions comparisons were 10.8, 9.2, and 27.6, respectively*

They also had shorter incubation periods (Table 4). On the other hand, cultivars 35/85, 74165 and 7440 had lower seedling infection levels with 28.8, 24.3 and 12.0%, respectively (Table 3); accompanied by longer incubation periods of 84, 108, and 112 mean number of days, respectively (Table 4).

Table 4. Incubation periods (number of days) of *G. xylarioides* isolates infection on seedlings of 9 Arabica coffee cultivars in the greenhouse at Jimma, 1997

Coffee	Gibberella xylarioides isolates *				
Cultivars	Gx12	Gx26	Gx31	Gx43	Mean
74165	0 h **	126 a - c	154 ab	154 ab	108.5 A
7440	0 h	133 a- c	168 a	147 ab	112.0 A
74304	0 h	35 gh	112 a - e	119 a – d	66.5 CD
F-17	0 h	84 c - g	98 b - f	63 d - g	61.2 CD
F-61	0 h	56 e - h	77 c - g	84 c - g	54.2 D
SN-5	0 h	98 b - f	77 c - g	105 b – f	70.0 C
35/85	0.h	105 b - f	133 a - c	98 b - f	84.0 B
24/85	0 h	49 f - h	49 f - h	28 gh	31.5 E
61/85	0 h	56 e - h	35 gh	28 gh	29.7 E
Mean	0 R	82.4 Q	100.3 P	91.8 PQ	

** Gx12, Gx26, Gx31, and Gx43, were G. xylarioides isolates obtained from Bebeka, Teppi, Jimma and Gera, respectively. ** 0 = indicates no incubation period (no external symptom was observed until end of the test). Means followed with the same letter(s) are not significantly (P < 0.05) different from each other according to DMRT. LSD values for the cultivars, the isolates and the interactions comparisons were 13.7, 17.5 and 55.6, respectively*

G. xylarioides isolates Gx26, Gx43 and Gx31 caused higher seedling deaths with 58.2, 53.4 and 52.2 mean percentages, respectively, than isolate Gx12 (Table 3). The isolate Gx26 induced wilting symptom within significantly shorter incubation period than Gx31 and Gx12 isolates (Table 4). In comparing the combined cultivar vs. isolate interaction, the isolate Gx26 was found to be fairly more aggressive than the isolate Gx43 on SN-5, 74304, 74165 coffee cultivars but less aggressive than the isolate Gx43 on cultivar F-61. The isolate Gx31 seems to be moderately aggressive while Gx12 was weakly pathogenic to all cultivars (Tables 3 and 4) (Girma and Mengistu, 2000).

Thus, this result substantiated the existence of variations both in resistance levels of coffee genotypes and in aggressiveness of the pathogen strains. Although the resistance was predominantly horizontal, the differential effect indicated by a significant cultivar x isolate interaction both in the seedling death rates and in the incubation periods presented some evidence for vertical resistance in Arabica coffee and *G. xylarioides* pathosystem. The variation among *G. xylarioides* isolates in some cultural characteristics such as growth rate and pigmentation along with the differential effect may suggest some kind of specialization in the fungus population (Girma and Mengistu, 2000).

Besides, as opposed to the recent experience in Zaire (Flood, 1996) and Uganda (Flood, 1997; Lukwago and Birikunzira, 1997), there has been no wilt disease encountered on Robusta coffee trees planted in a few hectars at Bebeka and in some plots adjacent to Arabica plots at Jimma. The absence of tracheomycosis on Robusta coffee most likely imply that *F. xylarioides* strain attacking Arabica trees in Ethiopia may be different from the isolates of alike species destroying Robusta coffee elsewhere in Africa. The strain on Robusta coffee may not be able to evolve in Ethiopia for the limited plantations of this *Coffea species*. Nevertheless, this speculation needs detailed research works involving a large number of isolates and genotypes from diverse environmental conditions, employing molecular and genetic techniques.

REFERENCES

Baker C. J. 1972. Root rots of coffee trees due to fungal infection. Kenya Coffee **37**: 255-261.

Booth C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute: Kew, Surrey, England. 237 pp.

Coste R. 1992. Coffee: The plant and the product. Macmillan Press Ltd: London. 328 pp.

Eshetu D., Teame G. and Girma A. 2000. Significance of minor diseases of *Coffea arabica* L. in Ethiopia: A review. Pp 58-65. In: Proceedings of the workshop on coffee berry disease (CBD) in Ethiopia, 13-15 August 1999, Addis Ababa, Ethiopia.

Flood J. 1996. A study of La Tracheomycosis or vascular wilt disease of coffee in Zaire. International Mycological Institute (IMI). UK. 13pp.

Flood J. 1997. Tracheomycosis or vascular wilt disease of coffee in Uganda. Report presented to Ugandan Coffee Development Authority (UCDA). Uganda. 12pp.

Girma A. and Mengistu H. 2000. Cultural characteristics and pathogenicity of *Gibberella xylarioides* isolates on coffee. Pest Mgt. J. of Ethiopia, **4**: 11-18.

Girma A., Mengistu H. and Hindorf H. 2001. Incidence of tracheomycosis, *Gibberella xylarioides* (Fusarium *xylarioides*), on Arabica coffee in Ethiopia. Journal of Plant Diseases and Protection. **108** (2), 136 - 142, 2001.

Kranz J. 1962. Coffee diseases in Guinea. FAO Plant Protection Bulletin **10**: 107-109.

Kranz J. and Mogk M. 1973. *Gibberella xylarioides* Heim & Saccas on Arabica Coffee in Ethiopia. Phytopath. Z. **78**: 365 - 366.

Lukwago G. and Birikunzira B. 1997. Coffee wilt disease (Tracheomycosis) and its implication on Uganda's economy. Pp 969 - 974. *In*: African Crop Science Conference Proceedings, Kampala, Uganda, **3**, 969-974.

Merdassa E. 1986. A review of coffee diseases and their control in Ethiopia. Pp 187-195. In: Tsedeke Abate.(ed.) Proceedings of the First Ethiopian Crop Protection Symposium. Addis Ababa, Ethiopia, 4-7 February 1986.

Mfwidi-Nitu P. 1994. The recrudescence of tracheomycosis (*Gibberella xylarioides*) of Robusta coffee in Zaire. African Coffee, Bulletin no. **40**: 9-12.

Pieters R. and Van der Graaff N. A. 1980. *Gibberella xylarioides* on Arabica coffee: evaluation of testing methods and evidence for the horizontal nature of resistance. Neth. J. Plant Path. **86**: 37-43.

Robinson R. N. 1974. Terminal report of the FAO coffee Pathologist to Ethiopia. Rome: FAO. AGO/74/443. 16 pp.

Stewart, R.B. 1957. Some plant diseases occurring in Kaffa province, Ethiopia. Imperial Ethiopian College of Agriculture and Mechanical Arts: Alemaya, Ethiopia. pp 15-16.

Van der Graaff N. A. 1979. Research on coffee pathology (Dec. 1974 - Nov. 1978). Assignment Report. AG: DP/Eth/74/ 002. FAO, Rome: 33 pp.

Van der Graaff N.A. and Pieters R. 1978. Resistance levels in *Coffea arabica* L. to *Gibberella xylarioides* and distribution pattern of the disease. Neth. J. Pl. Path. **84**: 117-120.

Wellman F. L. 1961. Coffee Botany, Cultivation, and Utilization. Leonard Hill (Books) Ltd.: London. 488 pp.

Contribution au développement d'un piège pour capturer le scolyte du café *Hypothenemus hampei* Ferr. en El Salvador

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SUMMARY

Mass trapping, which is currently being studied in El Salvador, is a promising alternative for controlling the coffee berry borer (CBB) *Hypothenemus hampei* Ferr. Following numerous field trials to perfect the method and assess its efficacy, priority was given to developing a large-capacity trap, culminating in industrial scale production and marketing. In this context, studies concentrated on the optimum rate of basic attractant diffusion and on comparing several models of traps. In light of the results obtained, and taking into account preliminary results and field observations, the first prototype traps specially designed to catch CBB were made. The BROCAP® model was finally developed combining the best characteristics of the prototypes, taking into account the following aspects: system functionality and simplicity, sturdiness of attachments and raw material quality.

Trapping trials with different attractant formulations were carried out in the middle of a CBB migration period, to check that trapping was environment-friendly. The basic attractant made up of alcohols did not prove to be totally specific to coffee berry borers, but was found to be extremely environment-friendly for useful fauna in coffee plantings. On average, *H. hampei* accounted for 97% of all the insects caught.

RÉSUMÉ

Le piégeage de masse, en cours d'étude en El Salvador, est une alternative prometteuse pour lutter contre le scolyte du café *Hypothenemus hampei* Ferr. A la suite de nombreux essais de terrain destinés au perfectionnement de la méthode et de l'évaluation de son efficacité, la priorité a été donnée à la mise au point d'un piège de forte capacité de capture avec comme finalité sa fabrication à échelle industrielle et sa commercialisation. Dans ce cadre, les études ont porté sur le taux de diffusion optimum de l'attractif de base et sur la comparaison de plusieurs modèles de pièges. A la lumière des résultats obtenus et en intégrant les résultats préliminaires et les observations de terrain, les premiers prototypes de pièges spécialement conçus pour la capture du scolyte ont été construits. Le modèle BROCAP® a finalement été mis au point à partir des meilleures caractéristiques de ces prototypes, et en tenant compte des aspects suivants: fonctionnalité et simplicité du système, solidité des éléments d'accouplement et qualité de la matière première.

Afin d'évaluer l'innocuité du piégeage pour l'environnement, des essais de capture avec différentes formulations d'attractifs ont été effectués en pleine période de migration des scolytes. Le mélange attractif de base composé de substances allélochimiques ne s'est pas révélé totalement spécifique pour le scolyte, mais a cependant manifesté un excellent respect pour la faune utile des caféières. En moyenne, *H. hampei* constitue 97% de l'ensemble des insectes capturés.

INTRODUCTION

Le scolyte des baies de café *Hypothenemus hampei* Ferr. est le plus important des ravageurs pour la culture du café en Amérique Latine. Son étonnant pouvoir d'adaptation et de dispersion ont fait que toutes les tentatives d'éradication sont restées vaines. L'unique façon de protéger la caféiculture contre les méfaits de ce ravageur est donc de pratiquer une lutte adéquate pour rabaisser les niveaux d'infestation à des valeurs économiquement acceptables (Decazy, 1989). Les contraintes socio-économiques de la filière café et les exigences en matière de protection de l'environnement, font que les alternatives non chimiques de lutte sont devenues le point de mire de toutes les Institutions de Recherche impliquées dans la mise au point de méthodes de lutte contre le scolyte. Depuis une décennie, la lutte biologique avec champignons entomopathogènes et parasitoïdes d'origine africaine a connu un grand essor dans de nombreux pays producteurs de café, elle n'a toutefois pas encore donné entière satisfaction quant à son efficacité et sa rentabilité (Baker, 1999). En El Salvador, la recherche sur le piégeage a vu le jour en 1997, après un bref essor dans d'autres pays (Gutierrez-Martinez et al., 1995; Mathieu, 1995; Mathieu et al., 1997, 1998; Mendoza Mora, 1991). Le principe de la capture du scolyte en période de post-récolte a permis d'envisager très rapidement l'élaboration d'une nouvelle méthode de lutte qui serait complémentaire des luttes biologique et culturale et donc compatible avec la lutte intégrée.

Les premières études sur le piégeage ont eu comme résultat, l'élaboration d'un mélange attractif efficace (non publié), la conception et l'amélioration d'un principe de capture (Dufour et al., à paraître) et à mise au point d'une méthode applicable directement sur le terrain (Dufour et al., 1999). Le but du travail présent est de montrer les principales étapes expérimentales qui ont abouti à la création du piège BROCAP® de type commercial, à haut rendement de capture et conçu pour une fabrication industrielle. Par ailleurs, la spécificité de l'attractif seul ou associé à d'autres composés, a été étudiée afin de connaître les éventuels effets du piégeage sur l'écosystème et son intérêt pour la conservation de la biodiversité.

MATÉRIEL ET MÉTHODES

Les pièges

Le premier piège expérimental "1B" a été conçu dans le seul but d'étudier les paramètres propres au piégeage (Figure 1). Il a donc été largement utilisé dans les essais de terrain. D'autres modèles tels que les "2A" (Figure 2), "3A" (Figure 3), "3B", ont été élaborés artisanalement puis testés à titre comparatif avec des pièges commerciaux "Multipher® A" et "Multipher® B" (Figure 4) normalement destinés à la capture d'autres espèces d'insectes. Par la suite, à partir des résultats de capture du scolyte, de son comportement de vol et plus précisément de son approche de la source attractive et de son atterrissage, deux prototypes du modèle définitif BROCAP® ont été créés (Figures 5, 6).

Le piège se compose de quatre éléments principaux:

- *Le récipient de capture* présente une ouverture dans sa partie supérieure permettant l'entrée des scolytes.
- *Le diffuseur* contient l'attractif qui diffuse de manière continue. Il se situe à l'intérieur des pièges pour tous les modèles autres que le BROCAP® et ses prototypes. Pour ces derniers, le diffuseur est placé à l'extérieur, juste au-dessus du cône d'entrée.
- La diffusion s'effectue directement par évaporation dans le cas de mélanges constitués de substances de volatilité semblable. Elle peut s'effectuer aussi grâce à une mèche plongeant dans le mélange, principalement lorsque la volatilité de ses composés est

différente. *L'attractif* est l'élément principal du système. L'intensité des vols vers le piège dépend de sa composition chimique.

- *Le liquide de capture* se compose d'eau et de quelques gouttes de savon liquide. Il a pour rôle de noyer les scolytes attirés puis capturés par le piège.

Figure 1. Modèle expérimental "1B"

Figure 2. Modèle "2A"

Figure 3. Modèle "2A"

Figure 4. Modèle “Multipher B”

Figure 5. Prototype “B” avec support en forme de croix

Figure 6. Prototype “A” sans support

Conditions et dispositifs expérimentaux

Les essais ont été mis en place dans la zone de café organique de l'exploitation "El Espino" située dans la Région Centrale du pays, à une altitude de 850 m. La variété Bourbon y est dominante et cultivée sous ombrage.

Cas des études de base

Toutes les études ont été conduites dans des parcelles de grande taille, composées généralement de 4 à 5 sous-parcelles de 3600 m^2 chacune (60 x 60 m. Les pièges s'accrochent sur des caféiers préalablement marqués, formant un réseau de capture homogène et systématique (Figure 7). Quel que soit le nombre de traitements, le dispositif comporte toujours 16 répétitions distribuées au hasard dans la parcelle. Chaque piège est une répétition.

Figure 7. Distribution des pièges dans les parcelles

4	8	12	16	20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80
3	7	11	15	19	23	27	31	35	39	43	47	51	55	59	63	67	71	75	79
2	6	10	14	18	22	26	30	34	38	42	46	50	54	58	62	66	70	74	78
1	5	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	77

<------- 60 m ------>

Le temps de chaque essai est défini en fonction de l'abondance des captures. Il peut donc se prolonger jusqu'à ce que les quantités de scolytes soient suffisantes pour l'analyse statistique. Les pièges sont ensuite retirés et les captures analysées au laboratoire. Si les quantités de scolytes sont faibles, elles sont évaluées directement par comptage. Dans le cas contraire, leur nombre est déterminé à l'aide d'une méthode volumétrique. Le volume d'attractif restant dans les diffuseurs est mesuré à la fin de chaque essai.

Les données de capture des différents essais sont analysées à l'aide de la méthode non paramétrique des rangs de Kruskal-Wallis.

Cas de l'étude des attractifs et de leur spécificité

L'étude a été mise en place dans une seule parcelle comportant 64 pièges "1B" fonctionnant 5 jours successifs toutes les deux semaines et pour une durée totale de 3 mois. Le dispositif se compose de 4 traitements et de 16 répétitions distribuées au hasard dans la parcelle: T1 représente l'attractif de base; T2, T3 y T4 comportent le même attractif associé respectivement à 1, 2 et 4 terpènes choisis parmi les plus représentatifs de l'ensemble des terpènes naturellement émis par les cerises de café *arabica* ou *canephora* lors de leur maturation (Mathieu et al., 1998). Ces terpènes émis par de petits diffuseurs indépendants, présentent des taux de diffusion très inférieurs à celui de l'attractif de base. Ce choix suppose que ces composés agissent dans certaines conditions sur la communication chimique du scolyte (Dufour et al., 1999). En plus du nombre de scolytes, sont pris en compte les autres espèces d'insectes capturées. Les résultats sont analysés à l'aide de tableaux croisés.

RÉSULTATS ET DISCUSSION

Diffuseurs et diffusion de l'attractif de base

Cet essai a pour objectif de définir la diffusion optimale de l'attractif, pour un piégeage réalisé dans des conditions de fortes migrations de scolytes. Deux facteurs sont étudiés: les modèles de diffuseurs (simple et à mèche) et les taux de diffusion.

Tableau 1. Effet du diffuseur et de la diffusion sur la capture des scolytes

	T1	T2	T3	T4
	3614	2727	5509	7308
	3705	5509	7461	4090
T1 = 0,124 g/ds*/jour	7111	1946	8517	3175
T2 = 0,204 g/ds/jour	1285	5804	3413	3041
T3 = 0,347 g/ds/jour	1803	1080	3210	3489
T4 = 0,132 g/dm**/jour	4950	7345	5587	5530
	4505	2880	17138	1503
Ds = diffuseur simple	1571	5483	9611	4521
Dm = diffuseur à mèche	4052	5900	7345	767
64 pièges "1B"	5939	6407	7798	4626
Attractif à base d'alcools	2999	3817	3772	5682
Hauteur de capture = 1,2 m	3251	4009	10471	1157
Durée du piégeage = 6 jours	2313	2325	1166	1891
	2317	2026	3780	1335
	3131	5904	3633	2985
	1436	2324	1316	7063
Moyenne	3373,88	4092,88	6232,94	
Somme des Rangs	425	522	680	
Ordre	4	2	1	
Kruskall-Wallis	A	A	A	
	H = 7,05	NS	DDL = 3	

Les résultats de l'analyse montrent que les deux systèmes de diffusion (T1/Ds et T4/Dm) sont d'égale efficacité pour des taux quasi semblables (Tableau 1). Par ailleurs les différents taux de diffusion testés (T1, T2, T3 et T4) ne jouent pas de rôle déterminant sur les captures. Il faut toutefois signaler que l'intensité des captures représentée par la somme des rangs, a tendance à s'accroître avec l'augmentation de la diffusion

Comparaison des différents modèles de pièges

Parmi les cinq modèles de pièges testés, les trois modèles expérimentaux comportant des ouvertures latérales, complètement libres (T1, T2, T3), présentent le même potentiel de capture (Tableau 2). Toutefois ce potentiel représenté par la somme des rangs, tend à diminuer au fur et à mesure que le diamètre des ouvertures se réduit.

Les deux modèles de pièges Multipher® (T4 et T5) dont les ouvertures sont situées juste au-dessous du couvercle qui les protège, s'avèrent beaucoup moins adaptés à la capture du scolyte que les précédents (Tableau 2). La forme des Multipher® a été conçue pour le piégeage d'autres insectes. Elle ne convient donc pas pour le scolyte du café qui a besoin de voler librement au-dessus des ouvertures avant de se laisser tomber dans le piège. Comme

précédemment, le potentiel de capture diminue lorsque le diamètre des ouvertures se réduit.

Elaborés à l'aide des résultats présents et des données obtenues antérieurement (Dufour et al., à paraître), les prototypes "A" et "B" se caractérisent d'une part, par la présence d'une ouverture horizontale très large, se réduisant vers le bas à la manière d'un entonnoir, et d'autre part par la position externe du diffuseur. Dans le cas du prototype "B" le diffuseur est porté par un support en forme de croix. Les quatre modèles testés présentent en commun la même couleur rouge, très attractive pour le scolyte (Dufour et al., à paraître) mais cependant, différemment appliquée selon les modèles.

Tableau 2. Première comparaison de modèles de pièges

T1 = modèle "1B" (ouverture : Ø = 4 cm)
T2 = modèle "3A" (ouverture : Ø = 3,5 cm)
T3 = modèle "3B" (ouverture : Ø = 2 cm)
T4 = modèle Multipher® A (grande ouverture)
T5 = modèle Multipher® B (petite ouverture)

80 pièges
Diffusion de l'attractif = 0,20 g /piège/jour
Hauteur de capture = 1,2 m
Durée du piégeage = 14 jours

	T1	**T2**	**T3**	**T4**	**T5**
	155	52	39	7	2
	192	58	92	7	5
	284	225	88	6	23
	225	110	105	3	5
	502	143	73	25	2
	435	54	73	80	6
	100	142	70	3	4
	175	125	102	48	3
	115	66	45	8	18
	112	36	99	13	43
	120	70	58	98	13
	165	275	97	6	3
	101	107	152	22	5
	163	11747	104	41	182
	304	122	114	44	11
	106	485	93	12	12
Moyenne	203.38	863.56	87.75	26.44	21.06
Somme des rangs	1049.5	869.5	718	344	259
Ordre	1	2	3	4	5
Kruskal-Wallis	A	A	A	B	B
	H = 53.13	Pr. < 0.05	ddl = 4		

Evaluation des prototypes du piège BROCAP®

Les résultats obtenus en période de migration moyenne (Tableau 3) ainsi que les observations faites *in situ* sur le comportement de vol des scolytes autour des pièges, indiquent que les paramètres communs aux prototypes "A" et "B" facilitent leur approche et leur atterrissage et par conséquent, améliorent considérablement le processus de piégeage. L'intensité des captures obtenue est représentée ici par la somme des rangs. L'avantage attribué au prototype "B" peut s'expliquer par la seule présence du support de diffusion en forme de croix, dont la couleur rouge génèrerait une attraction complémentaire.

Les autres modèles, beaucoup moins performants, présentent des différences entre eux, qui seraient dues à la répartition inégale de la couleur rouge.

Tableau 3. Evaluation des prototypes du piège BROCAP®

T1 = modèle "1B" (ouverture : Ø = 4 cm) T2 = modèle "2A" (ouverture : Ø = 4 cm) T3 = prototype "A" (ouverture : Ø = 17 cm) T4 = prototype "B" (ouverture : Ø = 17 cm) 64 pièges Diffusion de l'attractif = 0,21 g/piège/jour Hauteur de capture = 1,2 m Durée du piégeage = 10 jours	**T1**	**T2**	**T3**	**T4**
	474	249	3743	3483
	372	106	1804	3217
	333	292	1945	5827
	144	89	1610	5680
	243	288	1290	2115
	213	137	3023	2939
	182	284	5190	7747
	163	153	3826	1806
	488	96	3796	2603
	389	175	1408	3221
	310	63	1715	4594
	131	67	1798	2731
	47	69	1506	2938
	59	57	3578	3230
	192	85	2553	1046
	662	65	3635	812
Moyenne	275,13	142,19	2651,25	3374,31
Somme des rangs	327	201	750	802
Ordre	3	4	2	1
Kruskal-Wallis	B	B	A	A
	H = 48,94	Pr.<0.05	ddl = 3	

Création du piège commercial BROCAP®

Le piège BROCAP® a été élaboré à partir des meilleurs éléments identifiés sur les prototypes, avec une attention particulière pour la fonctionnalité et la simplicité du système, la solidité des pièces, la précision des zones d'ajustement et la qualité de la matière plastique (Figure 8). Ses principales caractéristiques physiques sont les suivantes:

Figure 8. Piège BROCAP®

- *Une ouverture* large et horizontale en forme d'entonnoir permettant d'optimiser la chute des scolytes dans le piège;

- *un diffuseur* placé au centre, juste au-dessus de l'ouverture, assurant une diffusion externe et de large rayon d'action;
- *un support* de diffusion contribuant à la solidité du système;
- *un récipient* de capture contenant le liquide destiné au noyage rapide des scolytes;
- *la couleur* rouge du cône et du support permettant d'augmenter l'effet attractif;
- *la transparence* du récipient de capture destinée à l'observation des insectes capturés et la présence d'un trop-plein dont le rôle est d'éviter les débordements en cas de fortes pluies.

Dans les conditions d'exploitation, la quantité optimale recommandée est de 17 pièges BROCAP®/ha. Les performances de ce modèle peuvent dépasser 10000 scolytes/piège/jour.

Etude des attractifs et de leur spécificité

Effets sur le scolyte

Contrairement aux résultats obtenus dans des essais antérieurs (Dufour et al., à paraître; 1999), le rôle synergique des terpènes ne s'est pas manifesté et n'a donc pas permis d'augmenter les captures.

Effets sur les autres insectes

Au cours des 35 jours de piégeage effectif de l'essai, 68 espèces d'insectes ont été capturées, sans compter les scolytes et sans inclure les 6 espèces d'arachnides et les 4 espèces d'arthropodes non identifiées. Les ordres les mieux représentés furent les coléoptères, les hyménoptères et les diptères.

L'interprétation des résultats de capture s'est réalisée en fonction de l'hypothèse suivante : le degré de spécificité d'une formulation attractive pour l'espèce étudiée, en l'occurrence le scolyte du café, trouve ses limites lorsqu'un élément du mélange attire de manière significative une ou plusieurs autres espèces. Un seuil de spécificité a donc été défini. Il correspond à 20 individus d'une même espèce par traitement, capturés lors de la période de piégeage. Ainsi, la comparaison du Tableau 4 présentant les espèces communes aux 4 traitements et le Tableau 5 indiquant seulement les espèces atteignant ou dépassant le seuil dans les différents traitements, montre que toutes les espèces manifestant une certaine attraction pour un traitement, sont également présentes dans les autres traitements. Ce résultat indique qu'il n'existe aucune relation spécifique entre les insectes capturés et les terpènes présents dans les différentes formulations.

