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BOOK OF ABSTRACTS

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Identification of *Hemileia vastatrix* candidate effectors reveals new ways of promoting pathogen variability through alternative splicing

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RATIONALE

Coffee leaf rust (CLR), caused by the biotrophic fungus *Hemileia vastatrix* (Hv), is the most important disease of *Coffea arabica*. The recent outbreak of CLR in central America highlighted the importance of the disease, and reinforced the need for better and faster breeding techniques in order to obtain new coffee resistant varieties. The identification of rust effectors has been single out as a shortcut to the identification of plant proteins that can guide the selection of new resistant genotypes.

METHODS

To identify candidate effectors we used a bioinformatic pipeline aimed at selecting putatively secreted protein sequences, followed by the application of the MEME algorithm to identify new motifs between the sequences (Talhinhas et al. 2014). Protein localization in coffee infected tissue was achieved by immunolocalization using specific antibodies raised against the candidate effectors (Kemen et al. 2005). Agroinfiltration of *Nicotiana benthamiana* leaves with different cDNAs sequences was used to identified the subcellular compartment target by the candidate effector proteins.

RESULTS

We choose four small proteins (<200 amino acids) predicted to be secreted, rich in cysteines and that share three amino acid motifs. The amplified genomic sequences also showed a similar structure. The transcript level of the four sequences was upregulated in infected coffee leaves, peaking seven days after inoculation, when haustoria were the predominant fungal structure observed. The candidate effectors were localized inside of intercellular hyphae and haustoria from *H. vastatrix* in infected leaves. Surprisingly, different transcripts were obtained for single candidate effectors when PCR amplified from infected coffee leaves, therefore revealing splicing variants. These sequences had a distinct transcription profile in leaves of *Coffea* spp. inoculated with different *H. vastatrix* isolates, and a different subcellular localization in agroinfiltrated *N. benthamiana* leaves.

CONCLUSIONS & PERSPECTIVES

The selected sequences showed the hallmark of true effectors, which needs to be supported by the ongoing effort of identifying the plant proteins interactors. The splicing variants identified can contribute to increase the 'arsenal' diversity that *H. vastatrix* employs in the 'arms race' against *Coffea* spp. immunity, and may contribute to the diversification of *H. vastatrix* virulence potential.

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References:

- Kemen et al. 2005 MPMI DOI: 10.1094/MPMI-18-1130.
- Talhinhas et al. 2014 Front. Plant Sci. DOI: 10.3389/fpls.2014.00088.