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IDENTIFICATION OF DISTINCTIVE TRANSCRIPTOMIC PROFILES AMONG *HEMILEIA VASTATRIX* PATHOTYPES THROUGHOUT KEY STAGES OF THE INFECTION PROCESS

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INTRODUCTION

Hemileia vastatrix (Hv), the pathogen responsible for Coffee Leaf Rust (CLR), has been spreading across the globe and causing devastating socio-economic consequences within coffee production. More than 50 races of Hv have been identified, but its virulence mechanisms are still poorly understood. In this study, we applied a transcriptomic approach to identify candidate virulence genes harbouring differential expression patterns, related to rust pathotypes during its compatible interaction.

MATERIALS | METHODS

1 RNA-seq data was obtained from five pathotypes during compatible interactions with the respective coffee genotype, at three key steps of the infection process, with a total of 45 samples.

Pathotypes	Stage of Infection
Hv1427 (control: v5)	**SI1 – 24h** (Fungal penetration)
Hv70 (v2, v4, v5)	**SI2 – 96h** (Haustoria >50% infection sites)
Hv178 (v2, v3, v5)	**SI3 – 8-11d** (Pre-sporulation)
Hv741 (v2, v5)	
Hv995 (v1, v5)	

2 Fungal RNA Extraction → RNA Sequencing (Illumina)

3 *de novo* transcriptome assembly (Trinity) → Transcript expression quantification (Salmon)

Functional Annotation ← Identification of differentially expressed genes (edgeR)

RESULTS | DISCUSSION

Transcriptome assembly enabled the identification of 27.679 unigenes and a total of 50.380 transcript isoforms. In addition, 2.347 potential secreted proteins with signal peptides were identified that may represent putative effectors. 1.596 transcripts were exclusively expressed in one of the five pathotypes across all infection time-points (**Fig 1**). **Fig 2** shows the GO terms of the unique isoforms exclusive to each pathotype.

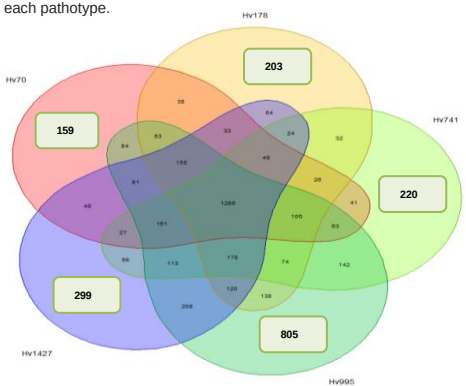

Figure 1- Venn diagrams of the expressed genes for all pathotypes (present in at least 2 or more replicates), which occur in all infection time-points (SI1/SI2/SI3).

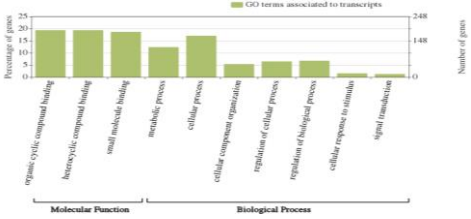

Figure 2- Gene Ontology (GO) plot of the unique transcripts of each pathotype.

We obtained 3.095 differentially expressed genes (DEGs) in all sample comparisons between pathotypes and infection time-points. The heatmap analysis of gene expression values grouped our samples according to the stages of infection (SI), and within each SI, all five pathotypes were separated by their distinct expression profiles (**Fig 3**). Comparisons between time-points and pathotypes revealed a higher number of DEGs in SI3 and for isolate Hv995, which seems to present the most contrasting transcriptomic profile (**Fig 4**). Most annotated DEGs from comparisons between pathotypes (**Fig 5**) were assigned to the binding GO category, related with metabolic/cellular biological processes.

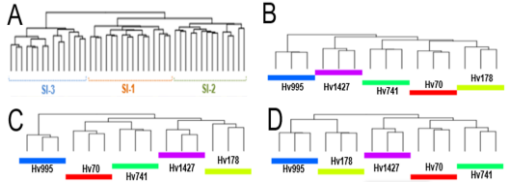

Figure 3- Phylogenetic trees obtained with heatmap analyses of the samples' gene expression patterns. Each branch is a replicate. We considered a DEG with a FDR<=1% and log(Fold Change >= 2). **A)** Comparison between all samples; **B)** Comparison between samples within SI1; **C)** Comparison between samples within SI2; **D)** Comparison between samples within SI3.

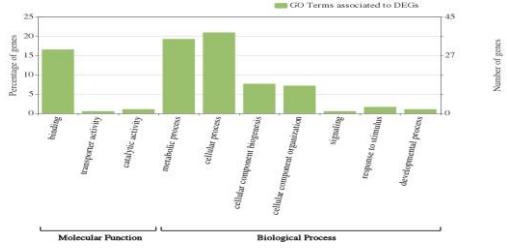

Figure 5- Gene Ontology (GO) plot of the DEGs between pathotype comparisons at same stage of infection.

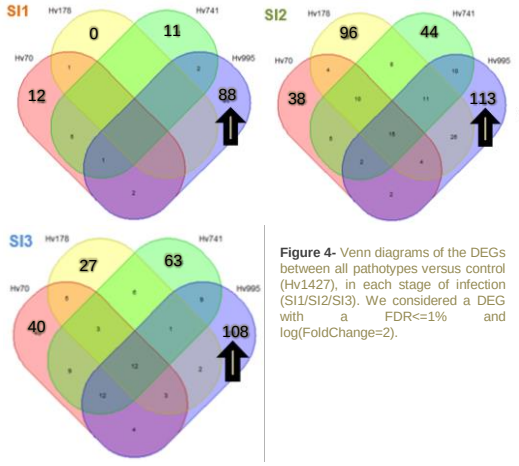

Figure 4- Venn diagrams of the DEGs between all pathotypes versus control (Hv1427), in each stage of infection (SI1/SI2/SI3). We considered a DEG with a FDR<=1% and log(FoldChange=2).

CONCLUSIONS | PERSPECTIVES

Our results show clear distinct gene expression profiles between rust pathotypes and/or infection stages, and pathotype-specific differential expression. Our study provides a deeper insight on the virulence mechanisms of Hv, unveiling vital information about candidate genes and differential expression patterns linked to rust pathotypes, which will allow future functional studies and to exploit diagnostic markers for Hv pathotypes.

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