



Pre-haustorial resistance of coffee HDT832/2 to leaf rust unveiled by transcriptome 454 pyrosequencing

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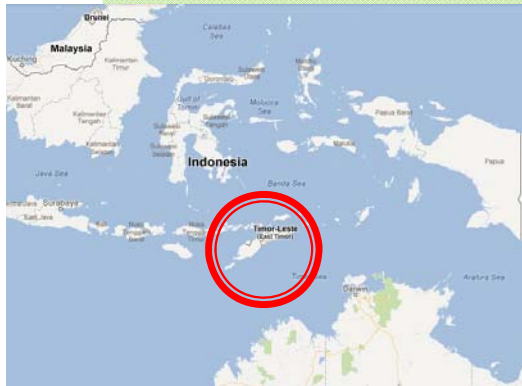
Coffee leaf rust – *Hemileia vastatrix*

- ▶ Leaf rust is the most devastating disease of Arabica coffee (*Coffea arabica*)
- ▶ Causes premature leaf fall and yield losses
- ▶ Is caused by the Basidiomycete fungus *Hemileia vastatrix*



The “Híbrido de Timor” (HDT)

- ▶ Rust **resistant** plants were identified in 1927, at East-Timor, within infected plants in an Arabica coffee plantation
- ▶ These arised from a natural cross between *C. arabica* and *C. canephora*



- ▶ Once shown its resistance to all known rust races, HDT was introduced into coffee breeding programmes and subsequently used in cultivation in 40+ countries

The “Híbrido de Timor” (HDT)

- ▶ However, after 40y cultivation, some of these resistances are beginning to be broken due to the appearance of new rust races, particularly in derivatives of HDT832/1 and HDT1343
- ▶ Still, HDT832/2 (and derivatives ‘Sarchimor’) maintains high levels of resistance, suggesting that its resistance may be more durable than in other HDT genotypes, raising the need to better characterise the resistance of this particular coffee genotype

Pre-haustorial resistance in HDT

- ▶ More frequently, incompatible coffee-rust interactions are characterised by post-haustorial resistance
- ▶ The resistance in HDT832/2 is mostly pre-haustorial, resembling non-host resistance
- ▶ To study the nature of the long-lasting resistance of the HDT 832/2 to *H. vastatrix*, this resistance was compared at the cellular and molecular levels with the non-host resistance of coffee to *Uromyces vignae*

Cytology and RT-qPCR

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Cellular and molecular analyses of coffee resistance to *Hemileia vastatrix* and nonhost resistance to *Uromyces vignae* in the resistance-donor genotype HDT832/2

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Abstract In Arabica coffee breeding, some of the most used sources of resistance to leaf rust (*Hemileia vastatrix*) are natural *Coffea arabica* × *canephora* hybrids (“Híbrido de Timor”). To decipher the cellular and molecular nature of that resistance, leaves of genotype HDT832/2, were challenged with *H. vastatrix* race II, and monitored using light microscopy and RT-qPCR expression analysis of genes involved in plant immunity mechanisms (*receptor-like kinase*, *WRKY* transcription factor 1, *phenylalanine ammonia-lyase*, *chalcone synthase*, *13-lipoxygenase*, *glycosyltransferase*, pathogenesis related *PR1b* and *PR10*). These were compared to

the nonhost resistance responses of HDT832/2 to the infection by the cowpea rust fungus (*Uromyces vignae*). *H. vastatrix* ceased growth more frequently after stomata penetration, forming few haustoria, inducing a hypersensitive-like response, phenol accumulation and haustorium encasement with callose. *U. vignae* could enter stomata but failed to form haustoria, while inducing hypersensitive-like responses and phenol accumulation. In host and nonhost interactions, activation of genes involved in signalling coincided with the differentiation of appressoria, and cellular responses (hypersensitive-like responses and accumulation of phenolic compounds) were recorded from the full appressorium or penetration hypha stages onwards. Similarly, a gene related to the JA pathway was first activated at the penetration hypha stage for both interactions, while genes related to the SA pathway were only activated in the host interaction, the latter being the single clear difference between host and nonhost interactions. The cellular and molecular resistance responses of HDT832/

▶ Cytological and RT-qPCR analyses showed that common immunity components are shared between host and non-host resistance

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454 pyrosequencing analysis

- ▶ Cytology and RT-qPCR enabled the selection of infection time-points corresponding to 50% host cell death (HR) and activation of defense/signalling genes
- ▶ Samples:
 - C – control leaves
 - H – leaves inoculated with *H. vastatrix* (host interaction)
 - U – leaves inoculated with *U. vignae* (non-host interaction)
- ▶ Over 1 million sequences were assembled into ca. 40000 contigs

454 pyrosequencing analysis

- ▶ Large proportion of genes expressed in both compatible and incompatible interactions
- ▶ Over 150 hits to rust genes
- ▶ 90% of genes with homology (NCBI or *Coffea* ESTs); 50% with a predicted function
- ▶ Over 100 PRs / defense proteins

Discussion

- ▶ This study highlights an extensive list of genes expressed in common between host and non-host resistances in coffee HDT832/2
- ▶ Opens the route for the identification of factors governing the gene-for-gene interaction with *H. vastatrix*
 - linking up with on-going projects addressing fungal gene expression and molecular diversity

Thank you

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