COMPARATIVE TRANSCRIPTOMICS OF TWO ISOLATES OF *S. scitamineum* WITH DIFFERENT LEVELS OF AGGRESSIVENESS IN SUGARCANE SMUT DISEASE

Pedro Fernando Vilanova Ferreira[1]

Renato Gustavo Hoffmann Bombardelli[1]

Claudia Barros Monteiro-Vitorello[1]

[1]College of Agriculture "Luiz de Queiroz"/University of São Paulo

pedrovilanova@usp.br

Objectives

The biotrophic fungus *Sporisorium scitamineum* is the causal agent of smut disease in sugarcane, responsible for significant yield losses. Cells carrying different and compatible mating-types are essential for cell fusion and recognition to produce the infective dikaryotic hyphae. Previous studies showed a difference in the aggressiveness of two isolates, SSC04 and SSC39, during sugarcane infection, despite genome similarities, evidenced by comparative analyses. Herein, for the first time, we sequenced the complete genomes of complementary mating-types of the more aggressive isolate SSC04B and less aggressive SSC39A to evaluate the transcriptional profile in search of genetic differences that may justify their distinct behavior in terms of the pathogen aggressiveness.

Materials and Methods

Haploid yeast-like cells from *S. scitamineum* isolates SSC04B and SSC39A stocked at -80ºC in glycerol were used. Genomic DNA extraction was performed following protocol for high quality DNA for long read sequencing. The complete genome sequencing was performed through the Oxford Nanopore MinION platform. Guppy v6.2.1 (Oxford Nanopore Technologies) was used for basecalling. Three different *de novo* genome assemblers were tested, Canu v1.8 (Koren et al. 2017), Fly v2.9 ((Kolmogorov, 2019) and QIAGEN CLC Workbench v.20.0 with the plug-in "Long Read Support" through the tool "De Novo Assemble Long Reads". Assembly polishing was performed through Medaka v1.2.2, Pilon and Racon. BUSCO v5.4.3 (Manni, 2021) was used to assess the assembly completeness using basidiomycota_odb10 database. Comparative analysis were performed by CLC Workbench v20.0 with the reference genome SSC39B (Taniguti et al., 2015). Transcripts from two different and independent experiments were mapped to the reference sequence using BWA-MEM (Li et al., 2013).

Results

The SSC39A genome assembly produced 30 contigs, while SSC04B produced 28. The more effective one in terms of genome completeness between the different tested assemblers was Flye, with 97.7% of Basidiomycota orthologs for the SSC39A genome and 96.8% for the SSC04B genome, after polishing. Furthermore, when aligned to the reference genome SSC04A, the SSC04B genome demonstrated 99.92% of average nucleotide identity and 98.10% of alignment percentage. When comparing two distinct mating-types, there were differences in collinearity regarding the adjacent region of loci *a* and *b* in chromosome 2 (Figure 1), where genes responsible for sexual mating are located.

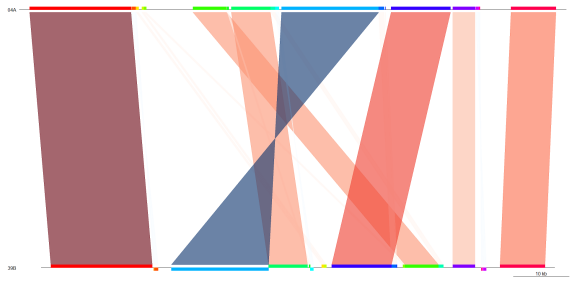

Figure 1. Inversions and relocations identified in the adjacent region of loci *a* and *b* in *Sporisorium scitamineum* chromosome 2 from different mating-types.

The transcript alignment results showed a variation in the number of reads obtained (Figure 2) due to sequencing depth of coverage (SSC04, 199.564 reads; SSC39, 99.341 reads). However, the preliminary results revealed variation in expression between alleles from different isolates and mating types.

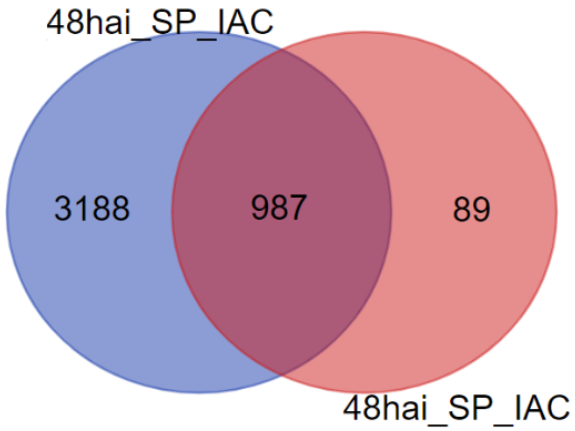

Figure 2. Venn diagram comparing the number of expressed genes by isolates SSC04 (blue) and SSC39 (red) during the infection of susceptible and resistant sugarcane varieties 48 hours after inoculation.

Conclusions

For the first time, we sequenced the complete genomes of two complementary mating-types of two different isolates with distinct levels of aggressiveness. The results suggest that there are changes in the adjacent region of the mating-type loci between genomes, and it is known that balanced structural variations are widespread between fungi species and may indicate positive selection since they may induce positive genetic changes (Gorkovskiy, 2021; Hartmann, 2021). In addition, transcript mapping revealed variation in allele expression, including among candidate effector genes expressed in both isolates 48 hours after inoculation, which will help further analyses of the transcriptional profile.

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