UNRAVELING FUNGAL AGGRESSIVENESS: PROFILING PATHOGENESIS-ASSOCIATED AND CANDIDATE EFFECTOR GENES IN SUGARCANE SMUT BRAZILIAN ISOLATES USING MULTI-OMICS APPROACHES

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Abstract:
Plants have evolved a complex array of molecules to defend themselves against pathogens. In contrast, pathogens have developed diverse sets of pathogenesis-related mechanisms to evade the plant immune response. In this context, we investigate sugarcane smut disease, a worldwide spread disease caused by the biotrophic fungus Sporisorium scitamineum. It has been found that the fungus expresses a specific set of genes during host colonization, yet the underlying mechanisms responsible for fungal aggressiveness and disease severity are still unknown. Herein, we have sequenced, for the first time, telomere-to-telomere genomes of both complementary mating-type cells from Brazilian isolates exhibiting different levels of aggressiveness to shed light on the role of genetic variability in determining isolate aggressiveness. To better understand gene expression dynamics, we have also sequenced transcriptomes from both isolates while growing in axenic culture and during infection of contrasting sugarcane genotypes, two days after inoculation. We used Oxford Nanopore MinION long-reads and Illumina short-reads platforms for DNA sequencing. For transcriptome data, we used the Illumina platform. We utilized Canu v.2.2 for genome assembly and Pilon v.1.24 for polishing. Variant calling between genomes was performed with GATK4. For differential gene expression analysis, we aligned sequences to the reference genome using Hisat2 v. 2.1.0 and obtained matrix counts with featureCounts v.1.6.0. Statistical analysis was conducted with edgeR v. 4.0.16, considering a gene expressed if it had at least one count per million per replicate for any treatment. Differentially expressed genes (DEGs) were identified with a p-value < 0.05 and an absolute log2 fold change ($|log2FC| > 1$. Finally, GO enrichment was carried out using topGO v. 2.54.0. Even though S. scitamineum reproduces mainly by selfing, which favors homozygosity, and has reportedly low variation, our analysis uncovered point mutation polymorphisms scattered through the genome both in coding and non-coding regions among the isolates. Additionally, non-synonymous mutations were found in pathogenesis-associated genes, along with structural variants located at mating-type loci. Through transcriptomic data, our findings reveal plant cell-wall degrading enzymes strongly induced in the aggressive isolate, alongside genes related to hyphal growth. We also found that xenobiotic transporter metabolism was significantly enriched in the aggressive isolate and induced in the resistant sugarcane genotype, coinciding with the time of the plant's oxidative burst, as highlighted by previous studies. Additionally, we observed different sets of candidate effector genes expressed between the isolates and distinct regulation patterns. In conclusion, our study provides valuable insights into the genetic variability and pathogenic mechanisms of S. scitamineum, the causative agent of sugarcane smut disease. Through comprehensive genome and transcriptome analyses, we identified key genetic differences between isolates with varying levels of aggressiveness. Our findings suggest that distinct isolates exhibit unique strategies during the initial stages of infection, which may contribute to differences in disease severity. Understanding these mechanisms is crucial for advancing plant breeding and disease management.
Palavras-chave: biotrophic fungi; plant-pathogen; virulence; sequencing; fungal genomics

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