L'abondance de quelques espèces de coléoptères et de diptères peut s'expliquer par l'effet attractif des alcools. Toutefois, aucune n'a été identifiée comme ravageur ou ennemi naturel d'importance économique pour la Région (King et Saunders, 1984). Les hyménoptères, abondants dans les caféières, sont représentés ici essentiellement par des Vespidés et des Formicidés. Ils ont été capturés lors des périodes de sécheresse, quand la recherche d'eau, essentielle à leur survie, les attire vers les pièges.

L'espèce n°68 identifiée comme *Chrysopa sp.* est l'unique espèce bénéfique capturée. Prédatrice d'Aphides et de Pseudococcines, elle est particulièrement active au cours de son stade larvaire. Cependant, sa présence est beaucoup trop aléatoire pour qu'elle puisse être influencée par les effets du piège. Elle fait donc partie de cette catégorie d'insectes capturés par hasard.

Tableau 4. Espèces attirées par l'un des traitements à partir du seuil de 20 individus

Ordre	Code de l'espèce	Nombre d'individus capturés pendant le piégeage			
		Traitement 1	Traitement 2	Traitement 3	Traitement 4
Coléoptères	1	42	59	83	91
	2	21	21	32	27
	3	13	14	29	20
	5	26	15	7	11
	12	6	26	11	16
Hyménoptères	32	144	194	121	51
	34	52	423	93	12
Diptères	44	22	23	35	41
	45	32	68	108	83
Total	8	358	843	519	352

Tableau 5. Espèces attirées par les 4 traitements

Ordre	Code de l'espèce	Nombre d'individus capturés pendant le piégeage			
		Traitement 1	Traitement 2	Traitement 3	Traitement 4
Coléoptères	1	42	59	83	91
	2	21	21	32	27
	3	13	14	29	20
	4	1	1	6	3
	5	26	15	7	11
	12	6	26	11	16
Hyménoptères	32	144	194	121	51
	34	52	423	93	12
	35	6	9	6	2
Diptères	43	1	3	6	2
	44	22	23	35	41
	45	32	68	108	83
Lépidoptères	48	10	2	11	1
	49	3	1	5	1
Hémiptères	54	5	1	6	5
	56	14	12	7	8
Homoptères	58	1	1	2	2
Neuroptères	68	18	9	13	13
Total	18	417	882	581	389

Finalement, les scolytes représentent 97% des insectes capturés dans l'ensemble de l'essai et pour toute la durée de piégeage.

CONCLUSION

Les résultats obtenus au cours de ce travail, sont une contribution très importante à l'élaboration du piège commercial BROCAP® destiné à la lutte contre le scolyte du café, dans le cadre de la lutte intégrée.

Il a été démontré que la variation de la diffusion de l'attractif de base (de 0,124 à 0,347 g/piège/jour) n'est pas un facteur déterminant pour le piégeage. L'attraction du scolyte semble dépendre beaucoup plus fortement de la nature de l'attractif que de la quantité diffusée. Il est

donc préférable d'adopter une faible vitesse de diffusion de manière à optimiser l'usage du produit. On sait déjà qu'un taux moyen de 0,21 g/piège/jour donne d'excellents résultats (Dufour et al., 1999).

La comparaison des différents modèles de piège expérimentaux et commerciaux a permis de mettre en évidence le rôle prépondérant de la taille et de l'emplacement des ouvertures des pièges. Contrairement à d'autres espèces d'insectes, le scolyte du café a besoin d'espace pour atteindre la source attractive. Il a dont fallu concevoir une ouverture large et accessible, faite dans une matière de couleur visuellement attractive (Dufour et al., à paraître) et l'intégrer au reste du piège. Cet élément a donc influencé la forme des prototypes qui sont à l'origine du piège commercial BROCAP®. En accord avec les résultats d'essais obtenus avec ces prototypes, le support de diffusion en forme de croix a été adopté, sachant qu'il contribue à la solidité du système et qu'il augmente l'attraction visuelle du scolyte. Tous les autres détails ajoutés au piège BROCAP® tels que les principes d'ajustement des pièces, la forme, la couleur et la taille du récipient de capture et du diffuseur, ont été choisies en fonction des exigences techniques du système. Il faut préciser aussi, que les différents éléments du piège ont été fabriqués en tenant compte des impératifs de moulage. Afin de garantir la qualité du produit commercial, le piège est actuellement en cours de validation multilocale.

L'étude de la spécificité de l'attractif de base et de ses dérivés, a révélé une forte relation allélochimique pour le scolyte mais non exclusive. En effet, d'autres espèces d'insectes sont capturées, mais leur nombre est généralement faible. Par ailleurs ces espèces n'ont pas d'importance économique notable pour l'agriculture. Il convient d'ajouter qu'aucun parasitoïde utilisé en lutte biologique tel que *Cephalonomia stephanoderis* Betrem n'a été rencontré parmi les insectes capturés. Les terpènes associés à l'attractif de base et considérés comme d'éventuelles substances allélochimiques, n'ont pas effet visible sur les autres insectes. Ce résultat présente donc un grand intérêt pour la recherche de nouvelles formulations attractives, plus efficaces et plus spécifiques. Le piégeage du scolyte se présente donc comme une méthode respectueuse de l'environnement et plus particulièrement de la biodiversité. Les premières observations faites sur les captures effectuées à l'aide du piège BROCAP® indiquent que ce modèle, compte tenu de sa forme et de sa conception, est plus sélectif que les modèles expérimentaux de type "1B" car il ne laisse pénétrer que les insectes de petite taille (Dufour, non publié).

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BIBLIOGRAPHIE

Baker P., 1999. The coffee berry borer in colombia. Final report of the DFID-Cenicafé-CABI Bioscience IPM for coffee project. 144 p.

Decazy B., 1989. Le scolyte des fruits du caféier *Hypothenemus hampei* Ferr.: considérations sur la lutte intégrée contre ce ravageur. In: annales XII Colloque International sur le café, Paipa, colombia, Ed. ASIC, 655-665.

Dufour B. et Frerot B.Optimisation du piégeage du scolyte du café *Hypothenemus hampei* Ferr. à paraître.

Dufour B., Gonzalez M. O., Frerot B., 1999. Piégeage de masse du scolyte du café *Hypothenemus hampei* Ferr. (Col. Scolytidae) en conditions réelles: Premiers résultats. XVIII Colloque ASIC 1999, Helsinki, Finlande.

Gutierrez-Martinez A., Hernandez-Rivas S.,Virgen-Sanchez A., 1995. Efectos de los diferentes extractos de café robusta *Coffea canephora* Pierre ex Froehner sobre la captura de la broca del café *Hypothenemus hampei* Ferrari (Coleoptera : Scolytidae). In memoria XVI simposio de caficultura latinoamericana, Managua, Nicaragua, oct. 1993, ed. IICA/PROMECAFE, Tegucigalpa, Honduras, **2**, 7 p.

King A. B. S. et Saunders J. L., 1984. Las plagas invertebradas de cultivos anuales alimenticios en América Central. Administración de Desarrollo Extranjero, Londres, 182 p.

Mathieu F., 1995. Mécanismes de la colonisation de l'hôte chez le scolyte du café *Hypothenemus hampei* (Ferr.)(Coleoptera: Scolytidae). Thèse de Doctorat, Université Paris VII, 134 p + annexes.

Mathieu F., Brun L. O., Marcillaud C., Frerot B., 1997. Trapping of the coffee berry borer *Hypothenemus hampei* Ferr. (Col., Scolytidae) within a mesh-enclosed environment: Interaction of olfactory and visual stimuli. J. Appl. Ent. **121**, 181-186.

Mathieu F., Malosse C., Frerot B., 1998. Identification of the volatile components released by fresh berries at different stages of ripeness. J. Agric. Food Chem., **46**: 3, 1106-1110.

Mendoza Mora J. R., 1991. Resposta da broca-do-café, *Hypothenemus hampei*, a estímulos visuales e semioquímicos. Tese Magister Scientiae, Universidade Federal de Viçosa,, Brasil, 44 p.

An Integrated Pest Management Strategy for the Control of the Coffee Stem Borer, *Bixadus Sierricola* White (Coleoptera: Lamiidae)

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SUMMARY

The larva of the coffee stem borer *Bixadus sierricola* White (Coleoptera: Lamiidae), is a very severe pest of coffee in the whole of West and Central Africa, reaching as far as Uganda. It has, in recent times, been recorded as a serious pest in all the coffee growing areas in Ghana. The adult beetle feeds on dewdrops on leaves and on the bark of green shoots and lays its eggs on the bark of the tree. Attacked trees are ring-barked by young larvae but older larvae, measuring up to 5 cm long x 8 mm wide, bore into the heartwood, producing large quantities of powdery frass which collect at the base of the tree. Borer holes occur exclusively on the main tree trunk up to a height of about 3 metres from ground level. In severe cases, as many as 80% of trees can be attacked. Young trees are completely ring-barked and die. Older trees survive very enfeebled and are often broken by wind or attacked by termites or fungi.

A four-replicated Randomized Complete Block Design (RCBD) experiment was conducted on a farmer's farm at Brofoyedru in the Ashanti Region of Ghana to investigate the effectiveness of Gastoxin (Aluminium Phosphide) paste, to control the larvae. The trapping of adult beetles using sugar-baited traps was also investigated.

Gastoxin was applied by squeezing the paste into fresh borer holes and sealing off the hole with plasticine. Ten transparent plastic bottles containing 10% sugar solution and having two holes on the sides were suspended on branches of randomly selected borer-infested trees to lure and trap the adult beetles.

100% control of the larvae was achieved within 15 days after the Gastoxin treatment. Only two of the total of 3,200 trees, one found two months and the other five months after treatment, were re-infested by larvae and were treated. Thereafter, no fresh re-infestations by larvae were recorded on the experimental farm nine months after the first Gastoxin treatment. On the other hand, the sugar-baited traps caught no adult beetles but rather caught several species of ants.

Other control methods, including the use of commercial formulation of the nematode *Steinernema* sp. to control the larvae and various designs of traps for catching adults, will be investigated in future experiments.

INTRODUCTION

The stem borer *Bixadus sierricola* White (*Coleoptera: Cerambycidae*) has been recorded as a serious pest of coffee in Ghana and other coffee growing areas in West, Central and East Africa (Padi, 1984, 1985; Padi and Ampomah, 1996; Le Pelley, 1968). Attacked trees are ring-barked by young larvae but older larvae, measuring up to 5 cm long x 8 mm wide, make their tunnels into the heartwood of the tree trunk, producing large quantities of powdery frass

which collects at the base the trees. Entrance holes occur exclusively on the main trunk within the lower 3 metres from ground level.. In extreme cases, as many as 80% of trees can be attacked. Young trees are completely ring-barked and die. Older trees survive very enfeebled and are often broken by wind or are predisposed to attack by termites or fungi. In Ghana, areas on the tree trunk that have been ring-barked were found to be associated with infestation by a Paraputo sp. (Homoptera: Pseudococcidae) (Padi, 1984). The adult beetle feeds on leaves, dewdrops on leaves and on the bark of green shoots and the exocarp of green and ripe berries (Le Pelley, 1968; Padi, 1984). The eggs are laid on the bark of the tree trunk.

In Ghana, serious damage has been recorded throughout the entire coffee growing area (Padi, 1984), particularly on old neglected farms. Recently, serious outbreaks of the borer were reported on large private-owned coffee plantations at Borofoyeduru and other localities in the Ashanti Region, and at Bibiani in the Western Region. At. Borofoyeduru, an outbreak on a 12.5 acre plantation resulted in considerable reduction in yield and caused over 400 dead trees to be removed

Insecticides used in the past to control *Bixadus sierricola* in Ghana were the highly toxic chemicals, Diazinon and Azodrin, applied as stem paint. Both chemicals are now banned because of their high mammalian toxicity and also because the method of application predisposed the user to contamination and poisoning. Moreover, there was the possibility of the chemical being washed from the tree trunk by rain to contaminate the soil and nearby streams. There is, therefore, the need to develop alternative control strategies that are user and environmentally friendly.

The objective for the present study was to develop an Integrated Pest Management (IPM) strategy, involving the use of Gastoxin (Phosphine) and the trapping of adult borers (beetles). Although *Bixadus sierricola* does not attack branches, lower branches that were dead or showed signs of attack by the twig borer *Xylosandrus compactus* Eich. (Coleoptera: Scolytidae) were removed and burnt, as a side issue.

Gastoxin is a fumigant insecticide paste of 57% Aluminium phosphide supplied by **Casa Bernardo Ltd. of Brazil** and is marketed through a local agent, Chemico Ghana. Ltd. It is packaged in 400 g cans of 8 tubes each weighing 50 g. Once inside the borer hole, Gastoxin comes into contact with the local humidity and starts releasing Phodphine (Aluminium Phosphide) gas that is deadly for borers. Although Phosphine gas is highly toxic to mammals, Gastoxin paste is safer to use since the method of application allows no contact with the user. The only protective clothing required are a respirator to prevent inhalation, rubber gloves and a pair of Wellington boots for protection against snake bites Aluminium Phosphide in a paste form does no damage to plants nor leaves any residue on fruits.

MATERIALS AND METHODS

A small-scale field trial was conducted on a coffee plantation at Brofoyedru in the Ashanti Region of Ghana to investigate the effectiveness of Gastoxin (Aluminium Phosphide) paste against *B. sierricola* larvae, combined with the trapping of adult beetles. The experimental design was a four-replicated Randomized Complete Block Design (RCBD), and included an untreated control. Each sub-plot measured 0.4 ha (one acre).

Prior to the application of Gastoxin, the treatment and control sub-plots were thoroughly inspected and the number of borer infested trees (i.e. trees having fresh borer damage as indicated by the presence of fresh powdery frass on the tree trunk). The number of trees

showing yellowing leaf symptom., as well as the number of borer holes per plot, were recorded on both treated and control plots. Treatment was applied as follows:

1. Affected trees were cleaned and the rind around each entrance hole on the trunk carefully removed.
2. Gastoxin was applied by squeezing out approximately 3g of the paste into each entrance hole. Thus one 50gm tube of Gastoxin paste was enough for treating approximately 16 borer holes.
3. The holes were closed with plasticine (substitute for glass putty recommended by the chemical company).
4. Dead lower branches and branches showing signs of *X. compactus* damage were removed and burnt.
5. Fifteen days after treatment, all the trees in each sub-plot were re-inspected and trees in the treatment sub-plots having fresh *B. sierricola* damage were treated with Gastoxin as described above.
6. Monthly post-treatment assessment of the numbers of infested trees, borer holes and trees with yellowing leaf symptom.

For the trapping of adults, ten transparent plastic bottles, each containing 10% sugar solution and having two holes on the sides were suspended on branches of randomly selected borer-infested trees in each treatment sub-plot to lure and trap the adult beetles.

RESULTS

Efficacy of Gastoxin against larvae

100% control of larvae was achieved within 15 days after the Gastoxin treatment in all cases (Table 1). Only two of the total of 3,200 trees, one found two months and the other five months after the first treatment, had fresh borer holes (Table 2) and were treated. Thereafter, no fresh damage was recorded on the treated plots eight months after the first Gastoxin treatmen. On the other hand, as many as 76 trees out of the initial 83 trees remained infested nine months after treatment on the control plots.

Table 1. Percent mortality of Bixadus sierricola larvae 15 days after treatment with Gastoxin insecticide paste (mean of four replicates): August 2000

Treatment	# of fresh holes (Pre-treated)	# of fresh holes	% borer mortality
Gastoxin	145	0	100%
Untreated control	79	109	-37.1%

The effectiveness of treatment was reflected in the number of trees with yellowing leaves which dropped from 14 in August to 7 (50%) only one month after the initial Gastoxin treatment on the treated plots whilst those on the control plots consistently increased from the initial 9 to 23 trees (Table 3). It was also observed that at the onset of the minor rainy season in March-April 2001, there was a reduction in the number of trees showing the yellowing leaf symptom on the treated plots (Table 3), indicating that the attacked trees were perhaps recovering from the borer damage.

Table 2. Efficacy of Gastoxin against coffee stem borers (Mean number of infested trees and fresh borer holes): August 2000-April 2001

Treatment	August		September		October		November		December		January		February		March		April	
	IT	IH	IT	IH	IT	IH	IT	IH	IT	IH	IT	IH	IT	IH	IT	IH	IT	IH
Gastoxin	125	145	0	0	0	0	1	2	0	0	0	0	1	2	0	0	0	0
Untreated Control	79	109	81	152	83	181	80	139	80	122	81	110	83	103	67	83	76	96

IT = #. of infested trees
IH = #. of fresh borer holes

Table 3. Number of infested trees per treatment showing yellowing/withering leaves (mean of four replicates): August-April 2001

Treatment	August	September	October	November	December	January	February	March	April
Gastoxin	14	7	6	6	6	5	1	0	0
Untreated Control	9	13	18	19	21	23	21	18	18

Trapping of adults

The sugar-baited traps caught no adult beetles throughout the period of three months for which they were tested. Instead, they caught several species of ants, notably *Oecophylla longinoda*, (Latr), *Crematogaster clariventris* (Mayr), *Crematogaster africana* Mayr, *Pheidole megacephala* F. and *Camponotus* spp.

DISCUSSION

Results from the present study show that the use of Gastoxin alone, without the trapping of adults, could effectively keep *B. sierricola* under control The results are consistent with results obtained for other insects on a variety of crops:. in recent studies in Ghana (Padi and Adu-Acheampong, 2000), Gastoxin paste effected 100% control of the cocoa stem borer *Eulophonotus myrmeleon* (Fldr.) (Lepidoptera: Cossidae) and other unidentified Coleopteran stem borers in small- and large-scale on farm trials. Information available in the Casa Bernardo Ltd. Information Manual on Gastoxin also indicates that Gastoxin has been effectively used for the control of a wide range of stem borers such as *Trachyderes thoracicus*, *Macropophora accentifer*, *Disploschema rotundicolle* which attack tree trunks and branches of citrus (oranges, lemons, tangerines) and pecans. It has also been effectively used on date trees, nuts, apple, peach and apricot trees as well as olive and tea trees/bushes.

When applied according to the recommended procedure, and treated borer holes are properly sealed, Gastoxin poses no user or environmental problems. One obvious advantage of this method is only a selected few selected protective clothing, hand gloves and respirators, are required. Nevertheless, since the small-scale peasant farmers who constitute the majority of cocoa farmers in Ghana tend not to adhere strictly to recommended procedures (Henderson et al., 1994; Padi et al., 2000), there is the need to exploit alternative less hazardous control methods such as the use of biological control agents. Thus, there are plans to test the possible use of a commercial formulation of the nematode *Steinernema* sp. marketed by Kollant S.p.A of Italy for biological control. This nematode is known to effectively control several species of Coleopteran and Lepidopterous stem borers. There are also future plans to identify locally available cheaper materials for sealing the borer holes after Gastoxin treatment. Preliminary

investigations on the use of a local “key soap” has proved quite promising. It is not advisable to use mud paste since it could promote the spread of fungal and other soil-borne diseases.

Since the traps used failed to catch any adults, there is the need to test other trap designs. Various trap designs, including sticky traps of different colours, will be tested in future experiments.

CONCLUSIONS

Results on the effects of Gastoxin on *B. sierricola* obtained so far have been remarkable but the trapping of adults has been disappointing.

Assessment of the number of infested trees and the number of borer holes will continue for another one year during which period yield on treated and control plots will be compared to determine the cost-effectiveness of treatment. It should also be possible to mpnitor the survival or death of attacked trees on the treated and control plots.

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REFERENCES

Henderson C. P., Owusu G. K., Jones A. P., Padi B. (1994). The importance to cocoa research of understanding farmers' rationale towards pest and disease control: with reference to capsid and black pod disease control in Ghana. Proceedings of the 11th International Cocoa Research Conference, 18th-24th July 1993, Yamoussoukro, La Cote d'Ivoire: 299-304.

Le Pelley R. H. (1968). Pests of coffee. Longman Green & co Ltd.: 590 pp.

Padi B. (1984). Coffee Entomology: Insect survey. Rep. Cocoa res. Inst. Ghana, 1976/77 – 1978/79: 99-105.

Padi B. (1985). Proposed integrated control approach to insect pests of coffee in Ghana. Proc. 11th Int. Scientific Conf. on Coffee (11th-15th February, 1985), Lome, Togo: 647-652.

Padi B. and Adu-Acheampong R. (2000). Preliminary measures for the control of the cocoa stem borer *Eulophonotus myrmeleon* Fldr. (Lepidoptera: Cossidae). Proc. 13th Int. Cocoa Res. Conf., Kota Kinabalu, Sabah, Malaysia, 9-14 October 2000 (in press): 12pp

Padi B., Ackonor J. B., Abitey M. A., Owusu E. B., Fofie A and Asante E. (2000c). A survey on insecticide use and residues in cocoa beans in Ghana. Ghana Cocoa Board Internal Report: 27 pp.

Padi B. and Ampomah E. (1996). Insect succession on three coffee types in Ghana. Ghana Journal of Agric. Science (in press).

Elaboration d'une stratégie de lutte durable et efficace contre l'anthracnose des baies du caféier Arabica dans les hautes terres de l'Ouest-Cameroun: bilan des connaissances acquises et perspectives

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SUMMARY

Arabica coffee berry disease (CBD) caused by *Colletotrichum kahawae* is one of the major factors limiting Arabica coffee production in Africa. Losses recorded in Cameroon are around 50% on average. Currently, the main objective for controlling this disease is to search for, create and disseminate varieties with sustainable resistance to CBD, which are adapted to the different ecologies and farming practices employed in smallholdings. To that end, greater knowledge of pathogen populations, the nature and degree of resistance in the host germplasm, and various epidemiological parameters of the disease is required. The genetic diversity of pathogen populations in Cameroon was assessed by determining vegetative compatibility groups (VCG), and subsequently compared to that in East African countries, using RAPD (Random Amplified Polymorphic DNA) markers. Host/parasite relations were characterized by artificially inoculating the hypocotyls of uprooted seedlings and unripe berries taken from Arabica coffee trees. The effect of certain abiotic factors on disease incidence was measured. Primary inoculum sources were sought.

A single vegetative compatibility group (VCG) was detected in cameroon population. A structural analysis of the Cameroonian population by RAPD suggested that this population was clonal with a limited structure. A comparison with the population from East Africa revealed one VCG comprising two sub-groups (East Africa and Cameroon) and the existence of substantial genetic differentiation between the two geographical populations, which suggested that there was no pathogen migration between these two regions and raised the problem of where the disease originated and how the pathogen evolved. Pathogenicity tests carried out with the different isolates studied did not reveal any specific resistance reactions. Nevertheless, various levels of isolate aggressiveness and different levels of resistance between genotypes were observed. Arabica coffee tree resistance seemed to be non-specific. The two resistance assessment methods usable in the laboratory showed that the genetic base of the tested accessions was narrow. The results obtained on hypocotyls seemed to be more reliable than those obtained on berries. However, the method most representative of tree performance in the field under high parasite pressure remains to be identified. The role of climate in development of disease was clearly established. However, the effect of each abiotic factor on disease development, for each farming system, remains to be determined. The investigation carried out into primary inoculum sources revealed that leaves and twigs bearing fruiting nodes are capable of harbouring the fungus. Nevertheless, it is still not known how this inoculum is conserved. Given the knowledge already acquired, an effective control method based on general and sustainable resistance and environmental management can be envisaged.

RÉSUMÉ

L'anthracnose des baies du caféier Arabica causée par *Colletotrichum kahawae* est l'un des facteurs limitant majeurs pour la production du café Arabica en Afrique, donc au Cameroun. Les pertes enregistrées sont de l'ordre de 50% en moyenne. Le principal objectif actuel de lutte contre cette maladie est de rechercher, de créer et de diffuser les variétés durablement résistantes à l'anthracnose et adaptées aux différentes écologies et systèmes culturaux pratiqués dans les petites exploitations paysannes. A cet effet, une meilleure connaissance à la fois des populations pathogènes, de la nature et du niveau de résistance du germoplasme hôte, et de divers paramètres épidémiologiques de la maladie est nécessaire. La diversité génétique des populations pathogènes du Cameroun a été évaluée par détermination des groupes de compatibilité végétative (GCV) et à l'aide des marqueurs RAPD (Random Amplified Polymorphic DNA) et a été ensuite comparée à celle des pays de l'Afrique de l'Est. La caractérisation des relations hôte/parasite a été réalisée à l'aide des inoculations artificielles sur hypocotyles de semenceaux déracinés et sur baies vertes détachées de caféiers Arabica. L'effet des quelques facteurs abiotiques sur l'incidence de la maladie a été mesuré. Les sources d'inoculum primaire ont été recherchées.

Un seul groupe de compatibilité végétative (GCV) a été mis en évidence. L'analyse de la structure de la population camerounaise à l'aide des RAPD suggère que cette population est clonale et peu structurée. Comparée à la population originaire d'Afrique de l'Est, les résultats montrent l'existence d'une forte différenciation génétique entre les deux populations géographiques. Ce qui suggère l'absence de migration de l'agent pathogène entre ces deux régions et soulève le problème de l'origine de la maladie et de l'évolution de l'agent pathogène. Des tests de pathogénie réalisés avec les différents isolats étudiés n'ont pas mis en évidence de réactions spécifiques de résistance. Toutefois, différents niveaux d'aggressivité des isolats et différents niveaux de résistance entre les génotypes ont été observés. La résistance du caféier Arabica semble de nature non spécifique. Les deux méthodes d'évaluation de la résistance utilisables en laboratoire ont montré que la base génétique des accessions testées est étroite. Les résultats obtenus avec des hypocotyles semblent plus fiables que ceux obtenus sur baies. Mais il reste à identifier la méthode la plus représentative du comportement des arbres au champ dans les conditions de forte pression parasitaire. Le rôle du climat sur le développement de la maladie est clairement établi. Par contre, l'effet de chaque facteur abiotique, de chaque système cultural sur l'évolution de la maladie reste à préciser. Les investigations menées sur les sources d'inoculum primaire ont permis de montrer que les feuilles, les branchettes porteuses des nœuds fructifères sont capables d'héberger le champignon. Les formes de conservation de cet inoculum reste inconnues. Sur la base des connaissances déjà acquises, une méthode de lutte efficace basée sur la résistance générale et durable, et la gestion de l'environnement est envisageable.

