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BOOK OF ABSTRACTS

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Evaluation of virus-induced gene silencing (VIGS) in coffee plants

Ribeiro de Barros Danielle[1], Diniz Inês[2, 3], Guerra-Guimarães Leonor[2, 3], Gil Azinheira Helena[2, 3], Silva Maria do Céu[2,3] (mariaceusilva@isa.ulisboa.pt), Batista Dora[2, 3]

[1] *Departamento de Fitossanidade, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil.* [2]*CIFC - Centro de Investigação das Ferrugens do Cafeeiro, Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Oeiras, Portugal*; [3]*LEAF-Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory. TERRA, ISA, Universidade de Lisboa, Lisbon, Portugal*

Rationale:
Virus-induced gene silencing (VIGS) is an effective method for gene silencing that uses a plant's antiviral defensive mechanism to suppress the expression of specific invasive viral transcripts. It downregulates endogenous genes by utilizing the posttranscriptional gene silencing machinery of plants and prevent systemic viral infections. VIGS is an RNA-mediated reverse genetics technology that has evolved into an indispensable approach for analysing the function of genes. VIGS has been adapted to many angiosperm species, allowing the analysis of gene functions in species whose genetic transformation is far from routine. With a vast amount of sequence information available for coffee, VIGS might provide a means to analyse the functions of candidate genes previously identified in large genomic or transcriptomic studies. To further elucidate the functional role of coffee resistance related-candidate genes, this study aims to test the tobacco rattle virus (TRV) vector system for coffee and tune the inoculation conditions and the efficiency of the transient expression process.

Methods:
In this study, the tobacco rattle virus (TRV1 and TRV2) vector carrying a *Coffea arabica* phytoene desaturase gene (*CaPDS*) was used. After sequencing, the TRV2:*CaPDS* construction was transformed into Agrobacterium *tumefaciens* (strain C58C1). Coffee plants at two developmental stages (hypocotyls and seedlings) were agroinfiltrated and root dipped with TRV1+TRV2:*CaPDS* using different bacterial culture concentrations. As a positive control, TRV1+TRV2:*CaPDS* construction was also agroinfiltrated in tobacco (*Nicotiana benthamiana, Nb*) as well as the TRV1+TRV2:*NbPDS* construction. Inoculated plants were regularly monitored for the detection of the albino phenotype resulting from the effectively silencing of *PDS* gene expression.

Results:
Coffee seedlings agroinfiltrated with TRV1+TRV2:*CaPDS* construction showed some chlorosis in the infiltrated leaves, but no albino phenotype was observed in the newly grown leaves (for all bacterial culture concentrations tested). No symptoms were observed when using agroinfiltration of hypocotyls or inoculation by dipping. In contrast, agroinfiltration of tobacco with both PDS constructions resulted in an albino phenotype, confirming that the TRV2:*CaPDS* construction is working.

Conclusions & Perspectives:
This was the first tentative approach to establish a functional gene study using VIGS in coffee plants. Since no systemic silencing of the *PDS* gene in coffee was observed and several conditions can interfere with the success of VIGS assays, other viral vector systems, bacterium strains, culture concentrations, and inoculation methods are under study.

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