

COFFEE (*COFFEA ARABICA* L.) BEAN TRANSCRIPTOME AFFECTED UNDER RUST (*HEMILEIA VASTATRIX* BERK. & BR) AND YIELD STRESSES

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Introduction

Coffee production is dramatically affected by several diseases and pests, all directly or indirectly influenced by environmental conditions. The oldest and most relevant disease is Coffee Leaf Rust (CLR) which is caused by *Hemileia vastatrix* Berk. et Br. CLR directly or indirectly affects the physiological condition of the plant which ultimately translates into cup (tasting) defects and bad coffee. To mitigate the CLR problem, resistant varieties need to be bred with improved market quality profiles. In addition to the lack of genetic knowledge of both plant, disease, and their interaction, the end use beverage chemistry and sensory perception are other barriers which need to be overcome in the short term. We previously showed that management practices affect cup quality and plant performance, but, to our knowledge, there is no report on how gene expression varies in response to CLR stress in coffee beans.

Materials/Methods

The experiment was located in the commercial coffee farm Hacienda Aquières, located in Turrialba, Costa Rica. Two coffee (*Coffea arabica* L.) plots of mature plants were selected. One plot was planted with an inbred (Caturra velho IAC 144, F8 originating from 'Caturra' x 'Mundo Novo') susceptible to predominant races of rust, and the other plot with a hybrid (H3, F1 of 'Caturra' x 'Ethiopian S31') with slight tolerance to the predominant races of rust. **Fruit thinning (T)** to 50% and **rust control (R)** with fungicides, were compared as management treatments against the control (C) (no preventive treatment) or using **fruit thinning and rust control (RT)**. Fruit samples were collected in the experimental plot once during the highest infection phase of CLR disease and fruit harvest (November). Mature and immature fruits were manually collected from each plant within a plot that represented a replication and treatment. The fruits were immediately hand-depulped and packed in aluminum envelopes. The samples were later transported and stored in a -80C freezer at TEC University. RNA was extracted using PureLink® RNA Mini Kit (LifeTechnologies Inc.). Dehydration and stabilization of the RNA samples for long term storage and normal temperature transportation were done using the RNAsable® solution (Biomatrix Inc.). The RNA samples were sent to Polar Genomics LLC (Ithaca, NY). Strand-specific RNA-seq library construction was performed using their own developed protocol compatible with the TrueSeq Stranded Total RNA Library preparation kit (Illumina®). Sequencing of the libraries were conducted with the Illumina HiSeq2500 (Illumina®), using a single-end, 101 bp strategy at the Institute of Biotechnology at Cornell University.

Conclusion/Perspectives

The transcriptome of the coffee fruit in two different cultivars and at two maturities was significantly influenced by two types of management practices intended to alleviate coffee leaf rust disease (CLR). The transcriptome and functional annotation of a total of 471 differentially expressed genes (DEGs) into 19 gene ontology (GO) terms, reflects that the coffee bean gene expression and volatile profiles, can be significantly modified by management practices. Further validation of the data and experiments will guide into which candidate genes may influence on pathways related to quality-associated chemical compounds.

References:

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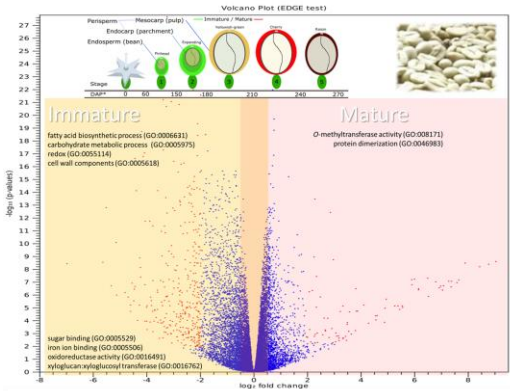
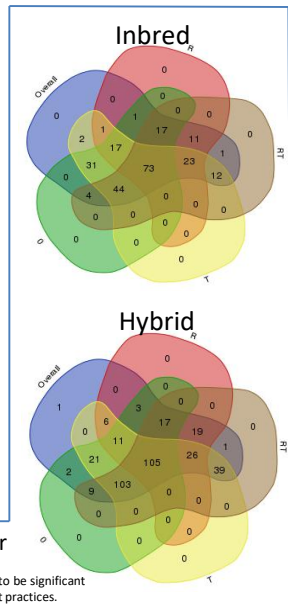


Figure 1: Immature samples were more active in lipid synthesis and carbohydrate metabolism.

Ninety percent (426) of the 471 DEGs corresponded to higher expression in the immature samples, showing that most metabolic processes were active before ripening. The maturity stage was selected to find treatment and cultivar significant differences of gene expression.

Figure 2: DEGs increased by treatments in each cultivar

The analysis showed that less than 49% (208) of those DEGs in immature beans were also found to be significant when considering the cultivar. These represent the core genes that were affected by management practices.



Results/Discussion

A total of 1,172,573,476 high-quality sequences were obtained from the 46 RNA samples. The 97% of the sequences were between 100-101 bp length after the trimming, with 44.5% GC content, and a phred score showing that 98% of the sequences were higher than 30 (99.9% of accuracy in base call).

On average, 82% of the sequences aligned to exons and over 93% of the fragments were uniquely mapped to the diploid *C. canephora* reference genome.

The gene expression varied increasingly larger between 1) different treatments of a single cultivar and maturity stage, 2) maturity stages within cultivars, and 3) cultivars.

A higher number of DEGs were found in the immature stage where synthesis of fatty acids and carbohydrates were most active.

The number of differentially expressed genes (DEG's) according to each treatment showed that the hybrid had between 6 to 7 times more DEGs than the inbred, 66% (271) of those DEGs unique between treatments.