Mots clés: Anthracnose, lutte, *Colletotrichum kahawae*, diversité, résistance, épidémiologie.

INTRODUCTION

Le caféier Arabica, de part ses exigences écologiques n'est cultivé au Cameroun que dans les hautes terres des provinces de l'Ouest et du Nord-Ouest, entre 1100 et 1800 mètres. Il couvrait dans les années 1990 une superficie d'environ 100,000 hectares. Aujourd'hui, les surfaces occupées par cette plante ont considérablement diminué. Le caféier Arabica est de plus en plus cultivé en association avec des cultures vivrières telles que: le maïs, les légumineuses à graines (haricot, soja, arachides), les tubercules, le plantain et les cultures maraîchères.

Depuis son apparition au Cameroun dans les années 1955, l'anthracnose des baies du caféier Arabica, encore appelé par les anglo-saxons Coffee Berry Disease (CBD), comme dans tous les autres pays africains producteurs du café Arabica, est considéré comme l'un des facteurs limitant le plus important pour la production. Les pertes enregistrées de l'ordre de 50% en moyenne peuvent atteindre 100% dans les zones de fortes pressions parasitaires qui sont généralement des zones les plus propices agronomiquement à la culture du café. Pour lutter contre cette maladie, augmenter les rendements et améliorer la qualité du café, un programme de sélection établi et mis en place à travers les essais comparatifs de variétés a identifié la variété Java parmi des centaines des variétés et accessions observées. La variété Java s'est montrée comme la mieux adaptée aux conditions de culture du caféier Arabica au Cameroun, la variété la plus productive, une des variétés les plus résistantes à l'anthracnose des baies et à la sécheresse, et végétativement, l'une des plus vigoureuses (Bouharmont, 1992; 1995). Par contre, elle s'est avérée un peu moins sensible à la rouille orangée que la variété Jamaïque partout cultivée dans le pays. Mais la diffusion du matériel végétal de Java n'a pas réellement abouti; par conséquent le niveau de résistance à l'anthracnose de la variété Java n'a pas été clairement établi au champ dans les conditions de cultures des petits paysans. En plus, les stratégies utilisées jusqu'à présent pour tenter de contrôler cette maladie sont essentiellement basées sur la lutte chimique. Cette approche a toutefois l'inconvénient d'être onéreuse et incompatible avec le seuil de rentabilité, en particulier dans le cas de l'arabicaculture camerounaise, généralement de type paysan.

Le coût de la protection phytosanitaire auquel il faut ajouter la dégradation du pouvoir d'achat offert par le prix au producteur, l'explosion démographique qui entraîne la réduction des surfaces cultivables, et l'importance des possibilités de revenus alternatifs obligent certains producteurs à abandonner la culture du café Arabica. Mais, pour de nombreuses familles des deux provinces et pour le Cameroun, le café Arabica constitue respectivement une source importante de revenus et une source de devises. Pour sauvegarder la culture de cette unique plante de rente pérenne de la zone, support depuis 70 ans de l'économie et du développement local, il faut proposer des systèmes de production durables permettant de maintenir la productivité et d'améliorer la rentabilité de la culture de l'Arabica; et limiter l'impact des contraintes parasitaires dont l'anthracnose des baies due à *Colletotrichum kahawae* constitue la majeure.

Le principal objectif actuel de lutte contre cette maladie est de rechercher, de créer et de diffuser les variétés durablement résistantes à l'anthracnose et adaptées aux différentes écologies et systèmes culturaux pratiqués dans les petites exploitations paysannes. A cet effet, une meilleure connaissance à la fois des populations pathogènes, de la nature et du niveau de résistance du germoplasme hôte, et de divers paramètres épidémiologiques de la maladie est nécessaire

En 1995, dans le cadre d'un projet financé par l'Union Européenne, l'Irad au Cameroun s'est associé à la Crf au Kenya, au Cifc au Portugal et au Cirad en France, pour élaborer des stratégies de lutte alternatives à la lutte purement chimique et basées sur la résistance variétale. Ce sont des résultats obtenus au Cameroun dans les composantes parasitaire, sensibilité variétale, épidémiologique et les perspectives dégagées au cours des cinq années du projet qui seront présentés dans ce papier.

STRUCTURE DES POPULATIONS PATHOGÈNES

Diversité génétique

L'étude de la diversité génétique a été abordée selon deux approches: la technique des Groupes de Compatibilité Végétative (GCV) et l'utilisation de marqueurs moléculaires neutres avec la technique de Random Amplified Polymorphic DNA (RAPD). Les tests de compatibilité végétative ont été effectués avec 17 isolats provenant de différentes zones de culture. L'analyse des marqueurs RAPD a été réalisée sur 65 isolats subdivisés en trois sous-populations régionales. Avec chacune des deux approches, la population pathogène camerounaise a été comparée à la population pathogène originaire des pays de l'Afrique de l'Est.

Tous les isolats confrontés se sont montrés intercompatibles. Les isolats de *C. kahawae* d'origine camerounaise étudiés forment un seul Groupe de Compatibilité Végétative. La population pathogène de *C. kahawae* semble être homogène. Les isolats camerounais confrontés à ceux de la population pathogène originaire d'Afrique de l'Est, se sont montrés partiellement compatibles. Les hétérocaryons issus des confrontations des isolats des deux populations géographiques (Cameroun d'une part et Afrique de l'Est d'autre part) intercompatibles ont été de très faible intensité par rapport aux hétérocaryons issus des confrontations des isolats de chacune des deux populations. La population camerounaise de *C. kahawae* semble être dissociée génétiquement de la population d'Afrique de l'Est.

L'analyse des marqueurs moléculaires RAPD a mis en évidence une très faible diversité génétique dans la population camerounaise. L'indice de diversité génétique de Nei (*H*) de l'ensemble des isolats étudiés a été de 0,05. La valeur de l'indice *Fst* de Weir et Cockerham estimé sur l'ensemble de la population camerounaise a indiqué que 11% de la variabilité observée est due à des différences entre les différentes sous populations. Une part de cette diversité observée résulterait de la différenciation des sous populations locales. En plus, l'analyse de la structure génétique de la population de *C. kahawae* a mis en évidence une forte proportion de loci en déséquilibre de liaison suggérant que la population de *C. kahawae* se maintient exclusivement par multiplication clonale. Le dendrogramme UPGMA construit à partir des indices de dissimilarité a montré que des isolats provenant de différentes localités appartiennent à un même haplotype. Ce qui confirme le caractère de multiplication asexuée des populations de *C. kahawae*.

En comparant la population camerounaise à celle de l'Afrique de l'Est, les deux populations se sont montrées bien que très proche génétiquement, fortement différenciées. Près de 2/3 de marqueurs étaient monomorphes. Les marqueurs spécifiques de la population camerounaise étaient de l'ordre de 21% tandis que ceux spécifiques de la population d'Afrique de l'Est étaient d'environ de 37%. L'estimation de *Fst* de l'ensemble des deux populations a indiqué que plus de 92% de la variabilité observée étaient dues aux différences entre les deux populations. La variabilité dans chaque population ne représentait que 8% de la variabilité totale. Cette différenciation des deux populations pose plus particulièrement le problème de l'origine de cette maladie au Cameroun. En effet, l'anthracnose des baies du caféier Arabica a été observée pour la première fois sur les hauts plateaux du Nord Ouest du Kenya en 1922. On pensait que cette maladie a été introduite au Cameroun à travers le matériel végétal importé du Kenya. Cette hypothèse n'étant pas vérifiée ou confirmée par les résultats cette étude de structure des populations pathogènes, l'origine de la maladie est à rechercher ailleurs.

Pouvoir pathogène des isolats et caractérisation de la résistance du caféier Arabica

L'analyse du pouvoir pathogène des isolats provenant de différentes localités a été effectuée sur une gamme de génotypes représentative de la diversité connue chez le caféier Arabica (Charrier et Eskes, 1998; Lasehermes et al., 1996). Certains de ces génotypes sont originaires du centre d'origine de l'hôte, situé en Ethiopie. D'autres sont des variétés cultivées de type Typica ou Bourbon. Le pouvoir pathogène a été analysé sur les hypocotyles de semenceaux et sur les baies vertes détachées.

Les analyses de variance des indices de pathogénie ont mis en évidence, quel que soit le type de test et la gamme d'hôtes utilisés, un effet " isolat" et un effet "génotype" très hautement significatifs. Les interactions "isolat x génotype" observées ont été très hautement significatives, toutefois elles n'expliquaient que moins de 10% de la variation totale.

Les résultats de l'évaluation du pouvoir pathogène n'ont pas mis en évidence de réactions différentielles, mais ils ont montré une variabilité dans l'agressivité de l'agent pathogène et différents niveaux de résistance chez l'hôte. L'origine des interactions observées était due en grande partie aux isolats très agressifs et aux isolats presque pas agressifs qui ne permettent pas de différencier les génotypes. Ceci contribue à suggérer que l'expression de la résistance du caféier Arabica est non spécifique et quantitative vis à vis de *C. kahawae*. Cette résistance est peut-être gouvernée par un nombre réduit de gènes, comme proposé par Van der Vossen et Walyaro (1980).

MÉTHODES D'ÉVALUATION ET IDENTIFICATION DES SOURCES DE RÉSISTANCE

L'identification et la caractérisation de nouvelles sources de résistance dans la gamme de matériel végétal existant dans les collections camerounaises ont été réalisées avec deux méthodes d'évaluation: test sur hypocotyles des semenceaux déracinés et test sur baies vertes détachées. Les résultats obtenus par chacune d'elles ont été comparés dans le but de définir la méthode la plus fiable et représentative du comportement des arbres au champ.

L'évaluation de la sensibilité des génotypes vis à vis d'une série d'isolats, à l'aide du test sur hypocotyles des semenceaux déracinés, a montré qu'il existe dans les collections du Cameroun, du matériel végétal, notamment quelques arbres d'origines éthiopiennes, présentant un bon niveau de résistance. Le classement des génotypes était globalement identique vis à vis de tous les isolats. Des coefficients de corrélation de rang ou de Spearman, très hautement significatifs, de l'ordre de 64% ont été enregistrés. Ces résultats constituent des éléments qui confirment le caractère non spécifique de la résistance du caféier Arabica à l'anthracnose des baies. Mais ces évaluations quantitatives effectuées sur des individus appartenant à une même origine ont révélé l'existence d'une certaine variabilité intra – origine. Cela implique une contrainte pratique dans l'exploitation de la résistance qui doit être considérée au niveau d'arbres individualisés et non d'une lignée considérée à priori autogame, donc homogène. Ce résultat amène à reconsidérer les modalités pratiques d'exécution des plans de croisements par exemple ou de sélection d'individus en vue de leur multiplication.

Les tests sur baies vertes détachées indiquent une grande variabilité dans les réponses et se révèlent peu fiables. Cette absence de fiabilité semble en partie liée au stade de développement des baies et aux conditions environnementales qui paraissent largement influencer l'expression des symptômes. Van der Graaff (1981) avait déjà mis en évidence l'effet date en évaluant la résistance du caféier Arabica sur baies vertes détachées. La variation de la sensibilité des baies en fonction de leur stade de développement ne peut être un élément non négligeable car elle a une incidence sur l'expression de la résistance des baies au

cours du temps et contribue à l'absence de corrélation des résultats obtenus à différentes dates. La faible répétabilité des résultats dans les conditions de l'étude rend difficilement exploitable les résultats obtenus et limite considérablement la comparaison avec les autres méthodes d'évaluation de la résistance.

Sur la base de ces observations, le test sur hypocotyles des semenceaux déracinés semble plus fonctionnel et adapté à l'évaluation de la résistance. Sa mise en oeuvre nécessite un contrôle strict des paramètres d'inoculation telle que la qualité de l'inoculum et requiert de nombreuses répétitions. La nature destructive de cette méthode constitue un facteur limitant pour l'étude de la reproductibilité. Par ailleurs, ce test ne permet pas l'évaluation du niveau de résistance d'individus F1. La mise en oeuvre d'un autre test sur des organes végétatifs renouvelables telles que les feuilles s'avérerait donc très utile. Les premiers résultats des travaux initiés au laboratoire de phytopathologie du CIRAD-CP dans cette voie ont montré qu'en conditions contrôlées, il est possible d'infecter des feuilles développées de caféier Arabica avec des isolats de *C. kahawae* jusqu'alors considéré comme pathogène exclusif des baies. De nouvelles méthodes d'évaluation de la résistance ont été décrites. La recherche des marqueurs moléculaires caractérisant la résistance au champ est l'une des voix à explorer. Suite aux travaux de Lashermes et al. (1996) sur l'utilisation des RAPD dans l'analyse de la diversité génétique des formes cultivées et sauvages d'Ethiopie du caféier Arabica, Agwanda et al. (1997), ont mis en évidence, à l'aide de cette même technique une grande variabilité dans les populations Catimor et Rume Sudan, et ont identifié trois marqueurs RAPD associés au gène T de résistance du caféier Arabica à l'anthracnose des baies.

Pour valider toutes ces méthodes d'évaluation potentielles visant essentiellement à mesurer la résistance intrinsèque, la mise au champ dans des conditions de forte pression parasitaire, des essais de validation est incontournable. L'évaluation au champ du matériel végétal permettrait aussi de s'assurer de la stabilité de la résistance dont la nature non spécifique pourrait rendre son expression sensible aux conditions environnementales. Cela serait surtout le moyen de vérifier si d'autres composantes de la résistance, liées aux conditions écologiques, à l'architecture de la plante ou la période de réceptivité des fruits par exemple, pourraient avoir une incidence sur l'expression de la résistance. D'après nos tests, la résistance intrinsèque vis à vis de l'anthracnose des baies semble, en effet, assez faible. C'est certainement ce qui permet d'expliquer qu'aucune corrélation forte ne se dégage entre la résistance au champ et l'évaluation par inoculations artificielles sur jeunes semenceaux ou sur baies vertes détachées.

Malgré les difficultés de comparaison des résultats obtenus avec les différentes méthodes d'évaluation, on observe que certains génotypes provenant de la collection de Foumbot et de la collection de Santa se sont montrés tolérants avec les deux tests d'évaluation en conditions contrôlées et avec l'évaluation au champ réalisée par Bouharmont (1995). Ces génotypes pourraient donc être employés dans un programme d'amélioration variétale.

ASPECTS ÉPIDÉMIOLOGIQUES

Sources d'inoculum

Il a été mis en évidence qu'on pouvait isoler le champignon à partir des branches des caféiers pendant les périodes d'absence des baies. L'écorce des plants de caféiers est considéré comme un réservoir potentiel d'inoculum primaire dans les zones de culture du Cameroun où le climat est de type tropical avec deux saisons: une saison sèche qui dure environ 5 mois et une saison de pluies.

Effet de différentes conditions agroécologiques sur le développement de la maladie

Les observations épidémiologiques réalisées sur deux sites situés dans deux altitudes différentes (1550 m et 1760 m) et sur des arbres situés d'une part à l'ombre et d'autre part en plein soleil ont montré que les dynamiques du développement de la maladie sont sous contrôle de plusieurs facteurs du milieu et des systèmes agro-culturaux . Par exemple, il a été mis en évidence qu'en haute altitude (1760 m), les pertes réellement dues à l'anthracnose variaient de 34 à 68% au soleil et de 38 à 55% à l'ombre selon les années. Et en moyenne altitude (1550 m), les pertes variaient de 9 à 32% au soleil et de 10 à 23% à l'ombre. Il apparaît essentiel de préciser davantage le rôle de chaque facteur du milieu et des différents types de systèmes culturaux sur les différentes phases du développement de la maladie. Une telle connaissance permettra en intégrant la composante variétale, d'adapter en conséquence les modes de conduite des plantations.

CONCLUSIONS: PROPOSITIONS SUR LA GESTION INTÉGRÉE DES RÉSULTATS OBTENUS ET PERSPECTIVES

A partir des résultats obtenus, quelques stratégies à moyen terme et quelques tactiques à court terme adaptées aux différentes zones de production sont proposées. La gestion de la résistance génétique est une composante importante des stratégies et des tactiques proposées.

Compte tenu des caractéristiques des populations pathogènes de *C. kahawae* étudiées: faible diversité génétique, populations à multiplication asexuée, la sélection de variétés de caféiers Arabica résistantes à l'anthracnose des baies devrait se révéler efficace. Cependant, les schémas de sélection devront tenir compte de la différenciation génétique des populations pathogènes. La sélection et les évaluations du matériel végétal en conditions contrôlées devront conjointement se faire avec les isolats provenant du Cameroun, des pays de l'Afrique de l'Est et si possible d'autres foyers isolés de la maladie. Cette stratégie nécessite une collaboration étroite entre les pays où sévit cette maladie et les pays du Nord travaillant sur la maladie, en vue d'une lutte génétique efficace contre ce fléau.

L'absence de spécificité permet d'envisager le développement d'une résistance générale et durable sous certaines conditions. La présélection de matériel végétal à l'aide d'inoculations artificielles nécessite un choix rigoureux des isolats testeurs. En effet, ces isolats doivent représenter la gamme d'agressivité rencontrée localement. Les évaluations de la résistance avec les tests de présélection devront être confirmées avec des évaluations du matériel végétal au champ dans différentes zones de forte pression parasitaire représentant les pools génétiques de la population pathogène.

La variabilité dans l'agressivité laisse aussi penser qu'une certaine pression de sélection pourrait s'exercer vis à vis de cette caractéristique. Pour vérifier cette hypothèse, les isolats devront être isolés de caféiers de différents niveaux de résistance, pour être inoculés de façon croisée sur les mêmes caféiers.

Dans l'état actuel des connaissances, l'utilisation du test précoce sur semenceaux constitue le moyen recommandé pour évaluer la résistance intrinsèque, les évaluations fournies par le test sur baies détachées se révélant aléatoires. Mais, compte tenu des limites du test sur hypocotyles de semenceaux, un test précoce d'évaluation de matériel reproductible, fiable et représentatif du comportement des variétés au champ sur un tissu végétal disponible en tout temps et en tout lieu, et renouvelable comme les feuilles (Nyassé et al., 1995) ou issu de culture de l'embryogénèse somatique doit être activement recherché. Un pareil test permettra d'analyser les caféiers issus des divers croisements qui contribueront à développer les

recherches sur les marqueurs moléculaires liés à la résistance du caféier Arabica à l'anthracnose des baies. Ces études doivent permettre une meilleure connaissance des bases génétiques de la résistance: nombre de QTL impliqués et niveau de la résistance apportée par chaque QTL. L'identification de QTL à partir des clones d'origines génétiques différentes et leurs hybrides F1 permettrait aussi de mieux orienter le choix de géniteurs dans les schémas de sélection.

Les premiers résultats portant sur les corrélations entre la résistance au champ et l'évaluation avec les inoculations artificielles sur semenceaux sont préliminaires et ne permettent pas de conclure. Un essai de validation à grande échelle est envisagé et constitue une action prioritaire. Des arbres individuels déjà évalués sur hypocotyles de semenceaux devraient être sélectionnés en fonction de leur niveau de résistance, multipliés par voies végétatives ou par autofécondations et plantés au champ dans les zones à forte pression parasitaire. Le degré d'attaque de l'anthracnose des baies de ces arbres et d'autres variables agronomiques seront notés durant au moins cinq années. Les résultats obtenus seront comparés à ceux obtenus avec les tests et des corrélations seront établies.

L'évaluation du matériel végétal sauvage présent dans les collections au Cameroun a permis de mettre en évidence des niveaux de résistance intéressants pour plusieurs lignées notamment les lignées d'origine éthiopienne. Ces populations constituent un réservoir de facteurs de résistance à l'anthracnose des baies exploitables pour l'amélioration du caféier Arabica. L'étude de cette source de résistance avec l'analyse des essais d'un plan de croisement factoriel incomplet des lignées éthiopiennes entre elles et/ou avec d'autres variétés commerciales (Caturra et Java) doit se faire. L'évaluation du niveau de résistance des individus de première génération F1 devra être entreprise en priorité. Par ailleurs, l'étude de l'héritabilité des caractères de résistance se fera avec des partenaires grâce à un essai de croisements entre des lignées d'origine éthiopienne, des Rume Sudan, K7 et hybride de Timor. Cela doit permettre l'identification et la sélection de variétés présentant des caractères de résistance à l'anthracnose et une bonne productivité.

RÉFÉRENCES

Agwanda O. C., Lashernes P., Trouslot P., Combes M. C. and Charrier A. 1997. Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee. Euphitica **97**: 241 - 252

Bouharmont P. 1992. Sélection de la variété Java et son utilisation pour la régénération de la caféière arabica au Cameroun. Café Cacao Thé **36** (4): 247-262.

Bouharmont P. 1995. La sélection du caféier Arabica au Cameroun (1964 - 1991). Document interne, Cirad - CP Montpellier: 85p.

Charrier A. and Eskes A. 1998. Les Caféiers. Pages 171-196 Dans: L'amélioration des plantes tropicales. Charrier, A., Jacquot, M., Hamon, S. and Nicolas, D. Eds., CIRAD, ORSTOM, Montpellier.

Lashermes P., Trouslot P., Anthony F., Combes M. C. and Charrier A. 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. Euphytica **87**: 59 - 64.

Nyassé S., Cilas C., Herail C. and Blaha G.. 1995. Leaf inoculation as an early screening test for cocoa (Theobroma cacao L.) Resistance to Phytophthora black pod disease. Crop protection **14** (8): 657 - 663.

Van der Graaff N.A. (1981). Selection of arabica coffee types resistant to coffee berry disease in Ethiopia. Meded. Lanbhogesh. Wag. **81** (11): 110 p.

Van der Vossen H. A. M. and Walyaro D.J. 1980. Breebing for resistance to coffee berry disease in *Coffea arabica* L. II. Inheritance of the résistance. Euphytica **29**: 777-791.

Coffea Canephora and *C. Congensis* Have Antixenosis Resistance to the Coffee Leaf Miner*.

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SUMMARY

The coffee leaf miner is the most important pest of *Coffea arabica* in Brazil, and all cultivars are susceptible to the insect. However, distinct reactions have been verified in *C. congensis* and *C. canephora*. Although some individuals are susceptible, others present 'resistance to the caterpillar', related to the type of reaction of the plants. In this work it was investigated the existence of the antixenosis in individuals of *C. canephora* and *C. congensis* species selected for the resistance to the coffee leaf miner in field and laboratory conditions. The Catuaí Vermelho cultivar of *C. arabica* was used as the susceptible standard. Experiments have been carried out in controlled laboratory infestations using Catuaí Vermelho and Obatã cultivars of *C. arabica*, Guarini cultivar of *C. canephora* and *C. congensis*. It was observed that *C. congensis* and the Guarini cultivar of *C. canephora* are less infested by the insects. The Obatã cultivar was the most infested variety and the Catuaí Vermelho cultivar *C. arabica* had an intermediate reaction. Thus, it can be said that in some plants of *C. congensis* and *C. canephora* cv. Guarini there are two distinct mechanisms of resistance: antixenosis and resistance to the larvae.

INTRODUCTION

Among the insects that cause economic damages to the Arabica coffee culture in Brazil, the coffee leaf miner, *Leucoptera coffeella* Guérin-Méneville, is distinguished as the most important pest (Parra, 1985). The injuries developed upon caterpillars infection and the premature fall of leaves restrict significantly the photosynthetic foliar surface of the plants (Crowe, 1964), leading to considerable losses in farming productivity and longevity (Souza et al., 1998). Although there is consensus in respect to insect susceptibility of *Coffea arabica* L., information regarding other *Coffea* species are perhaps contradictory. Guerreiro-Filho et al. (1991) described *C. congensis* Froehner as susceptible to the insect. However, highly resistant individuals of this species had been identified by Matos et al. (2000), in a wild *C. congensis* population, originated from the African Republic Center. Also, distinct reactions are observed in some cultivars of *C. canephora* Pierre. For instance, the damage observed in Robusta cultivar, either in field natural infestations (Medina-Filho et al., 1977), or at artificial laboratory conditions (Guerreiro-Filho et al., 1991), is very severe and similar to that verified in *C. arabica* cultivars. However, infestations at fields of Conilon cultivar are not very severe, and the use of defensives is practically dispensable (Ferreira et al., 1979). Lately, studies developed by Guerreiro-Filho and Mazzafera (2000), had evidenced that selected plants of Guarini cultivar present high level of resistance to the insect.

Most of the works regarding *Coffea* species resistance to the leaf miner, focus on the larval phase of the insect. However, some authors suggest that resistance can also be related to adults preference for oviposition at different parts of the plant. Studies carried out with susceptible coffee trees (Bigger, 1969) evidenced that females have a tendency to pound in young leaves about one third less than in oldest leaves. The intensity of the leaf green color also seems to influence the insect preference for oviposition. Then, the darkest leaves are more used for ovipositions than the lighter ones. In this work antixenosis resistance was investigated in distinct hosts, such as *C. arabica*, *C. congensis* and *C. canephora.* These were selected based on the 'resistance to larvae' presented in field and laboratory conditions (Matos et al., 2000; 2001).

MATERIAL AND METHODS

The preference of adult females of *Leucoptera coffeella* for oviposition in different hosts was evaluated in three *Coffea* species, in two distinct laboratory experiments.

