

28 June - 1 July

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28th Conference **Asic 2021**

BOOK OF ABSTRACTS

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- A close-up photograph of a coffee branch with several clusters of ripe, red cherries. The cherries are in various stages of ripeness, with some showing a slight white bloom. The background is a soft-focus green, suggesting a coffee plantation.
- Agronomy
 - Chemistry
 - Technology
 - Physiological effects
 - Sustainability, climate changes

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

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RATIONALE

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. CBD can cause losses up to 80% if no control measures are applied and its dispersal to other continents like America and Asia represents a serious concern. *Agrobacterium tumefaciens*-mediated transformation (ATMT) were applied to generated random mutagenesis to a Ck isolate. One transformant (Que2-441) was identified as non-pathogenic, as it was unable to produce symptoms on coffee green berries, even on wounded fruits (Cabral et al. 2016).

METHODS

To obtain the entire sequence of the gene disrupted by the ATMT in Que2-441, a bidirectional chromosome walking strategy was followed, according to the DNA Walking SpeedUp™ Premix Kit protocol (Seegene, USA). Amplified fragments in the third step of chromosome walking protocol were excised from the gel, purified and sequenced. A gene expression study was performed in order to characterize the expression of the identify disrupted gene along the infection process of the interaction Ck-Ca, on saprophyte mycelium, ungerminated conidia, conidia with melanized appressoria formed *in vivo* and *in vitro* (Vieira et al. 2016).

RESULTS

The chromosome walking protocol led to the identification of putative alkaline phytoceramidase 94.7% of identity and an e-value of 1e-140 to the accession KAF0322308 of *C. asianum* in the BlastX search. The KEGG Orthology (KO) of the predicted protein is the entry K04711, with definition dihydroceramidase [EC:3.5.1.-] that is involved in two pathways: sphingolipid metabolism (ko00600) and metabolic pathways (ko01100). The gene expression study revealed that the highest relative expression value was observed at 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). The relative expression of the *in vitro* and *in vivo* appressoria and ungerminated conidia did not differ statistically from the control.

CONCLUSIONS & PERSPECTIVES

This study showed that the gene alkaline phytoceramidase disrupted by the ATMT is up-regulated along the infection process and is a good candidate for gene knock-out, to validate his involvement in the pathogenicity of Ck to Ca. A better understanding of plant-pathogen interaction mechanisms will open new routes for the deployment of more informed plant protection strategies.

References:

- Cabral et al. 2016. In 26th International Conference on Coffee Science, ASIC, China, p.10.2
- Vieira et al. 2016 PLoS One DOI: 10.1371/journal.pone.0150651.