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Agrobacterium tumefaciens-mediated transformation revealed an alkaline phytoceramidase that is required in pathogenicity of *Colletotrichum kahawae* to *Coffea arabica*

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1. Introduction

The hemibiotrophic fungus *Colletotrichum kahawae* (Ck) is the causal agent of Coffee Berry Disease (CBD), the major limiting factor to the *Coffea arabica* (Ca) production in Africa, especially at high altitudes.

A deep knowledge of pathogenicity mechanisms involved in the infection process may contribute to more rational and effective breeding programs.

In this work, a gene disruption approach was used aiming to decipher the mechanisms of Ck pathogenicity.

2. Material and methods

Agrobacterium tumefaciens mediated transformation (ATMT) (Fig.1) were applied to generate random mutagenesis to a Ck isolate Que2 (from Kenya) aiming the identification of Ck genes involved in pathogenesis.

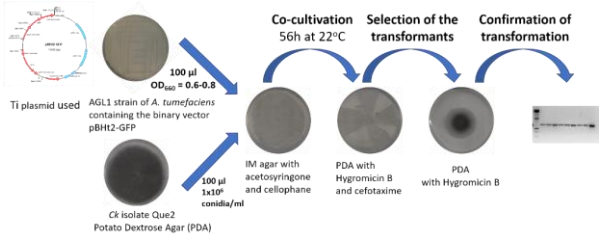

Fig. 1 - ATMT protocol used (Adapted from Talhinhas *et al.* 2008).

Genomic sequences flanking T-DNA were recovered by bidirectional chromosome walking strategy (DNA Walking SpeedUp™ Premix Kit, Seegene, USA).

3. Results

Using the ATMT protocol 173 transformants were obtained. These transformants were mitotically stable, resistant to hygromycin, express the GFP under fluorescence microscope (Fig. 2) and were able to germinate and produce melanized appressoria (Fig. 3).

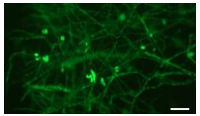

Fig. 2 - Visualization of the *GFP*-expressing under fluorescence microscope. Bar = 50 µm

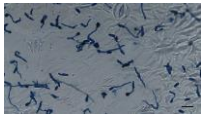

Fig. 3 - Conidial germination and appressoria formation. Bar = 20 µm

The pathogenicity tests reveal that 7% of the transformants obtained exhibit reduced aggressiveness and one of them was identified as non-pathogenic, as it was unable to produce symptoms on green coffee berries, even on wounded fruits (Fig. 4).

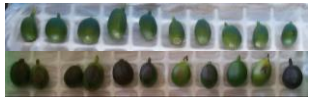

Fig. 4 - Symptoms 10 days after inoculation on green coffee berries of the non-pathogenic (up) and of the Ck isolate Que2 wild type (bottom).

The genomic sequencing flanking the T-DNA on the Left Boarder obtained by chromosome walking strategy for the non-pathogenic transformant suggest an insertion near the UTR5' of a putative **alkaline phytoceramidase**.

Inconclusive results were obtained for the T-DNA Right Boarder with this strategy.

The gene expression study for this gene revealed that the highest relative expression value was observed by 1 day after inoculation (dai), decreasing throughout the infection process (2, 5, 7 and 10 dai), although it is always up-regulated and with significant expression differences (t-student p<0,05) compared to the control (saprophytic mycelium) (Fig. 5).

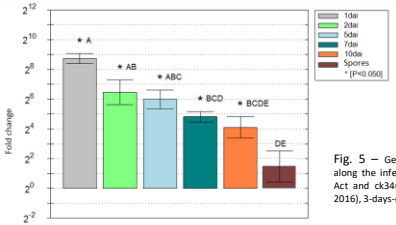

Fig. 5 – Gene expression of a phytoceramidase along the infection process. Normalization by PP1, Act and ck34620 as reference genes (Vieira et al 2016), 3-days-old mycelium was used as control.

4. Conclusions/Perspectives

Ongoing work, include the use of hiTAIL-PCR and/or inverse PCR to completely disclose the insertion of the T-DNA and the determination of the number of insertions by qPCR.

Nevertheless, these results suggest that this alkaline phytoceramidase can be a good candidate for gene knock-out, to validate its role in the pathogenicity of Ck to Ca.

A better understanding of plant-pathogen interaction mechanisms will open new routes for plant protection.

REFERENCES: Talhinhas *et al.* (2008). *Mol. Biotechnol.* 39: 57-67 doi 10.1007/s12033-007-9028-1
Vieira *et al* (2016) DOI:10.1371/journal.pone.0150651

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