In the first experiment, the *C. arabica* cultivars Catuaí Vermelho IAC 81 and Obatã IAC 1669-20 and the *C. canephora* cultivar Guarini IAC 1598 were evaluated. In the second experiment, we evaluated the species *C. congensis* IAC 4349, *C. canephora* cv. Guarini IAC 1598 and *C. arabica* cv. Obatã IAC 1669-20. The preference tests had been carried out disposing the cultivars two by two, with three replications of each one of the combinations presented in Table 1.

Table 1. Tests of preference for oviposition performed among *C. arabica* cultivars, *C. congensis* and *C. canephora* in two distinct experiments

Experiment I		Experiment II	
Catuaí Vermelho x Obatã	Catuaí Vermelho x Catuaí Vermelho	Congensis x Obatã	Congensis x Congensis
Obatã x Guarini	Obatã x Obatã	Obatã x Guarini	Obatã x Obatã
Guarini x Catuaí Vermelho	Guarini x Guarini	Guarini x Congensis	Guarini x Guarini

Five leaves belonging to the third pair of leaves from each cultivar were kept in a bed as described at Guerreiro-Filho et al. (1999). Plant material was displayed during 16 hours in cages of insects propagation (Katiyar and Ferrer, 1968), at high population density. Later, the number of eggs layered on leaves by females in each cultivar was determined. This number was then related to the total foliar surface of the parcel expressed in cm^2. The comparisons between cultivars were carried through by the χ^2 test (1%), using as significant variable the number of eggs/cm^2.

RESULTS

The results related to *P. coffeella* preference for oviposition in different cultivars of *Coffea* are presented in Tables 2 and 3. The results demonstrated that when leaves of a same cultivar were exposed to insects, the number of eggs layered by females was very similar among repetitions. In three combinations evaluated in the first experiment (Catuaí Vermelho X Catuaí Vermelho, Obatã X Obatã and Guarini X Guarini), and in the second experiment (C. congensis X C. congensis, Obatã X Obatã and Guarini X Guarini), the calculated value of χ^2 was very low and non-significant, indicating a perfect adjustment to the expected distribution. Relatively, in each comparison the frequency of oviposition was always close to 50% for each

treatment, being the differences never superior to 6%. The largest variations were observed in tests with cultivar Obatã, which displays differences of 4 and 6%, respectively in the second and third experiments.

Table 2. Oviposition frequency for *P. coffeella* in leaves of the species *C. arabica* cultivars Obatã and Catuaí Red and *C. canephora* cultivar Guarini

Tests	Frequency of Oviposition		χ^2; 1 df; $P_{(1\%)}$
	Eggs/cm^2	%	
Catuaí Vermelho x Obatã	2,36 x 5,63	30 x 70	91,88**
Obatã x Guarini	10,06 x 3,30	75 x 25	348,44**
Guarini x Catuaí Vermelho	1,67 x 5,67	23 x 77	270,06**
Catuaí Vermelho x Catuaí Vermelho	8,78 x 9,32	49 x 51	0,83 ns
Obatã x Obatã	9,55 x 8,39	53 x 47	5,83 ns
Guarini x Guarini	5,81 x 5,96	49 x 51	0.25 ns

However, when the leaf samples exposed to insects belonged to different cultivars, the values of χ^2 were significant, indicating the preference for one of the cultivars. The results of tests accomplished in the first experiment (Table 2) showed that *C. canephora* cultivar Guarini was less preferred for oviposition. This is true not only for the confront with cultivar Catuaí (23% x 77%), but also with the cultivar Obatã (25% x 75%). These data suggest the occurrence of antixenose resistance type in the cultivar Guarini. Also, simultaneous exposure of leaves of the two *C. arabica* cultivars to the insect revealed a larger oviposition frequency in the cultivar Obatã.

Table 3. Oviposition frequency of *P. coffeella* in leaves of the species *C. arabica* cultivar Obatã, *C. canephora* cultivar Guarini and *C. congensis*

Tests	Freqüência de Oviposição		χ^2; 1 df; $P_{(1\%)}$
	Eggs/cm^2	%	
Obatã x Guarini	1,04 x 0,84	55 x 45	15,22**
Guarini x Congensis	1,58 x 1,96	45 x 55	24,2**
Congensis x Obatã	1,67 x 2,78	38 x 62	136,46**
Obatã x Obatã	1,34 x 1,24	52 x 48	2,2 ns
Congensis x Congensis	1,78 x 1,76	50 x 50	0,085 ns
Guarini x Guarini	0,59 x 0,60	49 x 51	0,02 ns

Based on the results observed in this experiment, cultivars Guarini and Obatã were selected respectively as dissuasion and attractiveness standard to the leaf-miner for preference tests. These tests were performed on a selected specimen of *C. congensis*, which exhibited low infestation index at field conditions of high plague incidence (Matos et al., 2000). The results obtained are presented in Table 3.

The results confirmed that the cultivar Guarini has a higher dissuasion potential, when in presence of different germplasm. The ovoposition frequency in leaves of this cultivar was significantly lower than to those frequencies observed in leaves of *C. congensis* and cultivar Obatã. This cultivar was also preferred for oviposition in comparison to *C. congensis.*

DISCUSSION

In theory, insects site-preference for oviposition is related to an hierarchy established by the adult females when confronted to different hosts (Thompson and Pellmyr, 1991).

According to this criterion, it can be established here a classification of different hosts regarding the preference for oviposition by females of *P. coffeella*. Therefore, in presence of both *C. arabica* cultivars and *C. canephora*, the females ovipositioned preferentially in the cultivar Obatã, followed by Catuaí Vermelho, and lately in *C. canephora* cultivar Guarini. The species *C. congensis* exhibits intermediary preference between cultivars Obatã and Guarini.

It is worthwhile to notice that although *C. canephora* cultivar Guarini was less required for ovoposition than *C. arabica* cultivars, the cultivar Obatã, a resultant of the initial hybridization between these two species, is more attractive to leaf-miner females than the parental species. Also, observations at plantations confirmed an elevated incidence of lesions in leaves of this cultivar. However, this observation could be related to a higher foliar retention of Obatã plant, due to its resistance to the agent of the blight, *Hemileia vastatrix*.

So far, reasonable explanations why the females of *P. coffeella* display preference for different hosts are investigative. According to Cardenas (1981), the lighter green color predominant in *C. canephora* leaves is one reason for the low oviposition frequency of *P. coffeella* in this species. Although the lighter color is a common characteristic of young leaves, the cultivar Guarini used in this experiment has a lighter shade of green than the adult leaves of the cultivar Catuaí Vermelho, which by it turns has lighter leaves than cultivar Obatã. Interestingly, the density of eggs layered by females in the leaves of each cultivar decreased according this color gradient. Also, in plantations this association is frequently observed in leaves of a same plant, where either young or lighter leaves from the first pair are less attacked than the older and darker leaves (Bigger, 1969; Nantes and Parra, 1977).

Thompson and Pellmyr (1991) suggest that a differential concentration of specific chemical compounds in the leaves could play an important role in this color preference mechanism. Studies accomplished by Mazzafera (1991) regarding the role of caffeine acting as a repellent to *Atta spp*, suggest that this alkaloid could be classified as dissuader, responsible for a quantitative defense system in the plants. Then, the response would be dependent on the caffeine concentration in plant tissues.

P. coffeella is a leaf-miner and its caterpillar develops and feeds on the palissade parenchyma (Crowe, 1964). At biological conditions, lesions are abandoned by the larvae just before its transformation in chrysalides. However, atypical premature exit frequently results in elevated caterpillar mortality rates. The small lesions observed in resistant plants are due to high mortality of caterpillars that could not search for other host. This type of resistance is also observed in the fly of sorghum and is denominated 'resistance to the caterpillar' (Rossetto, 1984).

In face of this biological peculiarity of the leaf-miner, another consistent argument could be pointed out regarding resistance levels in plants. By this argument females would prefer to layer their eggs in susceptible hosts, where the offspring would have better survival chance. The significant correlation observed between the reaction type and the classification regarding preference for oviposition of the evaluated plants support this theory. Therefore, the posture frequency is smaller in leaves of species classified as resistant, such as *C. congensis* IAC

4349 (Matos et al., 2000), or moderately resistant such as *C. canephora* cultivar Guarini (Guerreiro-Filho and Mazzafera, 2000).

Nevertheless, it is important to mention that the total foliar surface damage caused by the caterpillars is not correlated to caffeine content in plant tissues. According to Guerreiro-Filho and Mazzafera (2000), *P. coffeella* is well adapted to the coffee tree and developed a tolerance mechanism to the potentially toxic effects of caffeine. Thus, one can suppose that plants resistant to the caterpillar could be also resistant in relation to preference for oviposition of *P. coffeella*. In other words, these plants would be also resistant by antixenose. However this theory needs to be further investigated.

Based on these information, it can be suggested the occurrence of different resistance mechanisms to the leaf-miner in coffee trees. A first type, denominated "resistance to the caterpillar", implies in an anomalous development and death of the caterpillars upon infection. A second type would be a defense mechanism related to dissuasion of the insects at the adult phase, leading to a lower demand by females oviposition for plants bearing specific genes.

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REFERENCES

Bigger M., 1969. Partial resistance of arabica coffee to the coffee leaf miner, *Leucoptera meyricki* Ghesq. (Lepidoptera-Lyonetiidae). East African Agricultural and Foresty Journal, **34** (4): 441-445.

Cardenas R.M., 1981. Caracterizacion histo-morfologica de daño del minador de la hoja, *Leucoptera coffeella* em especies e híbridos de *Coffea spp*. y observaciones sobre resistencia. Bogota, UNC, 69p. Tesis (MSc).

Crowe T.J., 1964. Coffee leaf miners in Kenya: II causes of outbreaks. Kenya Coffee, **29** (342): 223-231.

Ferreira A.J., Matiello J.B. and Paulini A.E., 1979. Comportamento de algumas progênies e linhagens de *Coffea arabica* em relação à infestação de bicho mineiro - *Perileucoptera coffeella* (Guérin-Méneville, 1842). In: Congresso Brasileiro de Pesquisas Cafeeiras, 7. Araxá. Resumos. Rio de Janeiro, IBC-Gerca, pp. 222-224.

Guerreiro-Filho O. and Mazzafera P., 2000. Caffeine does not protect coffee against the leaf miner, *Perileucoptera coffeella*. Journal of Chemical Ecology, **26** (6): 1447-1464.

Guerreiro-Filho O., Medina-Filho H.P. and Carvalho A., 1991. Fontes de resistência ao bicho mineiro *Perileucoptera coffeella,* em *Coffea spp.* Bragantia, **50**(1): 45-55, 1991.

Guerreiro-Filho O., Silvarolla M.B. and Eskes A.B., 1999. Expression and mode of inheritance of resistance in coffee to leaf miner *Perileucoptera coffeella*. Euphytica, **105** (1): 7-15.

Katiyar K.P. and Ferrer F., 1968. Rearing technique biology and sterilization of the coffee leaf miner, *Leucoptera coffeella* (Lepdoptera-Lyonetiidae). In: International Atomic Energy Agency, Vienna IAEA, pp. 165-175.

Matos, J.W., Adami M.J. and Guerreiro-Filho O., 2000. Resistência de *Coffea congensis* ao bicho mineiro do cafeeiro. Genetics and Molecular Biology, **23** (3): 494-495. Supplement.

Matos J.W., Ramiro D.A. and Guerreiro-Filho O., 2001. Variabilidade em *Coffea canephora* para resistência ao bicho mineiro. In: Congresso Brasileiro de Melhoramento de Plantas, 1. Goiânia. Resumos. Londrina, SBMP, pp. ?.

Mazzafera P., 1991. Análises químicas em folhas de cafeeiros atacados por *Atta* spp. Revista de Agricultura de Piracicaba, **66** (1): 33-45.

Medina-Filho H.P., Carvalho A. and Mônaco L.C., 1977. Melhoramento do cafeeiro. XXXVII - Observações sobre a resistência do cafeeiro ao bicho mineiro. Bragantia, **36**: 131-137.

Nantes J.F.D. and Parra J.R.P., 1977. Avaliação de danos causados por *Perileucoptera coffeella* (Guérin-Méneville, 1842) (Lepidoptera-Lyonetiidae), em três variedades de café (*Coffea spp.*). O Solo, **69** (2): 26-29.

Parra J.R.P., 1985. Biologia comparada de *Perileucoptera coffeella* (Guérin-Menéville, 1842) (Lepidoptera, Lyonetiidae) visando ao seu zoneamento ecológico no estado de São Paulo. Revista Brasileira de Entomologia, **29** (1): 45-76.

Rossetto C.J., 1984. Sorgum midge: host-plant resistance mechanisms. In: International Sorghun Entomology Workshop, Texas, USA, Proceedings. Patancheru, India, 1985. pp. 293-300.

Souza J.C., Reis P.R. and Rigitano R.L.O., 1998. Bicho-mineiro do cafeeiro: Biologia, Danos e Manejo Integrado. Boletim Técnico 54, EPAMIG, 48p.

Thompson J.N. and Pellmyr O., 1991. Evolution of oviposition behavior and host preference in Lepidoptera. Annual Review of Entomology, **36**: 65-89.

Microscopic Variations in Susceptible and Resistant Infections of *Coffea arabica* by *Colletotrichum kahawae*

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SUMMARY

Light microscopy examinations were carried out on seedling hypocotyls of *Coffea arabica* varieties of different resistance to *Colletotrichum kahawae*, the cause of coffee berry disease, for differences in the development of monoconidial isolates of the fungus. Resistant reactions were characterised by fewer infection sites, retarded fungal growth, cytoplasmic changes, plant cell and pathogen death, scab and cork barrier formation. The latter reactions were suggested likely to be pathogen non-specific and involving antimicrobial biochemicals.

INTRODUCTION

Understanding the mechanisms of interaction between *C. kahawae* and coffee resulting into coffee berry disease (CBD) is important in breeding for resistance and field management of the disease, which is of major economic importance in Africa (Masaba and Waller, 1992). In the past, many studies have been conducted on this subject as reviewed by Gichuru (1997). This study aimed at providing further information on this host-pathogen interaction focusing on microscopic differences during pathogenesis in different *Coffea arabica* varieties.

MATERIALS AND METHODS

Coffee tissues used were seedling hypocotyls inoculated by the method of van der Vossen et al. (1976). Seven arabica coffee varieties with different levels of resistance to CBD i.e. SL28 (susceptible), K7, Hibrido de Timor, Pretoria, Geisha 10, Mundo Novo (all medium resistance) and Rume Sudan (resistance) were inoculated with two monoconidial isolates of *C. kahawae*. The inoculated hypocotyls were sampled weekly for eight weeks (five seedlings per isolate x variety combination) and sectioned using Labline/Hooker plant microtome. The sections were observed without staining mounted in distilled water using light microscope for histological differences. Seedlings with inactive lesions were selected from the medium resistance and resistant varieties (susceptible ones had none), sectioned and the types of barriers recorded as type A (under raised scabs) and type B (under sunken lesions) (Masaba and van der Vossen, 1982). The percentages of each category were calculated.

RESULTS

In the susceptible varieties, the density of infected epidermal cells was higher making a rather continuous infection area while in the resistant varieties fewer cells were infected. The post-infection mycelial growth of the pathogen was rapid in susceptible varieties and slow or arrested in resistance varieties. Where there was growth of the pathogen in the resistant and medium resistant varieties, the growth was characterised by thickened hyphal cells. The infected cells and those ahead of the hyphae became granulated and they died thereafter. Cork barriers were formed beyond the infected areas and scabs formed. In the highly resistant varieties, most of the scabs were type A but as compatibility increased, larger scabs were

formed and ultimately type B barriers were formed in higher proportions (Table 1). Intermediate reactions were observed in all cases.

Table 1. Percentages of fully developed types A and B barriers under inactive lesions in moderately resistance and resistant varieties of *C arabica* inoculated with two isolates of *C. kahawae* eight weeks after inoculation

	ISOLATES			
	KNY 1		KNY 2	
Variety	**Type A**	**Type B**	**Type A**	**Type B**
Rume Sudan	70	30	98	2
Hibrido de Timor	34	66	44	56
K7	58	42	70	30
Pretoria	48	52	78	22
Geisha 10	12	88	10	90
Mundo Novo	10	90	6	94

DISCUSSION

The results show that resistance to *C. kahawae* by *C. arabica* is multilevel as also observed by other workers (Mwang'ombe and Shanker, 1994; Gichuru, 1997). The initial reactions appear to be mainly antimicrobial biochemicals and might be pathogen specific but those of cell death and cork barrier formation may be pathogen non-specific as also suggested by Masaba and van der Vossen (1982). The level of compatibility of the interaction determines the ultimate reaction type and the percentage of the types of barriers may indicate the differences in these levels. This may be due to high aggressiveness of the pathogen isolate or resistance of the host genotype.

ACKNOWLEDGEMENT

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REFERENCES

Gichuru, E K (1997). Resistance mechanisms in arabica coffee to coffee berry disease (*Colletotrichum kahawae Sp. Nov*.)-A Review. Kenya Coffee **62 (737):** 2441-2444.

Masaba D M and Van der Vossen, H A M (1982). Evidence of cork barrier formation as s resistance mechanism to berry disease (*Colletotrichum coffeanum*) in arabica coffee. Neth. J. Pl. Path. **88**: 19-32.

Masaba D M and Waller, J M (1992). Coffee berry disease: the current status. In J A Bailey and M J Jeger *eds. Colletotrichum*: Biology, pathology and control. CAB International, Wallingford, UK. pp 237-249.

Mwang'ombe A W and Shanker, M (1994). Pre-penetration events and sporulation of *Colletotrichum kahawae* Waller and Bridge (Syn. *Colletotrichum coffeanum* Noack) on different cultivars of Coffea arabica. E. Afr. Agric. For. J. **59 (4):** 327-336.

Van der Vossen. H A M, Cook, R T A and Murakaru, G N W (1976). Breeding for resistance to coffee berry disease caused by *Colletotrichum coffeanum* Noack (sensu Hindorf) in *Coffea arabica* L I. Methods of pre-selection for resistance. Euphytica **25**: 733- 745.

Observations on the Life History of the Coffee White Stem Borer, *Monochamus leuconotus* Pascoe (Coleoptera, Cerambycidae): Adult Emergence Patterns in Chipinge, Zimbabwe

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SUMMARY

The coffee white stem borer, *Monochamus leuconotus* is an important pest of Arabica coffee in Zimbabwe. Stems are damaged through ring barking and wood boring activities by the larval stage of the borer. Control of the borer used to be achieved by applying persistent insecticides such as dieldrin and aldrin onto the stems. However, this is no longer feasible because of their adverse effects on the environment. Emergence of the adults was monitored at the Coffee Research Station, Chipinge, Zimbabwe during the period November to October 1996/97, 1998/99 and 1999/2000. It has been observed that emergence starts in November and ends in April. The peak emergence period is January while the least is in April. This implies that it is important to monitor for stem borer emergence during the rainy season (November to April). Control of the pest can safely be achieved by handpicking of adults. A more environmentally friendly way of controlling the pest would be handpicking of adults of adults and/or spraying the adult with a non-persistent insecticide during the flight period of the adult. More work still needs to be done to determine the relationship between rainfall amount and adult emergence.

INTRODUCTION

The coffee white stem borer, *Monochamus leuconotus* is a serious pest of arabica coffee in Southern Africa (Hillocks et. al., 1999; Schoeman and Pasques, 1993). In Zimbabwe it used to be perceived as a minor pest attacking poorly managed coffee (Clowes and Hill, 1981). However, the decline of coffee prices in the world markets in 1989 and the 1992 drought led to a neglect of coffee plantations. This increased plant stress leading to an upsurge in the population levels of the borer. Currently it is now a serious pest in both the large scale and smallholder sectors. In the smallholder sector it ranks as the most important insect pest (Kutywayo, Unpublished).

The damage caused by white borer is mainly due to the ring barking and wood boring activities of the larvae (Tapley, 1960; Schoeman et. al., 1998). The white borer problem has become even more serious because of the banning of Dieldrin and aldrin which used be applied onto the stems. Schoeman and Pasques (1993) reported on the seriousness of the pest in South Africa since the banning of dieldrin. Currently, the pest is controlled by preventative application of chlorpyrifos that has to be on the stem before adult emergence and subsequent oviposition. However, there is a problem in timing the insecticide applications onto the stem and in addition, stem banding is laborious.

Therefore, the broad objective of this study was to monitor the emergence pattern of the pest with specific aims to find the start of emergence and the length of the emergence period.

METHODS AND MATERIALS

The study was done over three farming seasons – 1996/97, 1998/1999 and 1999/2000 seasons. The Coffee Research Station in Zimbabwe was used as the study site. The numbers of white borer adults captured on a daily basis were recorded. These were then totalled and plotted against time for the respective seasons. The start and end of adult emergence was noted.

RESULTS

Emergences first appeared in December, peaked in January and ended in March for the 1996/97 season (Fig 1). The lowest numbers were recorded in March.

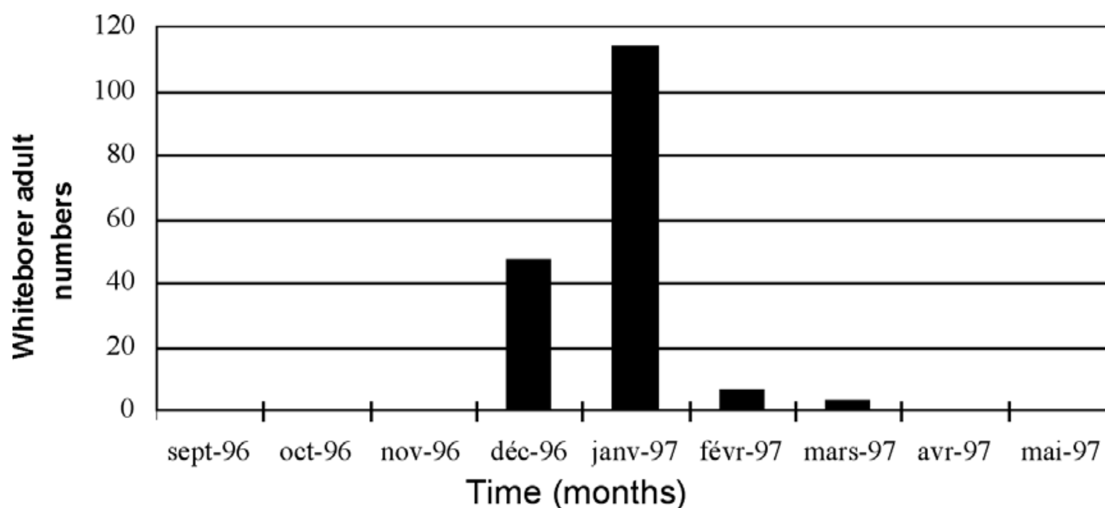

Figure 1. Whiteborer adult emergence pattern for the 1996/97 season

For the 1998/99 season, there were early emergences that occurred in late November (Figure 2). The peak was in January and emergence ended in April which had the lowest numbers.

Early emergences of late November were registered again for the 1999/2000 season (Figure 3). The peak was in January, with April being the last month of emergence as well as having the least numbers.

DISCUSSION

The new emergences seem to be induced by rain as the white borer adult distribution closely fits with the rainfall pattern. Studies carried out at Schoemansdal Coffee Estate in Mpumalanga Province, South Africa, during 1992-93 indicated that the main emergence period of adult beetles was during mid-December just after the first summer rains (Schoeman et. al., 1998). This eliminates reports of all year round emergences. Following these findings, stem banding has to be done well before the onset of rains to coincide with the emergence and oviposition occurring in late November. However, there is a need for a second application of an insecticide onto the stems to cater for the late emergences occurring in February and beyond. The currently used insecticide (Chlorpyrifos) has got short persistence.

The major emergence period is from November to April, with the latter being the last month for new emergences. It appears that the emergence period may shift with climate variations.

