

Characterization of the Early Response of Coffee to *Hemileia vastatrix* using Real-time Quantitative RT-PCR

D. GANESH¹, A-S. PETITOT², R. ALARY³, P. SANTOS⁴, A. RIBEIRO⁴,
M.C. SILVA⁴, L. GUERRA-GUIMARÃES⁴, D. FERNANDEZ²

¹Division of Plant Improvement, central Coffee Research Institute, Coffee Research Station
577 117, Chikmagalur District, Karnataka, India

² IRD - Institut de Recherche pour le Développement, UMR1097, BP64501, 34032
Montpellier Cedex 5, France

³ INRA - Institut National de la recherche Agronomique, UMR - PIA "Polymorphisme
d'Intérêt Agronomique", Equipe "Gènes, protéines du grain et qualité des blés", 2 place P.
viala, 34060 Montpellier Cedex 1, France

⁴CIFC - Centro de Investigação das Ferrugens do Cafeeiro, Quinta do Marquês, 2784-505
Oeiras, Portugal

SUMMARY

Arabica coffee (*Coffea arabica* L) varieties may be subject to high losses in potential production due to the orange rust fungus *Hemileia vastatrix*. In some naturally resistant varieties, a specific resistance response called «hypersensitive reaction» is early elicited by fungal infection. To identify host genes involved in the hypersensitive reaction, subtractive cDNA libraries constructed at specific stages of infection were screened by differential hybridization and semi-quantitative RT-PCR experiments. Among the differentially expressed genes were three cDNA clones displaying sequence homology to (i) a transcription factor of the WRKY plant specific family (*CaWRKY*), (ii) the *Arabidopsis* non-race specific disease resistance (NDR1) protein (*CaNDR1*) and (iii) a protein of unknown function (*CaR111*). Southern blotting experiments revealed that these genes are present as single-copy number in the genome of Arabica varieties and other *Coffea* species. mRNAs levels monitored during the early stages of the resistance reaction by real-time quantitative reverse-transcription Polymerase Chain Reaction (qRT-PCR) showed that the three genes were upregulated during pathogen infection. *CaWRKY* displayed the highest and earliest induction, starting as soon as 12 h after inoculation with a peak of induction around 18 h in the pathogen-treated resistant sample. Other genes were overexpressed within 12 h of inoculation, but to a lower extent than *CaWRKY*. These early responses begin prior to penetration of the host and may determine the outcome of the host-pathogen interaction. Further work will aim at understanding the role of selected clones in the mechanism of coffee resistance to parasites.