

Use of Leaf-Disk Technique for Gene Expression Analysis of the Coffee Responses to *Hemileia vastatrix* Infection

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SUMMARY

The most acknowledged method for coffee leaf-rust resistance evaluation uses leaf disks inoculated with *Hemileia vastatrix* and kept in moisture chambers. Besides an efficient control of inoculation conditions, this technique allows a simultaneous evaluation of innumerable plants, with diverse fungal race/coffee genotype combinations, and using low uredospore quantities. The objective of this study was to evaluate the suitability of this technique for the functional gene analysis of coffee responses to leaf-rust infection. A comparison of gene expression in the presence or absence of the pathogen was performed on intact leaves and on leaf disks. To avoid non-specific gene expression due to leaf injury, the leaf disks were prepared 24h and 48h before inoculation and kept moist. *Coffea arabica* plant samples of the resistant Obatã and the susceptible Ouro Verde cultivars were challenged with *H. vastatrix* race II and were collected 24 h after inoculation. Semi-quantitative reverse transcription (RT)-PCR and real time quantitative PCR were used to evaluate expression of several coffee genes. Genes known to be constitutively expressed such as the Glyceraldehyde 3-phosphate dehydrogenase gene or the Ubiquitin gene were used, as well as genes involved in disease resistance responses. Results demonstrated that overall there are differences in the gene expression patterns observed in leaves and disks, either prepared 24h or 48h before inoculation. The genes *PAD3* and *PR1b* showed induction in leaves and in the 48h-disks of Obatã upon rust fungus inoculation, and gene suppression in the 24h-disks treatment. The genes *WRKYs* were activated in leaves and suppressed in disks in the same cultivar. Opposite patterns of *WRKY* expression were detected in disks of Ouro Verde. Our results showed that most of the defense-related genes studied displayed altered patterns of gene expression compared to intact leaves. These results suggest that the leaf-disk technique cannot be successfully used for transcriptomic analysis of coffee-rust interactions.