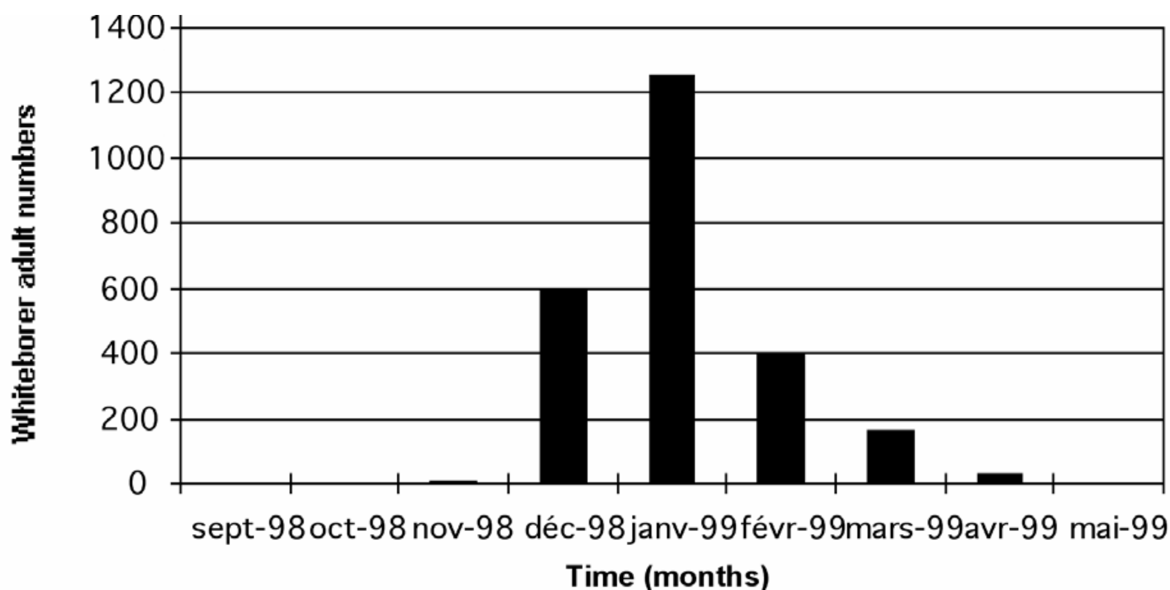

Figure 2. Whiteborer adult emergence pattern for the 1998/99 season

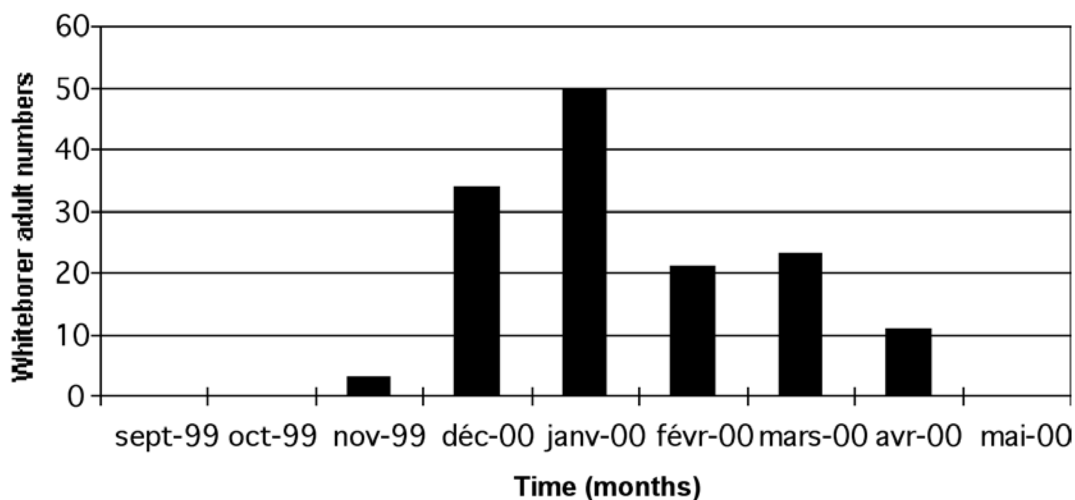

Figure 3. Whiteborer adult emergence pattern for the 1999/2000 season

Venkataramaiah and Rehiman, (1989) observed that the flight period of Xylotrechus *quadripes* Chev varied with rainfall patterns.

The occurrence of the peak emergence period in January was consistent for all the seasons studied. Schoeman and Pasques (1993) highlighted the importance of this period as a managerial tool in controlling the stem borer. Control could be achieved by handpicking of adults, which is environmentally friendly. There is also potential for using pheromones to lure the adults for control using the lure and kill or mating disruption techniques. Kutywayo (unpublished) conducted preliminary experiments on pheromones and found that there was some form of attraction between the male and female adults. However, more work still needs to be done to fully explore this potential. In addition, the relationship between adult emergence and rainfall needs to be studied.

REFERENCES

Clowes M. St. J. and Hill R. H. K. (1981) (eds). Coffee Handbook. 2nd edn Zimbabwe. Coffee growers Association 198 pp

Hillocks R. J., Phiri N. A. and Overfield D. (1999) Coffee pest and disease management options for smallholders in Malawi.

Schoeman P. S., Hamburg H. V. and Pasques B. P. (1998) The morphology and phenology of the white stem borer, *Monochamus leuconotus* (Pascoe) (Coleoptera: Cerambycidae), a pest of Arabica coffee.

Schoeman P. S. and Pasques B. (1993) Chlordane Stem Treatments to Combat White Coffee Stem Borer. SUBTROPICA **14** (5): 17-19

Schoeman P. S. and Pasques B. (1993) Chemicals Tested Against the White Coffee Stemborer. SUBTROPICA **252**: 16-18

Tapley R. G. 1960. The white stem borer Anthores leuconotus (Pasc) and its control. The East African Agricultural Journal **20**: 145-148.

Venkataramaiah G. H. and Rehiman M. U. (1989) Studies on the Control of White Stemborer of Coffee. Indian Coffee.

Some Cultural Characteristics and Pathogenicity of *Fusarium* Isolates from Fusarium Bark Disease on Coffee in Kenya

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SUMMARY

Two types of pathogenic *Fusarium* isolates with district cultural differences from those described for *Fusarium stilboides* Wollenw, the causal organism of Fusarium Bark Disease (FBD) of coffee in East Africa are increasingly being isolated from FBD cankers on coffee in Kenya. Their characteristics in culture and pathogenicity on mature coffee and seedling stems are illustrated. The possibility that FBD is caused by more than one type of *Fusarium stilboides* is discussed.

INTRODUCTION

Fusarium bark disease of coffee (FBD) caused by *Fusarium stilboides* is an important factor limiting arabica coffee production in the low and medium altitude coffee growing districts in Kenya. The symptoms of the disease were first noticed in Kenya in 1956 (Baker, 1970) but the presence of the fungus was not confirmed until 1964 when it was isolated from Taita Taveta district. The disease has also been reported in Madagascar (Dadant, 1960) Tanzania (Storey, 1932), Malawi (Siddiqi and Corbett, 1963) and Zimbabwe (Clowes and Logan, 1985). The symptoms of the disease have been fully described by Siddiqi and Corbett (1963). They include constrictions at bases of affected suckers and primary branches, cankers on the main stem especially around pruning wounds and primary branch bases and at or slightly above ground level on the main stem. The cankers enlarge to eventually girdle and affected region leading to death of distal portions. Other symptoms of minor importance include irregular dark brown yellow haloed leaf spots and sunken dark brown lesions on berries (Siddiqi and Corbett, 1963; Mignucci et al., 1985; Waller, 1993).

Cultures of *F. stilboides* in Potato Sucrose Agar have a characteristic carmine red pigmentation with white or pink floccose mycelium which becomes reddish brown with age (Booth, 1977). Sporulation is initially on aerial mycelium giving colonies a powdery appearance. Small scattered sporodochia are latter formed on agar surface. The fungus produces only macroconidia which are mostly 5-7 septate, thin walled and straight or slightly curved.

FBD continues to cause loss of production and investment to the Kenyan coffee farmer due to the need to frequently replace bushes lost to the disease but work to identify variations in culture and pathogenicity within the pathogen population have not been carried out. This was the aim of the present study.

MATERIALS AND METHODS

Culture media

Potato Sucrose Agar (PSA) and Coffee Leaf Extract Agar (CLEA) (coffee leaf - 250 g, agar - 20 g, water - 1 l) made by chopping and boiling clean coffee leaves in 500 mls water and sieving through fine sieve cloth, making up to 1 litre with water, adding agar and autoclaving at 15 psi for 20 min were used as growth media.

Isolations

Fusarium sp were isolated from coffee stems with characteristic cankers of FBD collected from three separate coffee growing areas i.e. Murang'a Makuyu and Meru during 1999 and 2000. Sections of the bark and woody tissue from the advancing edge of stem cankers were excised, surface sterilised in 70% alcohol and incubated on sterile glass slides enclosed in moist cottonwool lined plastic boxes for 5-6 days at room temperature. Each section was then put separately in 2 mls sterile distilled water in sterile tubes and agitated to suspend the spores. The suspension was observed for presence of macroconidia of *Fusarium sp.* and plated on coffee leaf extract agar. Plates were incubated at room temperature for 7 days and observed. Four apparently different cultures producing macroconidia of *Fusarium* were selected from each plate and transferred onto plates of PSA.

Colony Morphology and Growth

Colony morphology of isolates was assessed following growth on PSA for 10 days at room temperature. The mean colony diameter of three replicate cultures on PSA was measured after 4 days. Five isolates of each type were assessed for growth.

Pathogenicity

The pathogenicity of isolates was tested on stems of mature coffee and coffee seedlings. Inoculum was prepared by transferring single macroconidia from PSA cultures on to CLEA and incubating at room temperature for 7 days. Macroconidia were harvested by flooding plates with sterile distilled water and gently disturbing the agar surface with a sterile glass rod. Spore concentration was adjusted to 10^4 x 6 spores/ml. A spore suspension from each isolate was introduced onto 5 wounded mature and 5 wounded field size seedling stems. Inoculated portions were kept moist with aid of polythene sleeves moistened on the inside.

RESULTS

Isolations

A total of 126 isolates of *Fusarium sp* producing only macroconidia (42 from Murang'a, 54 from Makuyu and 30 from Meru) were obtained.

Morphology

Three types of cultures of *Fusarium* producing only macroconidia on PSA and coffee leaf extract agar were recognizable (Plate 1). Their appearance on PSA was as presented in Table 1.

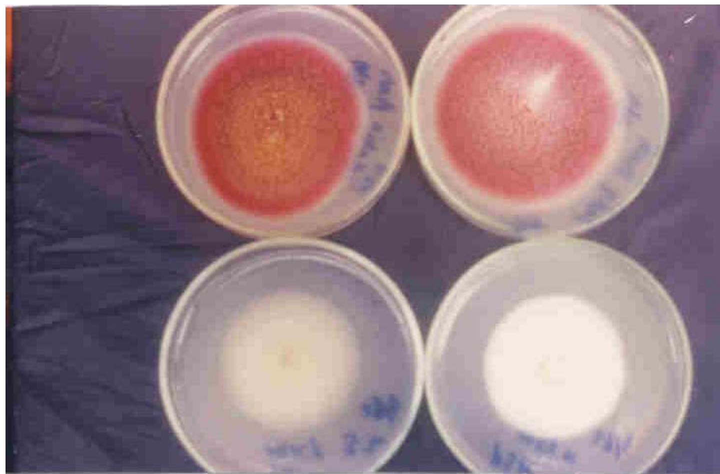

Plate 1. *Fusarium* isolates from FBD cankers

Table 1. Cultural characteristics of Fusarium isolates from FBD cankers

Type 1		
Pigmentation	-	Carmine red
Mycelium	-	Pink/White floccose
Growth rate	-	3.3cm/4 days
Macroconidia	-	Borne in sporodochia or formed from simple phialides, 3-7 septate, straight or slightly curved.
Type 2		
Pigmentation	-	Beige/white
Mycelium	-	White adpressed, non sporulating
Growth rate	-	2.5 cm/4 days
		Macroconidia - Formed in black hardened sporodochia scattered on agar surface in 10 days or older cultures. Irregularly shaped due to close packing in fruiting structures, 3-7 septate.
Type 3		
Pigmentation	-	Cream/light orange
Mycelium	-	Not visible, slimy appearance
Growth rate	-	2.4cm/4 days
		Macroconidia - Numerous, straight or slightly curved borne over whole agar surface, 3-7 septate.

Pathogenicity

All isolates tested were pathogenic to mature coffee and seedlings resulting in the characteristic sunken cankers (Plate 3). Type 3 isolates symptom progressed much faster girdling the seedling stems and killing them within 3 weeks. Type 1 isolates were relatively slow in developing symptoms. There was no disease symptom in all the uninoculated controls.

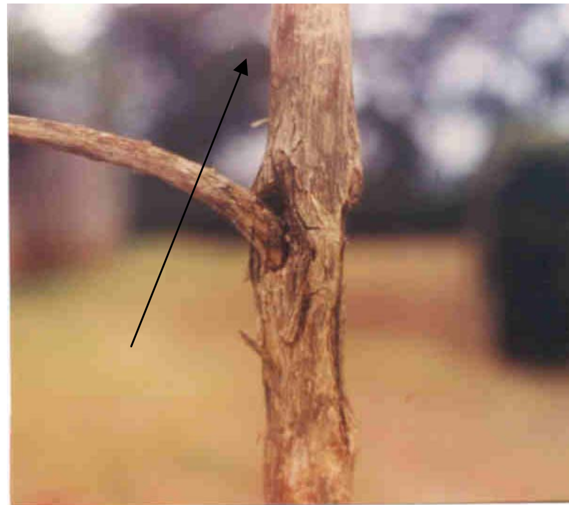

Plate 2. Field symptom of FBD

Plate 3. Artificial symptoms of FBD

DISCUSSION

Two previously undescribed types of *Fusarium stilboides* were isolated in this study. Type 2 characterized by white mycelial growth with macroconidial production restricted to hardened fruiting structures after 10 days growth on PSA was common to the three region sampled. Type 3 characterized by a slimy appearance, absence of aerial mycelium and prolific production of macroconidia was isolated from Makuyu and Murang'a. Its not being isolated

form Meru may be attributed to the smaller number of samples collected from the region. Both isolates were pathogenic on mature coffee and field age seedlings causing symptoms similar to those observed under field conditions (Plate 1). Successful inoculation on both mature stem and on seedlings was secured more easily and progressed at a faster rate with Type 3 isolate than with the typical cultures of *F. stilboides* (Type 1). The results suggested that more than one type of *Fusarium stilboides* are associated with FBD of coffee in Kenya.

ACKNOWLEDGEMENT

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REFERENCES

Baker C. J. (1970). Coffee Bark Disease in Kenya. Kenya Coffee, July 1970 226 pp.

Booth C. (1977) Fusarium. Laboratory guide to the identification of the Major Species. Commonwealth Mycological Institute.

Clowes M. St. J. and Logan W. J. C. (1985). A review of the current knowledge on Fusarium Bark Disease in Zimbabwe. In Advances in Coffee Management and Technology in Zimbabwe 1980 - 85. Cannon Press, Harare 176 pp.

Dadant R. (1960). Le dépérissement parasitaire du *Coffea arabica* sur les hauts plateaux de Madagascar. Agron. trop. Paris, **15**, 213-229.

Magnucci J. S., Hempperly P. R., Ballester J. and Rodriguez-Santiago O. (1985). Berry Anthracnose and Berry Disease of Coffee in Puerto Rico. Journal of Agriculture of University of Puerto Rico **69:** 1 pg 107-117.

Storey H. H. (1932). A Bark Disease of Coffee in East Africa. Ann. App. Biol. **19:** 173-184.

Siddiqi M. A. and Corbett D. C. M. (1963). Coffee Bark Disease in Nyasaland I: Pathogenicity, Description and Identity of the Causal Organism. Trans. Brit. Mycol. Soc. **46:** (1), 91-101

Waller J. M. (1993). Coffee Berry Disease: The continuing threat. Plant Protection Services I.M.I. No.15. In Shell Agriculture pg 32-37.

Further Evidence on the Occurrence of Coffee Berry Borer *Hypothenemus hampei* (FERR) in Ethiopia Significance for Ethiopia and the World

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SUMMARY

A survey carried out between 1997 and 1999 in the major coffee growing regions of Ethiopia has revealed that Coffee Berry Borer exists in all areas surveyed. Relatively higher population of CBB was observed on coffee growing at lower altitude. Except in few instances (less than 5%) it fed on dry coffee berries contrary to CBB existing in other parts of the world that is also to a large extent on green and red ripe cherries. This calls for proper identification of the pest. It is attacked by at least two unidentified parasitoids which if proven to be different from those existing in other parts of the world, then they can be used to combat CBB problem using a classical biological control approach, in areas of the world where it has become a limiting factor in coffee production.

INTRODUCTION

According to Million Abebe (1987) out of the over 45 species of arthropod pests recorded on Coffee in Ethiopia, there are only three Coffee Berry boring insects. They are Berry Moth *Prophantis smaragdina* (Butler), Berry Butterfly *Deudrox lorizona coffeae* and Berry worm *Cryptophlebia batrachopa* (Meyrick) that are all minor pests. With regard to the occurrence of the Coffee Berry Borer (CBB) there are conflicting reports made by a number of people who have visited various coffee growing areas of Ethiopia at different times and for various reasons. Samples of coffee berries from unspecified area in 1926, and from Sidamo, Amara and Eritrea in August of 1938 collected by Chiaramonte (1938), have not revealed any sign of the presence of coffee Berry Borer. Similarly Sylvian (1955) was not able to find Berry Borer himself but cites the result of unpublished information which claims the observation of damaged beans from Galla Sidamo and infested Berries from Arussi (the latter in 1939). Lejune (1958) and Greathead (1964) spent two years and seven weeks respectively in Ethiopia but both have not mentioned Berry Borer in their lists of Coffee pests. Davidson (1967) however, has observed damaged berries and live beetles on both drying trays and coffee bushes in the Southwestern Ethiopia (near Mizan Teferi) at altitudes of 1200 and 1600 m. In addition to this, samples of processed coffee containing beans showing damage characteristics of CBB was also found in Jimma area (1700-1800 m) and Shashemene (1600 m).

The coffee berry borer has not been of any concern to Ethiopia but in 1995 an unspecified degree of damage but very visible, was observed at Tepi coffee plantation, located at about 600 km. South West of Addis Ababa. This was obviously the first indication of an increase in the population of this pest the reason of which is not known. This sparked an interest to, at least, collect some information about this pest. A survey was therefore, initiated to find out its distribution in the country. This report summarizes the result of this survey carried out during the period 1997 to 1999 and covers some of the coffee growing areas in the South and the Southwest regions of Ethiopia.

MATERIALS AND METHODS

Dry Coffee berries were randomly collected from three localities in the south and six in Southwestern region of Ethiopia during the period 1997 to 1999. The number of berries collected depended upon the availability of berries that varied in this survey from 30 at Wonago testing site to 908 at Tepi state farm.

Then the number of berries attacked by berry borer were counted and recorded. The percentage damage was calculated to see the difference that exists among some of the locations particularly between locations of low and high altitudes. All the berries collected from each locality were then placed separately in glass cages and left for parasite emergence. The parasitoids were kept in vials, with proper label, for future identification.

RESULTS AND DISCUSSION

The number of damaged Coffee Berries collected from the various sites, are shown on Table 1. Generally, Coffee grown in the lower altitudes is more attacked than those grown in the higher altitudes.

Table 1. Number of coffee berries damaged by CBB at various locations in the South and South western Regions of Ethiopia

Date	Region	Sites	Altitude in m	No. Of Berries Collected	Damaged Berries**
Nov 20, 1997	South west	Bebeka State Farm	1000	789	391 (49)
Nov22, 1997	South west	Tepi State Farm	1200	908	388 (42)
Nov.23, 1997	South west	Tepi Research Sub-center	1200	579	424 (73)
Dec.01, 1997	South west	Gera Research Sub-center	1900	491	31 (6)
Feb.24, 1998	South west	Gera Research Sub-center	1900	520	50 (9)
Dec.17, 1997	South west	Jimma Research Center	1750	185	52 (28)
Feb.16, 1998	South west	Gera Research Sub-center	1900	196	91 (46)
Feb.24, 1998	South west	Goma II State Farm	1650	152	62
Feb.24, 1998	South west	Agaro Testing Site	1650	100	66
Feb.12, 1998	South	Awada Research sub-center	1750	66	9

***Numbers in parenthesis are percentage damaged coffee Berries.*

For example higher Berry borer damage was recorded at Tepi and Bebeka Coffee plantations that are situated at about 1200 and 1000 m asl respectively, than at Gera research sub center with an altitude of 1900 m. The pest was observed to exist at all locations. The identity of the parasitoids is not yet known but will be identified by experts abroad in the future. The result of this identification may have some practical significance for other coffee growing regions of the world where CBB is a problem and efforts are being made to control the pest using parasitoids and pathogens. The out come of this survey further strengthens previous findings that Coffee Berry Borer exists in the south and southwestern part of Ethiopia which are both the major coffee producing areas of the country.

ACKNOWLEDGEMENT

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REFERENCES

Chiaramonte A. 1938. Note di Entomologia Etiopica: l'Asseza di Stephanoderes hampei Ferr. nella Coltivazioni di Caffe. L'Agricoltura Coloniale 32:398 -399

Davidson A. 1967. The Occurrence of Coffee Berry Borer, Hypothenemus hampei (FERR.) in Ethiopia. A UNSF Report for the Project no. 200; Ethiopia

Greathead D. J. 1965. Draft Report on the Observation Made on Coffee Pests in Ethiopia. FAO coffee Mission of Ethiopia

Lejeune J. B. H 1958. Rapport au Gouvernement, Imperial d'Ethiopie sur la Production Cafeiere. Rapport No. 797, FAO, Rome

Million Abebe 1987. Insect pests of coffee with special emphasis on Antestia, *Antestiopsis intricata* in Ethiopia. Insect Sci., applic. 8,4-6: 977-980

Sylvian P. G. 1955 Some Observations on *Coffea arabica* L. in Ethiopia. Turrialba **5**: 37-53

Evaluation of Grafting and Nematicide Treatments for the Management of a Root-lesion Nematode, *Pratylenchus* sp., in *Coffea Arabica* L. Plantations in Guatemala

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SUMMARY

Root-lesion nematodes, *Pratylenchus* spp., are widely distributed on coffee in Guatemala, with a high negative impact on production. A field experiment was undertaken to assess damage by this parasite and to compare two control methods: grafting onto *Coffea canephora*, and nematicide treatments (terbufos). The experiment was carried out up to the third harvest (5 years). Nematode densities in the roots, coffee berry yield losses and plant mortality rates were highest on ungrafted *C. arabica*. This is the first clear experimental demonstration of the high degree of susceptibility of *C. arabica* to root-lesion nematodes in that country. Grafting onto *C. canephora* provided efficient control of nematode populations, and resulted in significantly much greater yields when compared to ungrafted plants. Nematicide treatments only suppressed nematode densities in the roots up to the second year after planting. As a consequence, plant mortality rates for ungrafted plants were significantly reduced. However, nematicide treatments did not result in significant yield increases, whether the plants were grafted or not. On grafted plants, a 10% increase in production was persistently observed over the three harvests when treated with nematicides, when compared with untreated plants. At the same time, this work provided evidence that shade may be a beneficial agronomic practice for controlling root-lesion nematodes. Analysis showed that grafting and nematicide treatments had no significant effects on bean size and the chemical quality of coffee beans, or on the organoleptic quality of the beverage. These results are important as grafting on *C. canephora* is increasingly being used to control root parasitism in Guatemala and in other countries of Central America.

RÉSUMÉ

Les nématodes des lésions, *Pratylenchus* sp., ont sur café au Guatemala, une large répartition géographique avec un impact important sur la production. Une expérimentation au champ a été menée pour évaluer les dégâts de ce parasite et comparer deux méthodes de lutte: le greffage sur *Coffea canephora* et les traitements nématicides (terbufos). Cette étude s'est déroulé jusqu'à la troisième récolte (5 ans). Les densités de populations de nématodes dans les racines, les pertes de production de café cerise ainsi que les taux de mortalité de plants étaient plus élevés dans les parcelles de *C. arabica* non greffé. C'est la première expérimentation permettant de démontrer clairement dans ce pays, le niveau élevé de sensibilité de *C. arabica* vis à vis d'un nématode des lésions. Le greffage sur *C. canephora* a permis un contrôle efficace des populations de nématodes se traduisant par une augmentation siginificative de la production en comparaison des plants non greffés. Les traitements nématicides n'ont prmis de réduire les densités de populations de nématodes dans les racines que jusqu'à la deuxième année après plantation. Cela a permis de réduire significativement les taux de mortalité des

plants non greffés. Toutefois, les traitements nématicides n'ont pas induit d'augmentation significative de la production tant pour les plants greffés que pour les plants de pied franc. Toutefois, pour les plants greffés, une tendance d'augmentation de 10% de la production, constante durant les trois récoltes, a pu être observée chez les plants traités en comparaison de ceux qui n'avaient pas été traités. En parallèle, cette étude a mis en évidence l'intérêt que peut présenter la pratique de l'ombrage pour la protection des caféiers contre les nématodes des lésions. Le greffage et les traitements nématicides n'ont pas eu d'effet significatif ni sur la granulométrie et qualité chimique des grains ni sur la qualité organoleptique de la boisson. Ces résultats revêtent une importance toute particulière dans le mesure où le greffage sur *C. canephora* est de plus en plus communément mis en pratique pour lutter contre le parasitisme radiculaire, au Guatemala et dans d'autres pays d'Amérique Centrale.

Key Words: chemical control, *Coffea* spp., coffee quality, grafting, Guatemala, pest management, *Pratylenchus*, root-lesion nematodes, terbufos.

INTRODUCTION

Root-lesion nematodes, *Pratylenchus* spp., are widely distributed on coffee in Guatemala and cause serious damage on this crop (Villain et al., 1999). A method of grafting *C. arabica* onto *C. canephora* was implemented in Guatemala by Reyna (1968) to control root parasitism. Its efficiency in specifically controlling root-lesion nematodes was evaluated and compared with that of nematicide treatments. Grafting onto *C. canephora* is increasingly being used in Guatemala and other countries of Central America. Since this region is keen to produce high quality coffees, this study was also aimed at evaluating the effects of grafting on *C. arabica* bean and beverage quality.

MATERIAL AND METHODS

Location

Southwest Guatemala, at an altitude of 900 m, in a 20-year-old *C. arabica* plantation infested with *Pratylenchus* sp. – identification is under way (Villain, 2000) – on volcanic soils (andisols). Average annual rainfall and temperature: 2900 mm (dry season from November to April) and 23°C respectively.

Cultivation

The *C. arabica* cultivar was Caturra. Planting in the field was carried out in June 1991 at a density of 5,000 plants per ha under shade trees (*Inga* sp.) spaced 8 m x 16 m apart.

Treatments

- Ungrafted without nematicide (control);
- Ungrafted with nematicide;
- Grafted without nematicide and
- Grafted with nematicide. Chemical treatments consisted of applications of terbufos 10 G twice a year (May and November), at a rate of 1 g per plant in the first 2 years and 2 g per plant thereafter.

Experimental design

Treatments replicated four times in a randomised complete block design. Each experimental plot contained 84 plants (seven rows). Observations were made on the 50 central plants.

RESULTS AND DISCUSSION

Pathogenicity of *Pratylenchus* sp.

C. arabica appeared highly susceptible and sensitive to this root-lesion nematode (Figure 1). Moreover, bean size was negatively correlated with nematode population densities ($r^2 = 0.62$; $P < 0.001$) for all plants (grafted or not and treated or not). The share of beans retained in 17/64"-aperture or larger sieves was reduced from 95% for the least infested plots to 65% for the most infested plots. Hence, the harm caused to coffee bean yields by *Pratylenchus* attacks may be not only quantitative but also qualitative.

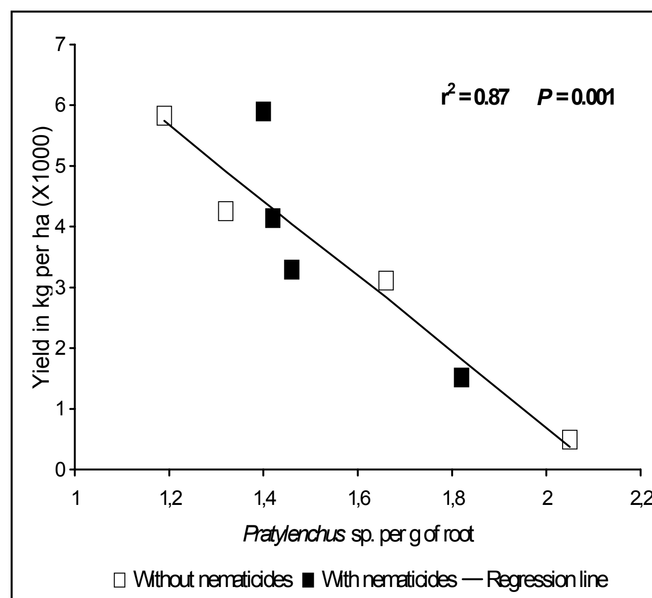

Figure 1. Relationship between average root-lesion nematode population density (mean of log[x+1] transformed nematode numbers per gram of roots from 1992 to 1995) and coffee berry yield at third harvest (1995) in plots of ungrafted *C. arabica*

Management of root-lesion nematodes

Nematicide treatments only suppressed nematode populations up to the second year after planting. This was sufficient to lower plant mortality rates in ungrafted plants (56% *vs.* 25%) but it did not result in significant yield increases, whether the plants were grafted or not (Figure 2).

Grafting onto *C. canephora* provided efficient control of nematode populations (< 16 per g of roots). This resulted in significantly lower plant mortality rates (6%) and much greater yields (Figure 2), whether the plants were treated or not. However, on grafted plants, a 10% increment in production was persistently observed over the three harvests when treated with nematicides in comparison with the untreated plants.

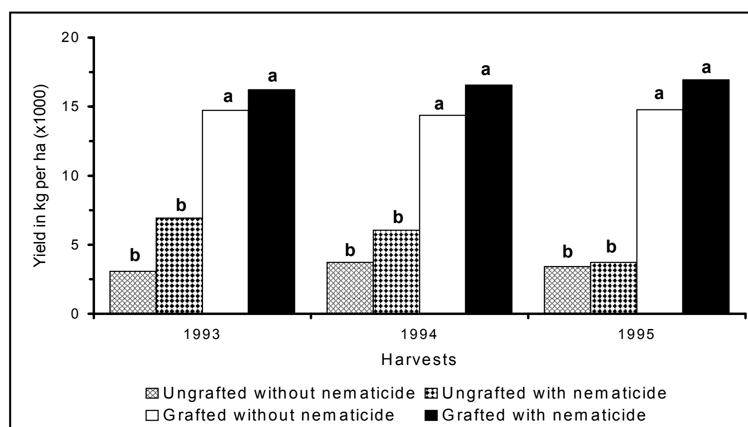

Figure 2. Average coffee berry yields of the four treatments, two, three and four years after planting. Bars with the same letter are not different according to the Newman and Keuls test at *P* = 0.05

Shade suppressed nematode populations on grafted plants. This could be in relation to a tendency for grafted plants to yield less with increased shade (Villain et al., 2000) in accordance with the already known negative effect of shade on yield when conditions are adequate (Cannell, 1985). Hence, this cultural practice may promote incomplete resistance to *Pratylenchus* through the plant's trophic status, as it regulates fruit yielding.

Effects of grafting and nematicide treatments on bean and beverage quality

Among the organic components of green beans, only sugar concentration at harvest 93 was significantly different between grafted and ungrafted plants (Table 1). There was no significant difference for caffeine, trigonelline, chlorogenic acids and lipids. Results for caffeine are in accordance with observations in Brazil of no rootstock effect on bean caffeine concentration, whatever the genotypes of both graft and rootstock (Melo et al., 1976).

Table 1. Effect of grafting onto *C. canephora* and nematicide treatments on concentrations of organic components in green coffee beans (%)

COMPONENT		Caffeine		Trigonelline		Chlorogenic acid		Sugars		Lipids
Harvest		93	95	93	95	93	95	93	95	95
Ungrafted	Without nematicide	1.23	1.05	1.07	0.99	7.45	7.01	**8.20 b**	7.05	13.95
	With nematicide	1.24	1.05	1.06	0.98	7.34	7.10	**8.09 b**	6.85	13.88
Grafted	Without nematicide	1.22	1.03	1.04	0.96	7.38	6.84	**8.64 a**	6.83	13.68
	With nematicide	1.21	1.06	1.03	0.94	7.25	7.05	**8.64 a**	6.85	13.48

Averages in columns without letters are not significantly different (P # 0, 05). Averages in the column with the same letter (bold type) do not differ according to the Newman and Keuls test (P # 0, 05).

Roasting results showed there was no significant difference for weight loss (Δ = 17.2%) or volume increase (Δ = 69.9%) of beans either between grafted and ungrafted plants or between plants with and without nematicide. The only significant differences detected for the organoleptic characteristics of the beverage were: 1) for harshness at harvest 95, with higher

values for ungrafted plants; 2) for the global preference score at harvest 93 with larger values for treated than for untreated plants. This could be related to the partial control of nematode populations obtained at the begining of the experiment in treated plots. There was no difference for the other characteristics evaluated: body, acidity, bitterness, astringency and preference (harvests 93 and 95) as well as aroma, sourness, metallic flavour and global preference score (harvest 95). On the whole, there were therefore no major and persistent differences in bean and beverage quality between grafted and ungrafted plants. However, the few differences observed, particulary for sugars, a key component of berry maturity and the most important aroma-related agent in the beverage (Clifford, 1985), need to be confirmed with different rootstock genotypes and different agronomical and ecological conditions. The increase in sugar concentration due to grafting onto *C. canephora* might result from the stronger physiological status of such grated plants.

CONCLUSIONS

The studied root-lesion nematode is very harmful on *C. arabica*. Grafting onto *C. canephora* appears to be an effective way of controlling this parasite, without compromising bean quality. This agricultural practice could therefore be useful in controlling complex phytoparasitic nematode communities in coffee as its merits for controlling highly pathogenic root-knot nematode populations has also been demonstrated (Bertrand et al., 2000). On the other hand, nematicide treatments did not provide effective protection for ungrafted *C. arabica* plants. Decision to apply or not nematicide treatments on grafted plants at planting in field should take into account local conditions, especially nematode communities.

REFERENCES

Bertrand B., Peña-Duran M. X., Anzueto F., Cilas C., Etienne H., Anthony F., Eskes A. 2000. Genetic study of *Coffea canephora* coffee tree resistance to *Meloidogyne incognita* nematodes in Guatemala and *Meloidogyne* sp. nematodes in El salvador for selection of rootstock varieties in Central America. Euphytica 113: 79-86.

Cannell M. G. R. 1985. Physiology of the coffee crop. In: Coffee: botany, biochemistry and production of beans and beverage, Clifford M. N. and Willson K. C. éd., New York, USA, Croom Helm, p. 108-134.

Clifford M. N. 1985. Chemical and physical aspects of green coffee and coffee products. In: Coffee: botany, biochemistry and production of beans and beverage, Clifford M. N. and Willson K. C. éd., New York, USA, Croom Helm, p. 108-134.

Melo M., Carvalho A., Monaco L.C. 1976. Contribução do porta-enxerto no teor de cafein em grãos de café. Bragantia **35** (5): 55-61.

Reyna E. H. 1968. La técnica de injerto hipocotiledonar del cafeto para el control de nematodos. Café (Lima) **17** (1): 5-11.

Villain L. 2000. Caractérisation et bioécologie du complexe parasitaire du genre *Pratylenchus* (Nemata: Pratylenchidae) présent sur caféiers (*Coffea* spp.) au Guatemala. Thèse de Doctorat. ENSAR, Rennes, France, 182 p.

Villain B., Anzueto F., Hernandez A., Sarah J.L. 1999. Los nematodos parásitos del cafeto. In: Desafíos de la caficultura en Centroamérica, Bertrand B. et Rapidel B. éd., San Jose, Costa Rica, ICCA-PROMECAFE, p. 327-368.

Villain L., Molina A., Sierra S., Decazy B., Sarah J. L. 2000. Effect of grafting and nematicide treatments on damage by root-lesion nematodes (*Pratylenchus* spp.) to *Coffea arabica* L. in Guatemala. Nematropica **30** (1): 87-100.

Le scolyte des fruits du caféier (*Hypothenemus hampei* Ferr.) au Togo: Etat actuel et perspectives

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RÉSUMÉ

Le scolyte des fruits du caféier est un important ravageur de par le monde. La lutte chimique contre cet insecte est difficile et est souvent freinée par des problèmes de résistance et des résidus insecticides dans le grain consommé. Le problème du scolyte date de très longtemps au Togo mais les études sur le ravageur n'ont débuté qu'en 1987. A partir de cette année, divers travaux ont permis de mieux connaître ce déprédateur. Les taux d'attaque atteignent 19% sur les plateaux et les pertes de production y sont évaluées à plus de 90 Kg/ha. Ces mêmes travaux ont permis de préciser le seuil de dégât économique avec utilisation de l'endosulfan. Dans les plantations, deux principaux parasitoïdes ont été répertoriés (*Cephalonomia stephanoderis* Betrem et *Phymastichus coffea* La Salle). Des méthodes d'élevage permettent de produire ces insectes au laboratoire. L'étude de leur efficacité a été abordée et a montré qu'il existerait un effet synergique des deux parasitoïdes pour la réduction des populations du ravageur. De nouvelles études sont actuellement initiées pour déterminer dans les plantations l'influence des facteurs climatiques et agronomiques sur les variations d'abondance des parasitoïdes dans les différentes zones de production.

Mots clé: Caféier, *Hypothenemus hampei, Cephalonomia stephanoderis, Phymastichus coffea*, Togo.

INTRODUCTION

Le scolyte des fruits du caféier *Hypothenemus hampei* est reconnu comme le ravageur le plus important dans les caféières. Les dégâts causés par cet insecte séminivore varient suivant les pays. Ainsi, son impact sur la production est moindre dans les pays d'Afrique d'où il est originaire mais très important en Amérique du Sud et du Centre où il est introduit de façon accidentelle (Morallo-Rejesus et Baldos, 1980; Baker, 1984; Barrera, 1992; Decazy, 1989).

Au Togo, le scolyte des fruits est présent dans les caféières depuis que le café est introduit vers 1930. Le problème n'est devenu important qu'avec l'intensification de la culture mais aucune mesure n'était prise pour lutter contre le ravageur. Les premières investigations de Borbon-Martinez (1989) ont montré que le problème n'était pas à négliger. Dès lors, plusieurs projets ont été mis sur pied pour connaître un peu plus ce ravageur (Wegbe, 1990; Brun, 1992; Feldhege, 1992; Wegbe, 1997).

L'objectif de cette communication est d'une part de faire le point des différents travaux réalisés sur le ravageur et son cortège parasitaire au Togo et d'autre part de présenter les perspectives pour mettre au point une lutte biologique ou intégrée prenant en compte non seulement les potentialités biotiques des parasitoïdes, mais aussi l'influence des facteurs climatiques et agronomiques dans les caféières.

TRAVAUX RÉALISÉS

Biologie du ravageur

La biologie du scolyte des fruits du caféier a été étudiée par Borbon-Martinez (1989).

Le cycle de développement du déprédateur est intimement lié à la phénologie du caféier.

Aire de dispersion, et importance

Les prospections réalisées dans toute la région caféicole du Togo en 1990 ont montré que le scolyte est présent dans toutes les zones agroclimatiques mais à des degrés différents. Les zones les plus attaquées ont été celles des plateaux de Dayes et d'Akposso-Akébou où les taux d'attaque dépassent quelque fois 19%. Les zones de plaine constituées par Agou, Kpalimé, Kpélé et Amou sont moins attaquées. Les taux d'attaque ne dépassent guère 8% dans ces zones. Cette différence est due à la l'humidité relative généralement élevée sur les plateaux, situation qui est propice à la multiplication des scolytes même en intercampagne. Dans les zones de plaines, la rigueur de la saison sèche en période de post récolte est un facteur défavorable au développement des scolytes.

L'estimation des pertes a été réalisée en adoptant une méthode d'échantillonnage prenant en compte les fruits scolytés tombés. Au cours de ces travaux, 50 caféiers ont été choisis au hasard dans une parcelle d'environ un hectare. Sur chaque caféier, un rameau fructifère a été retenu de façon aléatoire. Sous ce rameau, une gouttière en grillage moustiquaire été accrochée de façon a récupérer les fruits scolytés qui tombent entre deux observations. Ce mode d'échantillonnage a permis de connaître les taux d'attaque et les pertes réels dans les caféières. Au total, 15 parcelles à raison de 3 par zone agroclimatique définie ont été étudiées. Ces travaux ont montré aussi que les pertes sont fonction des taux d'attaque.

Seuil de dégât économique

La détermination du seuil de dégât économique a également été l'une de nos préoccupations. En 1997, il a été estimé à 2,3% trois , quatre mois après la floraison représentative. C'est-à-dire 3-4 mois après la grande floraison, si le taux d'attaque atteint ou dépasse 2,3%, la parcelle échantillonnée doit être traitée sinon, les pertes à la récolte seraient supérieures au coût du traitement insecticide. L'insecticide utilisé pour faire les calculs a été l'endosulfan. Le seuil de dégât économique étant une valeur qui varie en fonction du coût du traitement insecticide, du prix du kilogramme de café marchand et du rendement, il est important de l'actualiser chaque fois que l'un de ces facteurs varie (Wegbe, 1997).

Lutte chimique

En général, les planteurs togolais ne traitent pas leurs plantations contre le scolyte des fruits du caféier, mais en cas de pullulations massives, nous recommandons un à deux traitements à l'endosulfan par campagne. Dans les zones de plaines, une seule application suffit pour contrôler efficacement les attaques du scolyte. Les applications sont réalisées les après-midis, moment du vol colonisateur du ravageur à raison de 40 litres de bouillie par hectare.

Dans le souci de disposer d'une gamme d'insecticides à utiliser contre le scolyte pour éviter les phénomènes de résistance liés à l'utilisation répétée de l'endosulfan, chaque année, de nouveaux insecticides sont testés. Les différents insecticides essayés sont des groupes des organophosphorés et des pyréthrinoïdes. Mais il faut signaler qu'aucun des produits essayés

n'a donné de résultats satisfaisants. L'endosulfan a été toujours en tête dans le contrôle des scolytes.

La lutte chimique bien que permettant de limiter les dégâts de scolyte présente beaucoup d'inconvénients pour le planteur et pour l'environnement. Pour contourner ces inconvénients et produire du café à moindre coût et le plus naturellement possible, les travaux ont porté ces derniers temps sur l'utilisation d'ennemis naturels contre le ravageur.

Ennemis naturels du scolyte des fruits du caféier

Plusieurs ennemis naturels ont été répertoriés dans les caféières. Il s'agit d'un entomophyte et de trois hyménoptères parasitoïdes.

Beauveria Bassiana (Bals.) Vuil.

Ce champignon est très fréquent dans les plantations et attaque le scolyte par temps humide. Les insectes infectés se reconnaissent facilement à la moisissure blanche d'aspect velouté qui recouvre leur abdomen après leur mort.

Les travaux initiés sur ce champignon cosmopolite par en vue de déterminer les souches les plus efficientes sont en veilleuse.

Les hyménoptères parasitoïdes

Cephalonomia stephanoderis Betrem (Hymenoptera: Bethylidae) et *Prorops nasuta* Waterson (Hymenoptera : Bethylidae) sont tous deux des ectoparsitoïdes. Ces deux parasitoïdes se ressemblent du point de vue morphologique. La différence ne réside qu'au niveau de leurs têtes; *P. nasuta* a la tête terminée par une protubérance alors que *C. stephanoderis* a une tête arrondie.

Du point de vue biologique, les deux insectes ont des comportements parasitaires identiques. Les femelles déposent leurs œufs sur les larves de derniers stades et les nymphes du scolyte. Leurs cycles de développement sont également comparables (19 à 21 jours à 27,5°C) (Wegbe, 1990). *C. stephanoderis* est le plus abondant et est présent dans toutes les zones de production.

Phymastichus coffea, LaSalle, un nouveau parasitoïde est découvert pour la première fois au Togo par Borbon-Martinez (1989) et décrit par LaSalle (1990) comme un nouveau genre et une nouvelle espèce. C'est un endoparasitoïde des stades adultes. La femelle pond ses œufs directement dans le scolyte adulte à l'aide de son ovipositeur. Le scolyte parasité peut vivre encore 2 ou 3 jours et pénétrer dans le fruit. Le cycle complet du parasitoïde dure environ 30 jours à 27°C. Un *P. coffea* peut parasiter entre 4 et 7 adultes (Feldhege, 1992).

Les premiers parasitoïdes introduits dans les pays de l'Amérique du Sud et du Centre proviennent du Togo après une quarantaine à l'IIBC en Angleterre. Cette introduction a permis de réaliser d'importants travaux de lutte biologique au Mexique (Barrera, 1994).

Aujourd'hui, l'élevage de *Cephalonomia stephanoderis* est un acquis. Le parsitoïde peut être élevé en masse pour réaliser des lâchers. L'élevage de *P. coffea* est mis au point en utilisant un système de deux boîtes dont l'une contient de l'eau pour humidifier l'intérieur de la deuxième contenant les fruits et les scolytes parasités par *P. coffea*. Le rendement d'élevage a atteint 2,8. Ce système devrait être perfectionné afin d'augmenter le rendement d'élevage.

Des travaux préliminaires effectués au laboratoire ont révélé une synergie des actions des deux parasitoïdes.

Les travaux réalisés en 1992 ont montré la possibilité d'une lutte intégrée utilisant l'endosulfan et les parasitoïdes. En effet, au cours de ces études réalisées au laboratoire et en champ, il s'est avéré que l'endosulfan tuerait le scolyte mais ménagerait les deux principaux parasitoïdes (*C. stephanoderis* et *P. coffea*).

Les nombreux résultats obtenus permettent aujourd'hui de recommander une lutte biologique contre le scolyte des fruits du caféier cependant, plusieurs points d'ombre restent encore à éclaircir pour rendre plus efficace cette forme de lutte.

PERSPECTIVES

Pour trouver des informations complémentaires afin de mener une lutte biologique efficace, de nouveaux travaux sont mis en route et portent sur:

- la compétition entre les deux principaux parasitoïdes *C. stephanoderis* et *P.coffea*;
- l'influence de certains facteurs (agronomiques, climatiques…) sur la dynamique des populations du scolyte et de ses parasitoïdes;
- l'utilisation du piège à scolyte comme moyen d'estimation des attaques du scolyte.

DISCUSSION ET CONCLUSION

Plusieurs années de recherche ont permis de connaître un peu plus le ravageur et ses ennemis naturels. Il est possible aujourd'hui d'élever au laboratoire les deux principaux parasitoïdes *C. stephanoderis* et *P.coffea*. Les résultats préliminaires obtenus sur l'impact de ces parasitoïdes sur les populations du scolyte révèlent une synergie de leurs effets. Dans les pays d'Amérique Centrale et du Sud où le ravageur a été introduit de façon accidentelle, l'introduction de l'un des parasitoïdes *C. stephanoderis* n'a pas permis de réduire de façon significative les attaques du scolyte. Il serait nécessaire d'introduire dans ces pays les deux parasitoïdes pour optimiser les résultats.

Un des compléments nécessaires à la lutte contre le scolyte des fruits du caféier serait l'utilisation des pièges à scolytes mis au point au Salvador. Cependant, la spécificité du piège est à vérifier auparavant.

Dans tous les cas, certaines caractéristiques des plantations influent sur la dynamique des populations des parasitoïdes et de leur hôte. La connaissance des relations entre les facteurs climatiques et agronomiques des plantations et les variations d'abondance du ravageur et ses parasitoïdes permettra de proposer des stratégies de lutte différenciées selon les contextes.

RÉFÉRENCES

Barrera J. F., 1994. Dynamique des populations du scolyte des fruits du caféier, *Hypothenemus hampei* (Coleoptera: Scolytidae) et lutte biologique avec le parasitoïde *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae) au Chiapas, Mexique.Thèse de Doctorat de l'Université Paul Sabatier de Toulouse, France, 301 p.

Borbon-Martinez O. 1989.Bioécologie d'un ravageur des baies de caféier, *Hypothenemus hampei* Ferr.(Coleoptera:scolytidae) et de ses parasitoïdes au Togo. Thèse de Doctorat de l'Université Paul Sabatier de Toulouse, France, 185 p.

Damon A. 2000. A review of the biology and control of the coffee berry borer, Hypothenemus hampei Ferr. (Coleoptera: Scolytidae). Bulletin of Entomological Research. **90**. 453-465.

Decazy B. 1989. Le scolyte du fruit du caféier, *Hypothenemus hampei* Ferr. Considération sur la lutte intégrée contre ce ravageur. In: 13ème Colloque de l'Association Scientifique Internationale du Café. Paipa (Colombie) 21-25 août 1989, p. 655-665.

Feldhege M. R. 1992. Rearing tecniques and aspects of biology of *Phymastichus coffea* (Hymenoptera: Eulophidae) a recently described parasitoid of the coffee berry borer *Hypothenemus hampei* (Coloeoptera: Scolytidae). In Café Cacao Thé, **36**: 45-54.

LaSalle J. 1990. A new genus and species of Tetrastichidae (Hymenoptera : Eulophidae) parasitic on the coffe berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: scolytidae). Bulletin of Entomological Research, **80** (1): 7-10.

Morallo-Rejesus B., Baldos E. 1980.The biology of coffee berry borer *Hypothenemus hampei* (Ferr.) (Scolytidae: Coleoptera) and its incidence in the southern Tagolog provinces Philipp. Entomol., **4**: 303-316.

Wegbe K. 1990. Etude de la biologie de *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae) parasitoïde du scolyte des baies de caféier. Mémoire Ingénieur Agronome, option: Production Végétale, Université du Bénin, Ecole Supérieure d'Agronomie, Lomé, Togo. 54 pp.

Wegbe K., Decazy B., Cilas C., Djiekpor E. K. 1997. Le scolyte des fruits du caféier, Hypothenemus hampei Ferr. (Coleoptera: Scolytidae) au Togo. Méthodes d'estimation du niveau d'infestation, des pertes et du seuil de dégât économique. 17ème Colloque de l'Association Scientifique Internationale du Café. Nairobi (Kenya) p. 639-645.

Options for Durable Resistance to *Hemileia vastatrix*, the Causal Agent of Coffee Leaf Rust

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SUMMARY

Coffee Leaf Rust (CLR) caused by *Hemileia vastratrix* is endemic to most coffee growing countries world wide. Although genetic resistance to *H. vastratrix* has been known since early 1930s, efforts to obtain durable resistance were frustrated by proliferation of races of the fungus. To-date upto 39 races of the fungus have been reported in literature. Most of the races were compatible with the major SH genes of *Coffea arabica* origin. The observation that major genes derived from *C. canephora* confered resistance to most races of coffee rust shifted breeding priorities towards canephora derivatives. This important source of resistance could be enhanced if it operates in a minor gene or oligogenic background. In this paper, possible minor gene or oligogenic resistance has been reported and its deployment in the Kenyan commercial cultivar Ruiru 11 already carrying major gene resistance is discussed. The resistance is expected to be durable in nature.

INTRODUCTION

Coffee Leaf Rust (CLR) is a serious disease of the foliage causing premature leaf-fall, yield loss and even death of the tree under severe epiphytotics. The disease is distributed in most of the coffee growing countries of the world with higher incidence in the lower altitudes. With diminishing coffee prices and escalating costs of production, the use of inorganic fungicides to control coffee diseases is declining. A more economical and sustainable control is through breeding and selection for resistant varieties. Resistant cultivars so far developed carry major genes designated SH. The genes SH_1, SH_2, SH_4 and SH_5 are of *C. arabica* origin while SH_3 is derived from a natural cross between *C. liberia* and *C. arabica*. The genes SH_6- SH_9 are carried by Hibrido de Timor, an interspecific hybrid between *C. canephora* and *C. arabica*. Resistance derived from *C. arabica* are easily matched by the corresponding rust races (Eskes, 1983). The genes of interest are those derived from *C. canephora* which confer resistance to most common races. The physiologic group of genes (designated group A) occur in Hibrido de Timor and its hybrid derivative Catimor. Although group A varieties have not succumbed to rust, other physiologic groups of canephora origin, R(SH_6), 1($SH_{5,6,7,9}$), 2($SH_{5,8}$), and 3($SH_{5,6,9}$) have compatible rust races (Rodrigues et al., 1993). The work done at Coffee Rust Research Centre (CIFC) in Portugal indicates that there are a total of 39 physiologic races of rust already detected (Rodrigues et al., 1993). With the proliferation of the rust races, the few major genes which are still effective against most races of the fungus are continuously being threatened. In this paper, possible minor gene resistance is elucidated and its deployment in a commercial cultivar with major gene resistance is discussed.

DETERMINATION OF QUANTITATIVE NATURE OF RUST RESISTANCE IN RUME SUDAN VARIETY

Five components of resistance including infection %, days to infection, sporulation %, days to sporulation and days to 50% sporulation were recorded on the parental, F1, F2 and backcross

generations of a cross between Rume Sudan variety (Resistant) and SL 28 variety (Susceptible). The leaf disc inoculation procedure described by Eskes (1983) and van Dongen (1979) was used for disease screening. The means of parents, F1 and F2 generations were used to compute the mid-parent value (m), the additive (d) and the dominance (h) components to determine the nature of gene action. The degree of dominance was calculated as h/d (Allard 1960) while broad sense heritabilities were computed according Allard (1960) and Simmonds (1979).

The number of segregating genes were estimated according to Lande (1981) and Wrights (1968). The estimation was based on the assumption that, the genes are in one parent only, not linked, have equal effects, have equal degree of dominance, act in the same direction and that interaction components are not important. The additive (d), dominance (h) degree of dominance (h/d) and scaling tests were used to determine the validity of the assumptions. Estimates of number of genes can be heavily biased if the assumptions are not met. Individuals scaling tests were performed on two components of resistance (infection % and sporulation %) which had high heritabilities and therefore useful in selection for resistance to rust. The scaling tests were performed by determining the deviation from zero of four equations; a = 2 x B1-P1-F1; b = 2 x B2-P2-F1, c = 4 x F2-2x F1-P1-P2, d = 2 x F2-B1-B2 where P1 = mean of susceptible parent (1), P2 = mean of resistant parent (2), F1 = mean of F1 cross, F2 = mean of F2 generation, B 1 = mean of backcross generation to parent 1 and B2 = mean of backcross generation to parent 2. Incase of absence of interaction components (epistasis), the equations should equal to zero (Mather and Jinks, 1982).

RESULTS AND DISCUSSION

Broad sense heritability estimates indicated that infection % and sporulation % were controlled by strong genetic effects (Table 1).

Table 1. Midparent values (m), additive (d), dominance (h), degree of dominance (h/d) and broad sense heritabilities of five components of resistance to leaf rust

Resistance component	Broad sence heritability[1]		Midparent value (m) (P1+P2)/2	Additive value (d) (P1-m)	Dominance value (h) (F1-M)	Degree of dominanc e (h/d)
	A	S				
Infection %	0.73	0.46	42.34	–15.67	12.22	–0.78
Days of infection	0	0.07	18.39	3.94	–4.39	–1.11
Sporulation %	0.74	0.38	32.45	–20.85	15.88	–0.76
Days to sporulation	0	0	26.84	7.72	–5.95	–0.77
Days to 50% sporulation	0	0	32.22	8.22	–2.22	–0.27

[1]Broad sense heritability was computed according to h^2 = (VF2-VE)/VF2 with column A:VE =½ (VPI + VP2) (Allard, 1960) and Column S: VE = 1/3 (VP1 + VP2 +VF1) (Simmonds, 1979)

The components are therefore suitable for selection since high heritabilities are indicative of high rates of success in recovering desired genes in future generations. The remaining components were omitted from further analyses. The degree of dominance was –0.78 for infection % and –0.76 for sporulation % indicating that the genes were recessive in inheritance (negative sign) with partial or small effects (>–1.0) which act by reducing

infection and sporulation. Individual scaling tests for the two important components of resistance revealed that all the terms did not differ significantly from zero (Table 2).

Table 2. Results of individual scaling tests for infection % and Sporulation %

Scaling Test	Infection %		Sporulation %	
	Value	Deviation from Zero	Value	Deviation from Zero
a=2 x B1-P1-F1	–25.0± 63.0	Not significant	–27.6±64.0	Not significant
b=2 x B2-P2-F1	18.1± 60.0	Not significant	5.3±59.3	Not significant
c=4xF2-2xF1-P1-P2	7.5± 55.0	Not significant	6.8±135.0	Not significant
d=2xF2-B1-B2	7.1± 65.7	Not significant	14.4±68.0	Not significant

This indicates that only additive and dominance effects contribute to the gene action. The assumption that there was no interaction effect is therefore valid. To large extent, the remaining assumptions were also met.

The number of genes in the resistant Rume Sudan parent were calculated using four different methods in Table 3 subscript. Using method one derived from Lande (1981), one to seven genes were estimated for infection % and one to six genes for sporulation % (Table 3). Methods 2, 3 and 4 derived from Wright (1968) estimated one gene for infection % and one to six genes for sporulation %. These results therefore confirm that there could be upto seven (7) genes with incomplete recessive inheritance controlling resistance to leaf rust in Rume Sudan

Table 3. Number of independently segregating genes controlling rust infection and sporulation in Rume Sudan x SL 28 cross

Method	Infection %		Sporulation %	
	Value	Estimate	Value	Estimate
1 (a)	2.64	3	0.76	1
(b)	0.52	1	1.23	2
(c)	6.46	7	0.56	1
(d)	0.27	1	5.98	6
2	0.68	1	1.65	2
3	0.56	1	5.77	6
4	0.01	1	0.02	1

Method 1: (a): $N = (P2\text{-}P1)^2/8\ (VF2\text{-}VF1)$ (b) $N = (P2\text{-}P1)^2/8\ (VF2\text{-}[VF1/2+VP1/4+VP2/4])$, (c): $N = (P2\text{-}P1)^2/8\ (2VF2\text{-}VB1\text{-}VB2)$, (d) $N = (P2\text{-}P1)^2/8\ (VB1+VB2\text{-}[VFI+VP1/2+VP2/2])$ where, VP1 = Variance of P1, VP2 = Variance of P2,VF1 = Variance of F1, VF2 = Variance of F2, VB1 = Variance of B1 and VB2 = Variance of B2.

Method 2: $N = (P2\text{-}P1)^2(1.5\text{-}2h(1\text{-}h)/8(VF2\text{-}VE)$ where P2 is the average of the resistant parent, P1 is the average of thesusceptible parent, h = (F1-P2)/(P2-P1), VE = ¼(VPI+VP2+2VF1)

Method 3: D = (F1-P1)2/4 (VB1-VE), Where D is the number of genes by which F1 differs from the resistant patent, VE = 0.5 (VF1+VP2)

Methods 4: $D = (F1-P1)^2/4\ (VB1-VE)$, where D is the number of genes by which F1 differs from the susceptible parent, VE = 0.5 (VFI-VP1

Durable resistance to leaf rust may be achieved by combining in one variety, the major gene resistance present in Hibrido de Timor and its derivatives like Catimor with minor gene resistance available in Rume Sudan. The Ruiru 11 cultivar developed at the Coffee Research Foundation in Kenya has both Catimor and Rume Sudan in its pedigree. It is suggested that one generation of selfing and selection for highly resistant types may fix the recessive rust resistance genes. These genes will provide possible supplementary resistance to major genes derived from the catimor parents. All other desirable characters of Ruiru 11 will be maintained in the cultivar during the selection process.

REFERENCES

Allard R. W., 1960. Principles of Plant Breeding. Wiley, New York and London.

Dongen L. L. Van, 1979. Selection for horizontal resistance to Coffee rust. CRS, Ruiru Mimeo 12 p 14 Tables, 4 figs.

Eskes A. B., 1983. Incomplete resistant to Coffee Leaf rust (*Hemileia vastatrix*). PhD Thesis, Wageningen University, The Netherlands.

Lande R., 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99: 541-553.

Mather K. and Jinks J. L., 1982. Biometrical genetics. The study of continues variation. Third edition. University press. Cambridge.

Rodrigues Jr. C. J., Varzea V. M. P., Godinho I. L., Palma S., Rato R. C., 1993. New physiologic races of *Hemileia vastatrix*. 15 ASIC Colloquium, Montpellier, France. pp 318-321.

Simmonds N. W., 1979. Principles of crop improvement. Longman, London Limited.

Wright S., 1968. Evolution and genetics of populations. Volume 1. Genetic and biometric foundations. The University of Chicago Press, Chicago and London, 469 pp.

Impact de deux parasitoïdes (*Cephalonamia stephanoderis* Betrem et *Phymasticus coffea* LaSalle) sur les populations de scolytes (*Hypothenemus hampeï* Ferr.) au laboratoire

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SUMMARY

The fight against coffee berry borer (CBB), *Hypothenemus hampei* Ferrari, is mainly chemical and is especially carried out with endosulfan. The phenomena of resistance of the pest with respect to this insecticide and the problems of residues in the consumed grains led the researchers to think of an alternative to the chemical fight by the use of the natural enemies. Two principal parasitoids of CBB cohabit in the coffee plantations in Togo. A feasibility study at the laboratory made it possible to appreciate the individual effectiveness of each of both parasitoids but also their combined effect. Resulted obtained show that *Cephalonomia stephanoderis* Betrem and *Phymastichus coffea* La Salle reduce in to a considerable way the populations of CBB. Moreover a synergistic effect of both parasitoids was observed.

Key words: Coffee tree, *Hypothenemus hampei*, *Cephalonomia stephanoderi*s, *Phymastichus coffea,* parasitoïdes, Togo.

RÉSUMÉ

La lutte contre le scolyte des fruits du caféier, *Hypothenemus hampei* Ferrari, est essentiellement chimique et est surtout réalisée avec de l'endosulfan. Les phénomènes de résistance du ravageur vis-à-vis de cet insecticide et les problèmes de résidus dans les grains consommés ont amené les chercheurs à penser à une alternative à la lutte chimique par utilisation des ennemis naturels. Deux principaux parasitoïdes du scolyte cohabitent dans les caféières togolaises. Une étude préliminaire au laboratoire a permis d'apprécier l'efficacité individuelle de chacun des deux parasitoïdes mais aussi leur effet conjugué. Les résultas obtenus montrent que *Cephalonomia stephanoderi*s Betrem et *Phymastichus coffea* La Salle réduisent de façon considérable les populations de scolytes. En outre un effet synergique des deux parasitoïdes a été observé.

Mots clé: Caféier, *Hypothenemus hampei*, *Cephalonomia stephanoderi*s, *Phymastichus coffea,* parasitoïdes, Togo.

INTRODUCTION

Insecte cosmopolite, le scolyte des fruits, *Hypothenemus hampei* Ferr. cause de graves préjudices aux récoltes dans la plupart des pays producteurs de café (Morallo-Rejesus and Baldos, 1980; Baker, 1984; Barrera, 1992). Le Pelley (1973) rapporte des pertes globales de 40-80%. Au Togo, ces pertes dépassent 9% dans certaines plantations du plateau de Dayes (Wegbe, 1997).

Mises à part quelques méthodes de lutte culturales telles que la récolte sanitaire ou la taille des caféiers, le contrôle des pullulations du ravageur est essentiellement chimique et réalisé surtout avec l'endosulfan (Decazy, 1989). Cette forme de lutte bien qu'efficace pour abaisser rapidement les niveaux d'infestation présente plusieurs inconvénients dont la résistance des scolytes vis-à-vis des insecticides utilisés.

Pour pallier ces inconvénients et dans le souci de produire du café à moindre coût et le plus naturellement possible, d'autres moyens de lutte sont étudiés. Au cours de ces dix dernières années, les efforts ont particulièrement porté sur la lutte biologique par utilisation de parasitoïdes. En effet, deux principaux parasitoïdes répertoriés dans les caféières africaines font objet d'importantes études. Il s'agit de *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae), parasitoïde des stades larvaire et nymphal du scolyte et de *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae), endoparasitoïde de l'adulte.

Le deuxième parasitoïde a été découvert pour la première fois au Togo par Borbon-Martinez en 1989 et est décrit par LaSalle en 1990. Des études de biologie et d'écologie ont été largement initiées sur ce nouveau parsitoïde au Togo (Borbon-Martinez, 1989; Wegbe, 1990; Feldhege,1992). Les deux parasitoïdes cohabitent dans les caféières et ont tous pour hôte les différents stades de scolyte. L'introduction de *C. stephanoderis* dans les pays d'Amérique du Sud et du Centre n'a pas permis de réduire de façon significative les pertes causées par le scolyte (Barrera, 1994; Damon, 1999).

L'objectif de cet article est d'étudier au laboratoire les performances individuelles de chacun des parasitoïdes mais surtout leur effet conjugué sur les populations de scolyte.

MATÉRIEL ET MÉTHODES

Des fruits noirs scolytés contenant les différents stades du ravageur et de ses parasitoïdes ont été répartis dans des caisses d'émergence. Ces caisses sont constituées d'une partie en contre plaqué et d'une autre de tubes à essais abouchés à des trous. Les insectes attirés par la lumière émergent et viennent se loger dans les tubes à essais. Les différentes espèces d'insectes (scolyte et parasitoïdes) ont été récoltées chaque après-midi. L'élevage de scolyte a été réalisé sur milieu artificiel dont le composé principal est la poudre de café vert moulu (Villacorta, 1993). Le milieu, coulé dans des tubes à essais (200 x 22 mm), a été ensemencé de femelles adultes, préalablement lavées à l'eau de javel 2 pour mille. Au bout de 25-30 jours, les nouveaux adultes émergé ont été récoltés pour servir dans les élevages de parasitoïdes, dans les études d'efficacité de parasitoïdes ou encore dans d'autres élevages de scolytes.

Elevage de Cephalonomia stephanoderis

Des *C. stephanoderis* femelles provenant des caisses d'émergence ont été réparties dans des boîtes d'élevage de scolytes mis en place depuis 4 à 6 semaines. Au bout de 20 à 25 jours, les adultes émergés sont récoltés pour réaliser les tests d'efficacité.

Elevage de *Phymastichus coffea*

Des femelles de *P. coffea* ont été réparties sur des femelles de scolyte provenant des élevages et préalablement installées dans des boites. Les deux espèces d'insectes ont ainsi été mises ensemble pendant 2 à 3 heures pour provoquer l'attaque des scolytes par les *P.coffea.* Les insectes (scolytes parasités et *P. coffea*) ont été répartis à leur tour sur des fruits de café jaunes ou rouges qui ont été préalablement passés au soleil afin de diminuer leur teneur en eau. Les

scolytes parasités ont pénétré dans les fruits. Les jeunes parasitoïdes ont commencé leur sortie au bout de 30 jours.

Les élevages en laboratoire nous ont permis de disposer de jeunes insectes ayant encore toutes leurs potentialités biologiques.

Photo 1. Cephalonia stephanoderis en cours de ponte (B. Dufour)

Photo 2. Femelle et Mâle

Les tests d'efficacité de parasitoïdes

C. stephanoderis

Les scolytes émergés des élevages précédents ont été utilisés pour réaliser d'autres élevages de scolyte dans les proportions 100 fruits sains pour 100 scolytes femelles. Quatre à six semaines après la mise en place de ces nouveaux élevages, des femelles de *C. stephanoderis* ont été réparties dans chaque boîte. Deux traitements ont été réalisés, 5 et 10 *C. stephanoderis.*

P. coffea

Les scolytes et les *P. coffea* provenant des élevages en laboratoire ont été utilisés pour réaliser cette étude d'efficacité. L'essai est réalisé de la même façon que l'élevage de *P. coffea* décrit plus haut.

C. stephenoderis et ***P. coffea***

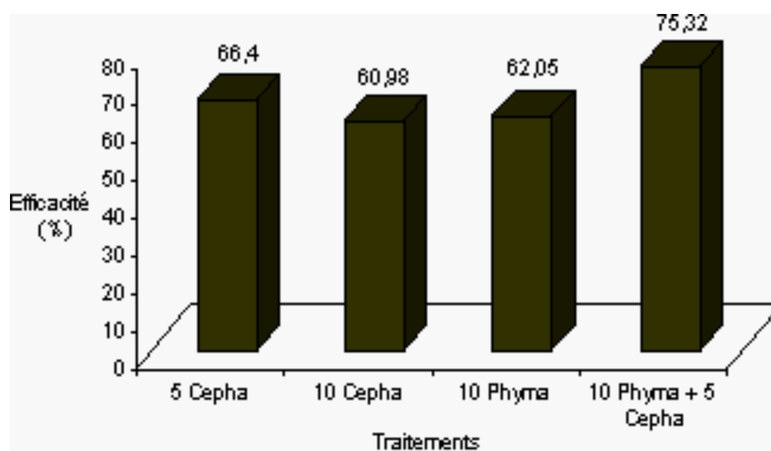

Figure 1. Effets comparés des différente traitements

Cette combinaison a pour but de mesurer l'efficacité conjuguée des deux parasitoïdes sur les populations du ravageur. Ainsi, 3 à 4 semaines après attaque des scolytes par *P. coffea*, et leur mise en salle d'élevage, 5 *C. stephanoderis* ont été introduits dans chaque boîte.

Tous les traitements ont été répétés 5 fois.

Les élevages et les tests d'efficacité ont été conduits dans des boîtes plastiques (142 x 102 x 87 mm). Les conditions dans les salles d'élevage ont été 27,5°C, 70-80% d'H.R et une photopériode de 12 heures de lumière et 12 heures d'obscurité.

L'effet de chaque parasitoïde a été déterminé par la formule de Koch (1973):

100 (N-N')/N où **N** est le nombre de scolytes vivants sortis du traitement témoin et **N'** le nombre de scolytes vivants émergés d'un traitement au parasitoïde.

L'émergence cumulée de scolytes de chaque traitement, portée sur un même graphique, a permis de visualiser les différences.

RÉSULTATS ET DISCUSSION

Tous les parasitoïdes ont eu un effet sur les populations de scolytes quelque soit l'espèce ou le nombre d'insectes utilisés (Figure 1). Tous les traitements ont donné des efficacités supérieures à 50%. Mais ces résultats sont insuffisants pour le contrôle efficace des pullulations.efficacités enregistrées avec les *C. stephanoderis* sont inférieures à celle signalée par Barrera (1994) pour des essais réalisés en champ (89-96,5%). La comparaison des résultats relatifs au même parasitoïde révèle qu'il existerait une légère compétition intraspécifique; l'effet obtenu pour 5 *C. stephanoderis* et 10 *C. stephanoderis* étant respectivement 66,40% et 60%. Ce phénomène signalé par Barrera (1994) peut constituer un frein à la multiplication rapide du parasitoïde dans les élevages en laboratoire. Néanmoins, il faut signaler que dans la nature, cette gêne mutuelle des parasitoïdes est sûrement moindre.

L'utilisation de 10 *P. coffea* et 5 *C. stephenoderis* a donné le résultat le plus intéressant. La combinaison améliore donc l'efficacité. Cette situation prévaut dans la nature où les deux parasitoïdes partagent la même niche écologique et attaquent le même ravageur.

La lutte biologique contre le scolyte des fruits devrait donc se faire avec les deux principaux parasitoïdes. En effet, les actions des deux parastoïdes se complètent. *P. coffea* agirait plus au moment de la colonisation de la nouvelle fructification alors que *C. stephanoderis* réduirait considérablement les populations de scolytes pendant la maturation des fruits quand les stades larvaire et nymphal de l'hôte sont présents. Il agirait également en période post-récolte sur les populations résiduelles de scolytes.

La mise en place d'une lutte biologique passe par la maîtrise des élevages de parasitoïdes. L'élevage de *C. stephanoderis* est au point (Barrera, 1994); celui de *P. coffea*, longtemps considéré difficile, est actuellement réalisé en Colombie où plus d'un million de parasitoïdes sont produits par mois (Baker, 1999). L'un des points essentiels pour la réussite de la lutte biologique contre le scolyte est la détermination du nombre de parasitoïdes à lâcher pour avoir une efficacité suffisante. Un bon équilibre entre le nombre de *C. stephanoderis* et de *P. coffea* doit être déterminé pour augmenter l'efficacité de la lutte. Les périodes propices de lâcher de chacun des parasitoïdes doivent aussi être recherchées.

La lutte biologique contre le scolyte des fruits du caféier *Hypothenemus hampei* Ferr. par utilisations de parasitoïdes est possible si les deux principaux parasitoïdes (*Cephalonomia stephanoderis* Betrem et *Phymastichus coffea* LaSalle) sont utilisés dans les bonnes proportions et aux périodes propices.

RÉFÉRENCES

Baker P. S. 1984. Some aspects of the behavior of the coffee berry borer in relation to its control in southern Mexico (Coleoptera: Scolytidae) Folia Entomol. Mex., **61**: 924.

Baker P. S. 1999. The coffee berry borer in Colombia. Chinchina Colombia 154 P.

Barrera J. F., 1994. Dynamique des populations du scolyte des fruits du caféier, *Hypothenemus hampei* (Coleoptera: Scolytidae) et lutte biologique avec le parasitoïde *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae) au Chiapas, Mexique.Thèse de Doctorat de l'Université Paul Sabatier de Toulouse, France, 301 p.

Borbon-Martinez O. 1989. Bioécologie d'un ravageur des baies de caféier, *Hypothenemus hampei* Ferr.(Coleoptera: Scolytidae) et de ses parasitoïdes au Togo. Thèse de Doctorat de l'Université Paul Sabatier de Toulouse, France, 185 p.

Brun L. O., Marcillaud C., Gaudichon V. and Suckling D. M. 1989. Endosulfan resistance in *Hypothenemus hampei* (Coleoptera: Scolytidae) in New Caledonia. J. Econ. Entomol., **8**: 1311-1316.

Damon A. 2000. A review of the biology and control of the coffee berry borer, Hypothenemus hampei Ferr. (Coleoptera: Scolytidae). Bulletin of Entomological Research. **90**. 453-465.

Decazy B. 1989. Le scolyte du fruit du caféier, *Hypothenemus hampei* Ferr. Considération sur la lutte intégrée contre ce ravageur. In: 13ème Colloque de l'Association Scientifique Internationale du Café. Paipa (Colombie) 21-25 août 1989, p. 655-665.

Feldhege M. R. 1992. Rearing tecniques and aspects of biology of *Phymastichus coffea* (Hymenoptera: Eulophidae) a recently described parasitoid of the coffee berry borer *Hypothenemus hampei* (Coloeoptera: Scolytidae). In Café Cacao Thé, **36**: 45-54.

Koch V. J. M. 1973. Abondance de *Hypothenemus hampei* Ferr., Scolyte des graines de café, en fonction de sa plante hôte et de son parasite *Cephalonomia stephanoderis* Betrem, en Côte d'Ivoire. Meded. Landbouwhogesch. Wageningen, 73-16, 85 pp.

LaSalle J. 1990. A new genus and species of Tetrastichidae (Hymenoptera: Eulophidae) parasitic on the coffe berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: scolytidae). Bulletin of Entomological Research, **80** (1): 7-10.

Le Pelley R. H. 1973. Coffee Insects. Ann. Rev. Entomol. **18**: 121-142.

Morallo-Rejesus B., Baldos E. 1980. The biology of coffee berry borer *Hypothenemus hampei* (Ferr.) (Scolytidae: Coleoptera) and its incidence in the southern Tagolog provinces Philipp. Entomol., **4**: 303-316.

Reid J. C., Mansigh A. 1985. Economic losses dues to *Hypothenemus hampei* Ferr. during processing of coffee berries in Jamaïca. Trop. Pest Manag. **31**: 55-59.

Wegbe K. 1990. Etude de la biologie de *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae) parasitoïde du scolyte des baies de caféier. Mémoire Ingénieur Agronome, option: Production Végétale, Université du Bénin, Ecole Supérieure d'Agronomie, Lomé, Togo. 54 pp.

Wegbe K., Decazy B., Cilas C., Djiekpor E. K. 1997. Le scolyte des fruits du caféier, Hypothenemus hampei Ferr. (Coleoptera: Scolytidae) au Togo. Méthodes d’estimation du niveau d’infestation, des pertes et du seuil de dégât économique. 17ème Colloque de l’Association Scientifique Internationale du Café. Nairobi (Kenya) p. 639-645.

Annual Epidemic Model of Coffee Leaf Rust (*Hemileia vastatrix*) Severity in Hainan Island, PRC

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SUMMARY

An annual epidemic model of Coffee Leaf Rust Severity (CLRS) in Hainan Island, PR China, was investigated from September 1993 to April 1994, the months of the year where the rust is of high field incidence. For this purpose a 4-year coffee plantation was chosen at Danzhou county and 24 trees were used. Leaves of eight branches, 4 from upper and 4 from lower layers of the canopy, were assessed for leaf rust severity. Results showed that temperature, rainfall and dew duration (in days) were three key correlation factors affecting the disease epidemics. Temperature was a limiting factor for the epidemics with an optimum between 20ºC and 25 C. At this range of temperature, the monocyclic process of the disease only needed 23-28 days. The infected leaves after removing the urediospores could produce spores again within 1-3 days. When the field average temperature was higher than 26 C or lower than 19ºC, the monocyclic process took more than 30 days or could not complete at all. During the rust epidemic early periods, heavy rains and dews of long duration provided enough free water for spores germination and penetration and also helped spores dispersal. These three factors worked together to build up the rust epidemics. No significant differences were noted for the CLRS between the upper and lower assessed branches and the orientation of the branches. However the CLRS at the South oriented branches was lower than in the other directions in the first three months of the disease epidemics, a fact that was correlated with the prevailing winds blown from the Southeast, East and Northeast directions during the early period of the epidemics. By statistic analysis all possible climate factors correlated to CLRS. The following regression equation was developed to predict CLRS: $Y = 215-3.98X_1-0.138X_4-3.46X_6$ ($S = 13.4$, $R^2 = 86.01$, $F = 36.9 > F_{0.01} = 5.09^{**}$), where Y is CLRS, X_1 is the average temperature of 30 days before prediction, X_4 the amount of rainfall in millimeters and X_6 the number of dew days during the same period.

Key words: Coffee leaf rust, *Hemileia vastatrix* Berk & Br, Severity, Epidemic model, Hainan, China.

INTRODUCTION

Coffee leaf rust (*Hemileia vastatrix* Berk & Br) is a very serious disease at coffee plantations in China, occurring from September to next March in Hainan. According to Yu (1987) rust epidemic occurs in days with a temperature between 19°C and 23°C at 1-2 o'clock in the early morning.

Coffee rust epidemic is a polycyclic process, and each cycle involves three subprocesses: infection, sporulation and spore dissemination. The infection process comprises: urediniospore germination, host penetration and hyphal colonization.

Multiple regression analysis has been used to predict the development of coffee rust (Kushalappa and Ludwig, 1982; Kushalappa et al., 1982; Kushalappa, 1981; Kushalappa and Lafesse, 1981) and of other plant diseases (Eversmeyer and Burleigh, 1970; Burleigh et al., 1972; Fit and Mccartney, 1986; Broome et al., 1995). Kushalappa et al. (1981; ~ and Ludwig, 1982; ~ et al., 1982; ~ and Lafesse, 1981) have developed several methods to predict coffee leaf rust infection rates. The present work tries to reveal the correlation between climatic factors and Coffee Leaf Rust Severity (CLRS) and tries to establish a prediction model for Hainan Island.

MATERIALS AND METHODS

Twenty four trees of 4-year old *Coffea arabica cv.* Mundo novo were selected at random on a 2 ha plantation, at Danzhou County (latitude N: 19º26', altitude 18.6 m), Hainan Island, PR China. Trees were growing (1,0 x 2,0 m apart) in the West-East direction. Eight branches from the upper and lower layers of 4 different directions were marked in each tree. Data from individually tagged leaves were collected at 7-day intervals since September 1993 to April 1994. The data included the number of leaves on given nodes, and presence and absence of rust. The colonization process was quantified as latency period (LP: the time between inoculation and 50% lesion sporulation) and monocyclic process (MCP: the time between inoculation and full lesion sporulation). In parallel with natural field epidemic, inoculations were made in the field with rust isolate II according to the method of d'Oliveira and Rodrigues (1961) at different seasons and climatic locals. Rust degree assessment followed d'Oliveira's (1954-57) scales. Rust severity assessment was evaluated with the formula:

$$Y = (b + 2*c + 3*d + 4*e)*100/(4*(a + b + c + d + e))$$

in which:

- Y is CLRS;
- a, is the number of 0 degree infected leaves;
- b, the number of 1 degree infected leaves, including the present and fallen leaves;
- c, the number of 2 degree infected leaves, including the present and fallen leaves; etc.

All meteorological factors (temperature, rainfall, number of dew days, relative humidity, sunlight, wind speed, etc.) were obtained from a meteorological station situated 100 m from the site of experiment.

CLRS was evaluated by using the regression procedure of Minitab Data Analysis Software (Minitab Inc., State College, PA). All possible combinations of different climatic factors were regressed for the CLRS data. Regression equations were evaluated according to significance of regression coefficients, coefficients of determination (R^2) and R^2 adjusted for degrees of freedom (R_a^2), and pattern and distribution of residuals (Neter et al., 1985).

RESULTS

Relation of LP and MCP with the temperature (field inoculations)

Table 1 shows that LP usually took 23-28 days, and MCP needed 28-30 days when the average temperature was 20-26ºC. When the average temperature was lower than 20 C or higher than 26 C, the first symptoms appearance took longer or could not be detected during this epidemic period. However, when the temperature became suitable, the inoculated leaves produced lesions and later pustules.

Table 1. Relation of temperature with the rust (*H. vastatrix*) LP and MCP

Av. temp(°C)[1]	Temp. range (°C)	FSAI[2] (days)	FSA[3] (days)	LP[4] (days)	MCP[5] (days)	Local of inoculation	Date of inoculation
27.1	23-33	20-26	\#	\	\	Guangxi Province	Jun. 6
26.6	23-29	17-22	24-29	28-34	35-44	Guangxi Province	May 3
25.4	21-28	16-19	23-26	26--31	30-35	Hainan Province	Sept.17
23.9	23-25	14-16	21-23	25-28	28-30	Hainan Province	Oct. 13
22.4	21-23	14-15	20-22	23-25	25-28	Hainan Province	Oct. 28
20.8	18-24	20-25	28-32	30-35	31-38	Hainan Province	Nov. 13
18.0	15-21	25-29	33-39	36-43	42-56	Hainan Province	Dec. 3
17.0	15-19	29-30	35-38	37-43	45-58	Yunnan Province	Nov 3

[1]Av. temp: average temperature at the MCP periods
[2]FSAI: The first symptoms after inoculation
[3]FSA: The first spores after inoculation. #: The inoculated leaves produced lesion and pustules in mid August with average temperature of 23ºC

Relation of temperature and relative humidity with secondary sporulation

Under field conditions, the secondary sporulation of the lesions after previous removal of the urediniospores, responded differently according to temperature (Table 2). When the average temperature was 20-25°C, the reproduction of urediniospores only took 1-3 days. When the average temperature was higher than 30° C or lower than 15° C, the lesions did not form any further urediniospores. The relative humidity (RH) had little influence on the lesions secondary sporulation. These results, obtained in natural field condition confirmed the results obtained with artificial inoculations (Table 1), demonstrating the great influence of temperature on the urediniospores lesion secondary sporulation.

Comparison of CLRS in different branch growing directions

Statistical analysis of the data of CLRS (data not shown) collected from 8 different branches growing directions indicated that there were no significant differences related to the branch growth orientation. However, the CLRS was lower on south orientation at the early three months epidemic.

Regression analysis of the climate factors

Table 1 shows that MCP took about 28-30 days. The average MCP at the whole epidemic season was about 30 days. Therefore, the data of different climatic factors 30 days before the date of prediction should be considered to be the best to construct the regression epidemic model. The following climatic factors of 30 days before the prediction day were used in stepwise regression analysis:

- X_1: The average temperature.
- X_2: The average highest temperature.
- X_3: The average lowest temperature.

- X_4: The amount of rainfall.
- X_5: The average relative humidity.
- X_6: The number of dew days.
- X_7: Average temperature.
- X_8: The highest temperature.
- X_9: The lowest temperature.
- X_{10}: Total sunlight time. Additional to the above climatic factors, three other factors at the prediction day were used.

With the aid of Minitab, the following regression equation of CLRS for climatic factors was developed:

$$\mathbf{Y = 215 - 3.98 * X_1 - 0.138 * X_4 - 3.46 * X_6}$$
$$(S = 13.4, R^2 = 0.86, F = 36.9 \{F_{0.01} = 5.09\})$$

where Y is the CLRS. Further analysis of variance is given in Table 3.

The table showed that the standard residual is in reasonable range, except for the day of April 3.

Table 2. Relation of temperature and relative humidity (RH) with rust spore reproduction

Date of observation	Temp. range	Av. temp.	RH (%)	BSS* (days)	FPS** (days)
5 Aug.-3 Sept.	29.3-32.7	31.1	74-81	No spore formed	\
2-16 Sept.	26.1-31.0	28.8	77-81	6-7	14
1-17 Nov.	24.4-26.8	25.3	83-85	2-3	6-8
1-9 Oct.	21.4-25.6	23.1	66-78	2-3	8
26 Oct.-27 Nov.	20.1-22.3	21.0	85-89	1-2	6
9-12 Dec.	19.0-20.1	19.2	70-80	3-4	12
1 Jan.-1 Feb.	15.4-20.0	17.0	83-86	7-9	19-21
3 Nov.-20 Dec.	13.0-15.0	14.0	66-84	No spore formed	\

**BSS: Beginning of Secondary Sporulation; **FPS: Full Pustule Sporulation*

DISCUSSION

CLRS is a S-type curve

Figure 1 shows that CLRS is a traditional S-type curve, i.e. of the type $Y = k/(1+ae^{-bx})$.

By extending down the first observed point of the curve in Figure 1, we could roughly infer that September 5 was the best time to represent the beginning of the epidemics. When using the epidemic weeks to represent the timetables of CLRS, we found that k, a and b are constant coefficients. Using the CLRS data and related timetables provided to the S-type computer program, we concluded that the model equation was :

$$\mathbf{Y = 97.283/(1+35.4048e^{-0.2454x})}$$
$$(F = 170.97^{**}\ [F_{0.01} = 8.18])$$

where Y was CLRS, x was the number of epidemic weeks beginning in September 5.

Table 3. Further analysis of variance explained by each variable when entered in the order given

Date	Average Temp.	CLRS	No. of dew days	Rainfall (mm)	Standard Residual	Predicted Y value	Residual
Sept. 19	26.8	2.57	21	151.3	1.1	15.01	–12.44
Sept. 26	26.7	3.78	21	151.3		13.51	–9.73
Oct. 3	26.6	4.99	22	166.6	–0.45	10.24	–5.25
Oct. 10	26	6.72	20	277.5		3.83	2.89
Oct. 17	25.4	8.45	17	389.2	0.56	1.55	6.90
Oct. 24	24.8	10.79	16	391.5		6.75	4.04
Oct. 31	24.2	13.12	15	395.0	0.07	12.44	0.68
Nov. 7	24.1	15.93	18	286.5		17.09	–1.16
Nov. 14	24	18.73	21	177.5	–0.3	22.55	–3.82
Nov. 21	23.4	27.8	22	166.7	0.38	22.97	4.83
Nov. 28	22.3	36	21	45.5	–0.92	47.56	–11.56
Dec. 5	20.2	42.74	19	55.7	–1.46	61.43	–18.96
Dec. 12	19.1	47.47	21	35.6	–1.13	61.67	–14.20
Dec. 19	18.2	56.42	23	35.4	–0.17	58.37	–1.95
Dec. 26	17.6	63.66	20	52.3	–0.42	68.8	–5.14
Jan. 2	18.7	68.29	20	34.4		68.89	1.40
Jan. 9	19.4	76.67	18	20.9	0.11	72.88	3.79
Jan. 16	19.8	81.65	16	25.3	–0.3	77.59	4.06
Jan. 23	19.1	84.1	15	21.0		84.34	–0.24
Jan. 30	18.4	86.54	14	18.6	0.38	91.01	–4.47
Feb. 6	17.3	92.22	14	29.1	–0.92	93.94	–4.05
Feb. 13	17.6	93.38	15	16.5	–1.4	91.03	2.35
Feb. 20	18.8	93.65	20	33		66.22	27.43
Feb. 27	19.4	93.92	18	49.5	–1.13	62.01	31.91
Mar. 6	19.1	94.6	16	58.2	–0.17	75.84	18.76
Mar. 13	19.1	92.23	10	58.5		96.21	–3.98
Mar. 20	19.1	89.85	10	71.1	–0.42	94.8	–4.95
Mar. 27	18.8	88.64	9	58.8	0.11	101.15	–12.51
Apr. 3	23.6	84.61	17	18.2	2.21R	59.98	24.63

R: indicates an observation with a large standard residual

Further analysis made at Table 4 confirmed CLRS was an S-type curve.

CLRS epidemic could be recognized as four different phases

According to Figure 1, we could roughly identify CLRS as being constituted by 4 phases: (1) A period of slow multiplication or lag phase: from early September to the end of October, during which time the lesions slightly increased in size and its average number lesions per leaf less than 5. (2) A period of fast incidence disease or logarithmic phase extending from November to the end of February, during which time the infection rate increased quickly and the severity reached to maximum due to adequate temperature and dew, favorable for spore germination, penetration and colonization. (3) A period of post-logarithmic phase or extensive defoliation phase beginning on early March up to mid April. During this phase, there was an extensive defoliation. Practically all infected leaves fell off, and some branches appeared with

dieback. (4) A phase of very little new infected leaves, associated with the rapid new leaf formation from May to August.

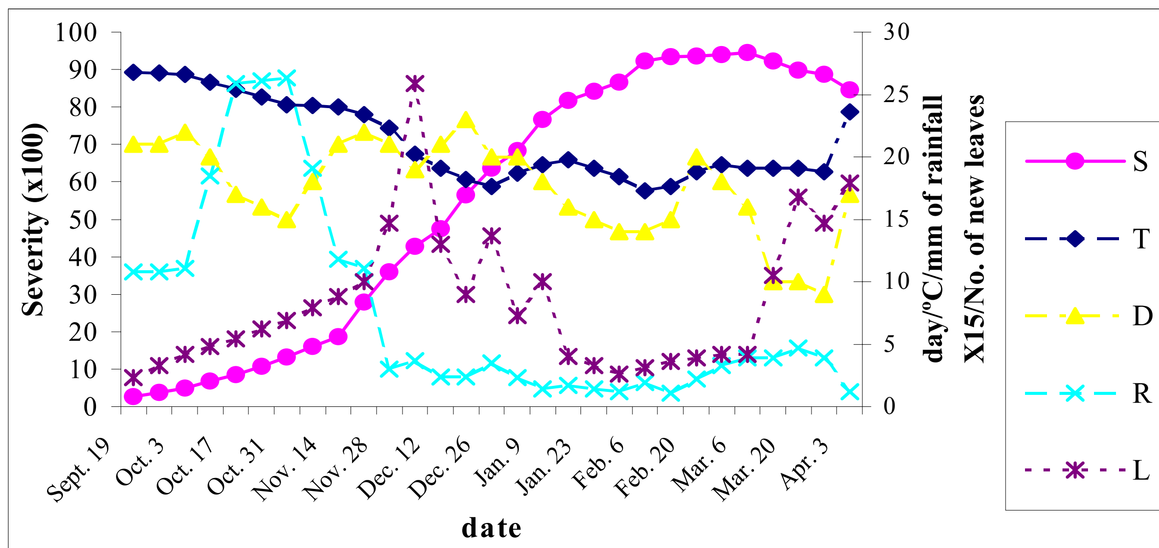

Figure 1. Coffee rust annual epidemic curves as a function of the temperature, number of dew days and amount of rainfall, in Hainan Island. S: CLRS (x 100); L: average number of newly formed leaves per day; R, D and T: the amount of rainfall (mm x 15), numbers of dew days and average temperature at 30-day intervals, respectively

Table 4. comparison of the residual predicted Y with the field CLRS value

Date	Sept 19	Oct. 3	Oct. 17	Oct. 31	Nov 14	Nov 28	Dec. 12	Dec. 26	Jan. 9	Jan. 16	Jan 30	Feb. 13	Feb. 27	Mar 6	Mar. 20	Apr. 2
Ep. Wks*	2	4	6	8	10	12	14	16	18	19	21	23	25	26	28	29
CLRS	2.6	5.0	8.5	13.1	18.7	36.0	48.5	63.7	76.7	81.7	86.5	93.4	93.9	94.6	89.9	84.6
Pred.Y**	4.3	6.8	10.7	16.3	24.1	34.0	45.5	57.3	68.2	72.9	80.8	86.5	90.4	91.8	93.8	94.6
Residual	-1.7	-1.8	-2.2	-3.2	-5.4	2.0	3.0	6.4	8.5	8.6	5.7	6.9	7.5	2.8	-4.1	-10.0

**Ep. Wks: epidemic weeks; **Pred. Y: predicted Y value*

Coffee leaf rust epidemics results from factors such as temperature, rainfall and number of dew days; wind may also play key roles

Our result shows that the monthly average temperature, rainfall and number of dew days have a good correlation with CLRS.

From Table 3 it can be seen that the average temperature always has correlation with CLRS index. From August to September, the monthly average temperature decreased from 27.9ºC to 26.5ºC, the latent lesions formed new spores and the rust epidemics developed slowly. From October to December, the average monthly temperature decreased to 19.4ºC and the CLRS index increased rapidly. From January to February, the average monthly temperature was 17.3-19.8ºC, and this low temperature made the rust epidemics slow down. From March to April, the average monthly temperature increased rapidly from 22.1 to 26.1ºC, and the great amount of newly formed leaves also helped CLRS to slow down (Fig 1). In mid April, no new infected leaves were found, but previously infected ones had an enlargement and increasing number of lesions.

Kusalappa et al. (1983) shows that the infection rate of the inoculated plants at 26ºC in the laboratory conditions was reduced to about 20% as compared with the maximum infection rate at the optimum temperature. With 30ºC, no infection was found. Our results also confirm that when the average temperature was 27ºC, no sporulation could be found (Table 1), and at 31ºC no secondary sporulation occurs (Table 2). Danzhou is a tropical area and the rainy season is from May to October, with 84.2% of the year rainfall. However, despite 51% of the total rainfall occurs from May to August, during this period there is no epidemic, because the temperature is not enough. Therefore, temperature is in fact a conditioning factor affecting the epidemics.

Rainfall showed correlation with CLRS because heavy rainfall helped urediniospores splashing within each tree and from the infected trees to the neighbor ones at an early period of the epidemics. Although from December to March there is a dry period in Hainan, there is sometimes a drizzle during those months, beneficial for the rust spores germination and penetration. Dews has a similar function to rain. From September to November, heavy dews usually formed in non-raining nights, starting around midnight and drying out at 10-12 o'clock of next morning, providing thus more than 10 hrs of wet conditions for spore germination and penetration. From mid February to March, owing to shorter or no period at all of dews at night, associated with low temperature in February, spore germination and penetration is rare and the epidemic slows down in March, and completely stops in April (Figure 1).

Previous authors have demonstrated that urediniospores are mainly wind disseminated (Kushalappa, 1989; Martins et al., 1977; Pauvert, 1986; Willocquet et al., 1998; Bawden et al., 1971). Our results suggested that wind contributed to the CLRS. The CLRS in south directions was lower than in other directions at the early 3 months epidemics (data not shown). Prevailing wind and the other following reasons may be responsible for this: First, the wind did not provide the same opportunity to disperse spores to different directions. More than 60% of wind was from the direction of East-East-South, East and East-East-North in October and November (Data not shown). The wind provided more opportunity for spore dispersal from the leaves of those directions to the leaves of the same or opposite directions. Second: Influence of the plant density on spore dispersal. The coffee plant canopies covering the interrows, did not leave much possibilities for spore dispersal from North to south. Third: At the early epidemic period, the available spores for dispersal was very little and the dissemination opportunity was negligible. When CLRS was higher than 20, like in mid November, the proportion of diseased leaves allowed a better spore dissemination. The disease incidence reached to about the same level.

REFERENCES

Agrios G. N. 1988. Plant Pathology. Third edition. Page 466.

Bawden J., Gregory P. H. and Johnson C. G. 1971. Possible wind transport of coffee leaf rust across the alantic ocean, Nature (London), 229, 500.

Broome J. C., English J. T., Marois J. J., Latorre B. A. and Aliles J. C. 1995. Development of an infection model for Botrytis bunch rot of grape based on wetness duration and temperature. Phytopathology, **85**: 97-102.

Burleigh J. R., Eversmeyer M. G. and Roelfs A. P. 1972. Development of linear equations for predicting wheat leaf rust. Phytopathology. **62**: 947-953.

D'Oliveira B. Revista do Cafe Portugues, 1954-57, Separata, n. **3**: 61 pp.

D'Oliveira B. Rodrigues Jr. C. J. 1961. O problema das ferrugens do cafeeiro. Revista do Cafe Portugues, **8** (29): 5-50.

Eversmeyer M. G. and Burleigh J. R. 1970. A method of predicting epidemic development of wheat leaf rust. Phytopathology. **60**: 805-811.

Fit B. D. L. and Mccartney H. A. 1986. Spore dispersal in splash droplets. Pages 87-114 in: Water, Fungi and plants. P.G. Ayres and L. Boddy, eds. Cambridge University Press, Cambridge.

Kushalappa A. C. and Ludwig A. 1982. Calculation of Apparent Infection Rate in Plant Disease: Development of a Method to Correct for Host Growth. Phytopathology. **72**: 1373-1377.

Kushalappa A. C., Akutsu M. and Ludwig A. 1982. Application of Survival Ratio for Monocyclic Process of Hemileia vastatrix in Predicting Coffee Rust Infection Rates. Phytopathology. **73**: 96-103.

Kushalappa A.C. 1981. Linear models applied to variation in the rate of coffee rust development. Phytopathol. Z., **101**: 22-30.

Kushalappa A. C. and Lafesse R. M. 1981. LEAFAL: a computer program for quantitative analysis of leaf fall in coffee principally from rust. Phytopathol. Z. **101**: 97-105.

Kushalappa A. C. 1989. Biology and Epidemiology. In Coffee rust: epidemiology, resistance, and Management. Eds. by Kushalappa A. C. and Eskes A. B. CRC Press. P 13-80.

Kushalappa A. C. and Eskes A. B. 1989. Advance in coffee rust research. Annu. Rev. Phytopathol. **27**: 503-31.

Martins E. M. F., Palazzo D. A., Karazawa M. 1977. Importance fo the wind in the release and dissemination of spores of *Hemileia vastatrix.* Fitopatol. Bras. **3**: 25-42.

Neter J., Wasserman and Kutner M. H. 1985. Applied linear Statistical Models. 2nd ed. Richard D. Irwin, Inc., Homewood, IL. 1127 pp.

Pauvert P., 1986. Les paramètres Epidémiologiques de l'Oodium de l'Orge (*Erysiphe graminis D.C. f. Sp. Horewi).* Paris, France: Université de Paris VI, PhD thesis.

Willocque L., Berud F., Raoux L. and Clerjeau M. 1998. Effects of wind, relative humidity, leaf movement and colony age on dispersal of conidia of *Uncinula necator,* causal agent of grape powdery mildew. Plant pathol. **47**, 234-242.

Yu H. 1987. Coffee leaf rust (Hemileia vastatrix) and its control. Guangdong Agricultural Bureau Press. PP 77.

Closing Address ASIC 2001

O. VITZTHUM

In the name of ASIC I would like to thank all the organisers and sponsors for the excellent realisation of this Symposium and to thank for the great hospitality in this wonderful ambience here.

Special thanks should be given to

- ICO, the International Coffee Organisation
- NCA the US National Coffee Association
- to the international Coffee Companies Douwe Egberts, ICT, Kraft Foods, Nescafé, Probat, Tchibo and Mellita
- to the local Companies
- Corsîni, Demus, Illycaffè. Lavazza, Pacorini, Scuolari, and Qualicaf

and – not to forget – special thanks to Daniela Candelari, the “allround assistant” from Illycaffé.

I personally remember very well the first ASIC Symposium 1967 in Trieste. It was the first ASIC conference outside of France after its foundation in 1965 by René Coste, Ernesto Illy and Pierre Navellier.

As this time here we have not given summary reviews of the presentations at the end of the symposium, I would like to try shortly to stress which progress ASIC has made since the last meeting here some 36 years ago. I will also give some highlights of the meeting.

In **Human Physiology** ASIC and other associations like PEC and COSIC had been influential fl convincing the scientific community that only serious research at elevated standards could give clearance to many questions. By that it can be concluded today that reasonable coffee consumption (~5 cups a day) does not have adverse effects on human health. Additional current research demonstrated that coffee consumption may well have beneficial effects on health - due to its antioxidant activity, particularly in the areas of brain degenerative disease, cancer of the colon and heart disease.

Results from **Coffee Chemistry** and **Food Processing** research in the mentioned time period contributed to facilitate a better production of the coffee product today.

New analytical methods for

- aroma elucidation
- off flavour analyses
- origin determination
- characterisation of shelf life and others

have helped to optimise processes like

- roasting
- soluble coffee manufacturing
- decaffeination
- quality upgrading
- and packaging

Especially in this symposium we have heard that the carbohydrate composition of coffee nearly is elucidated now. Excellent reports on packaging and roasting will stimulate new developments and thus will yield a further benefit for the consumer as well. First substantial reports on the composition of the non-volatile substances in coffee will help to better understand the coffee taste in the future.

Newly incorporated into the ASIC conference had been **Workshops** like "Mould prevention in coffee plantations". Here a progress was reported by identifying all the risk factors: Guidelines for strict moisture management in the producing countries and separation of husks from coffee beans have been developed.

In **Agronomy** good results have been achieved over the last 30 years to fight major coffee diseases – like leaf rust, coffee borer etc. – by increasing control activities through breeding programs for resistance using new germplasm and the development of new agronomic practices like high density plantations.

Advances in **Biotechnology** were applied in the producer countries to the development of new tools like micropropagation by somatic embryogenesis, molecular markers etc. This conference was dedicated also to the new area of coffee genomics, that rapidly has developed after the elucidation of model genome.

Finally I will try to give you an outlook for the next 10 to 20 years.

In **Chemistry** the time for identifying new components of the odour of the coffee aroma has come to an end after 30 years. Skilful laboratories now can mimic artificially the smell of the coffee aroma. Due to existing strict coffee regulations this knowledge will only be used for quality control. Future research will study the non-volatile coffee components like the melanoidins or Maillard products, the brown colour of the roast and will better elucidate and understand the role of the antioxidants especially for shelf life, The deciphering of sensory items like – still unclear – bitterness will result finally out of this research as well.

Concerning **Food Processing** my personal view is that in some future we no longer will roast as we do today. New research results about the roasting dynamics – we have got some good impressions from the roast session this week – and a future better understanding of the roast process itself will yield in new technologies within the next couple of years after more than 300 years of conventional roasting.

New ways for determination of geographic coffee origins like DNA analyses or inductively coupled plasma spectrometry will be perfected.

Biological control will replace chemical pesticides in future to the benefit of the farmer and the consumer. Coffee plants will be modified so as to better attract parasitic insects that fight the diseases. Green coffee processing may be adapted better to agronomic and socio-economic aspects, being more environmentally friendly. We may say that the coffee will be more "tailored" to the needs or the farmer, roaster and consumer.

Integration of emerging technologies like genomics, cryopreservatîon$_1$ information technology, in particular molecular techniques will be used to identify useful genes and to manage and increase access to genetic diversity.

In February of this year I met Monsieur Coste, our honorary president being absent from here today due to his high age of 94. He expressively stated that he is very happy that the "Institution ASIC" has become a world-wide well respected meeting place for all scientists and engineers that are active in the world of coffee.

I hope that all of you had a chance to enjoy the wonderful landscape of Trieste with its delightful harbour. We all have been very much impressed by the kindness and amiability of the Italian people and we will leave from here with a great remembrance of “la bella Italia”.

Herewith I officially close the 19th Symposium 2001 in Trieste, wish you all a good return to your country or hometown and would like to say.

“Farewell until next ASIC conference in 2 years”.