Multi-omics to identify components of the sugarcane-smut molecular cross-talk

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Sugarcane smut disease
**Complex Saccharum spp.**

- S. robustum
- S. spontaneum
- S. officinarum
- S. sinense
- S. barbieri
- S. edule
- Narenga sp.
- Sclerostachya sp.
- Erianthus sp.
- Miscanthus sp.

Genetic background of 20 S. officinarum, two S. spontaneum and a couple of S. barbieri and S. sinense clones.

Modern sugarcane cultivars

Narrow genetic base

Nucelar genome ~10Gb

~10% S. spontaneum

Smut fungi produce teliospores

S. scitamineum gfp-expressing (GFP filter – 505 nm)

Smut Whip

Smut susceptible genotype

20 μM

Biotrophic fungus


**GENETIC VARIABILITY** of *Sporisorium* obtained in different sugarcane regions and infected sugarcane varieties

Microbiology Letters 363 (24), fnw277 (2016)

**METABOLOMIC** profile of resistant and susceptible sugarcane genotypes

Frontiers in plant science 8, 882 (2017)

**FUNCTIONAL ANALYSIS** profile of resistant and susceptible sugarcane genotypes


**COMPARATIVE GENOMICS** of various smut fungi

Frontiers in Microbiology 9, 660 (2018); Molecular Plant-Microbe Interactions 34 (4), 448-452(2017)

Characterization of *S. scitamineum* plant **IMMUNE SUPPRESSING EFFECCTOR PROTEINS**


**MODEL BIOLOGICAL SYSTEMS** to study early events of host responses to smut fungi

Phytopathology (2022)

**Sporisorium BIOLOGICAL NETWORK ANALYSIS**

**TRANSGENIC & GENOME EDITED** smut-resistant plants

**ESALQ**

**PROTEOMIC** profile of resistant and susceptible sugarcane genotypes

IS-MPMI XVIII Congress (2019)

**3rd SET OF TRANSCRIPTOMIC CANE ENERGY** → smut disease

**DUAL TRANSCRIPTOMIC** profile of susceptible sugarcane genotypes

PLoS One 11 (9), e0162237 (2016); Scientific reports 9 (1), 1-12 (2019)

**2nd SET OF TRANSCRIPTOMIC** profile of resistant and susceptible sugarcane genotypes

BMC genomics 20 (1), 1-17 (2019)

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BMC genomics 20 (1), 1-17 (2019)
TF
RGA proteins

host component
effector
PAMP
S/T Kinase
LRR
TM
NBS
CC

transmembrane domain

Signal peptide

intercellular space
apoplast
plasma membrane

PTI
Signaling cascades

ETI
Signaling cascades

ROS

6, 48, and 72 hai (SP80-3280)
RESISTANT X SUSCEPTIBLE

Sorghum bicolor

COMPGG: 72,269 (Cardoso-Silva, 2014) + 16,219 = 88,488
Table 2 Overview of clusters of RGAs predicted within three genome references of sugarcane

<table>
<thead>
<tr>
<th>Statistics</th>
<th>R570</th>
<th>AP85-441</th>
<th>S. bicolor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of clusters</td>
<td>79</td>
<td>136</td>
<td>179</td>
</tr>
<tr>
<td>Total number of RGAs arranged in clusters</td>
<td>308</td>
<td>556</td>
<td>749</td>
</tr>
<tr>
<td>Largest number of RGAs in a cluster</td>
<td>10</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Maximum cluster length (bp)</td>
<td>359,057</td>
<td>742,308</td>
<td>570,975</td>
</tr>
</tbody>
</table>

Fig. 4

A. R570

B. AP85-441

C. S. bicolor

* Enrichment of RG-DAE orthologs
Model organism: *A. thaliana*

**metabolic networks**

**PPI networks**

Uniprot model organisms: Viridiplantae

Experimental and text mining interactions

Text mining: Immune system-enriched networks

**Search terms**

“Plant defence”

“Plant resistance”

- Houses ~1.93 million manually curated protein and genetic interactions from 70 species;
- Includes low-throughput and high-throughput;
- Can be used to build complex networks;
- Free access
Sugarcane Biological Network (COMPGG)

- 9,186 nodes (proteins + reactions)
- 42,499 edges
- Largest component size: 8,548
- 355 RGA nodes (1,829 orthologs)

RGAs (Resistance Analog Genes)

(A) IAC DEGs, (B) SP DEGs, (C) IAC GSEA leading-edge genes, (D) SP GSEA leading-edge genes

Gene Set Enrichment Analysis (Subramanian et al., 2005)
Number of nodes having RGADEs within 18 subgraphs predicted as densely connected regions by MCODE algorithm wrapped in Cytoscape v3.8. Nodes harboring RGADE are depicted according to legend.
RGA Densely connected regions comparison

- Interacts with BAK1

Densely connected subgraph 42 predicted by MCODE. a) SP80-3280. b) IAC66-6.

<table>
<thead>
<tr>
<th>Centrality group</th>
<th>Number of RGA CV nodes</th>
<th>Number of sugarcane orthologs</th>
<th>Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>45</td>
<td>AHK3, CESA6, AT4G21380, AT2G42620, AT2G45140, AT4G23180, AT1G04960</td>
</tr>
<tr>
<td>B</td>
<td>22</td>
<td>126</td>
<td>EFR, COI1, AB36G, AHK4, CESA3, CEPR2, CLV1, AHK2, MIK1, PXL1, 2.4.1.43, AT3G56370, AT5G56750, AT5G4560, AT3G05710, AT3G24660, AT5G47910, AT4G34220, AT5G67280, AT4G04570, AT5G49770, AT4G23270</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>61</td>
<td>BAK1, FLS2, BRI1, Y5838, SUVH2, BRL2, AT5G46860, AT1G45145</td>
</tr>
</tbody>
</table>

CV, cutting vertices; RGA, resistance gene analogs.
**PRR/co-receptors**

- **FLS2**
- **CERK1**
- **BKK1**
- **BAK1**
- **BIK1**

**PAMPs**

- Flagelin
- EF-Tu
- Chitin

**Signaling cascade**

- **MAPK**
- **Phosphorylation**
- **Transphosphorylation**

**NADPH production**

- **Oxidative burst**
  - $\text{O}_2^-$ to $\text{H}_2\text{O}_2$

**WRKYs**

- Defense genes expression

**RESISTANCE**

- **(CDPKs) Calcium-Dependent Protein Kinases**

**ERF**

**BKK1BAK1**

**BIK1**

**BKK1**

**BAK1**

**BIK1**

**RLB1**

**Ca**

**H**

**O**

**O**

**Oxidative burst**

- **NADPH oxidase/respiratory burst oxidase homolog (RBOHs)**
3rd transcriptomic data: Energy cane – smut

Gustavo Crestana,
PhD candidate

RGA-related modules

GSEA Analysis of MCODE Modules

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of MCODE Modules</th>
</tr>
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<tr>
<td>Resistant</td>
<td>18</td>
</tr>
<tr>
<td>Susceptible</td>
<td>14</td>
</tr>
</tbody>
</table>

Positively enriched
Negatively enriched

6 RGA-related modules

Gene expression
- up-regulated
- down-regulated
- not DEG

K-means degree centrality groups
- D
- OC
- B
- A

Resistant

Susceptible

Gustavo Crestana, PhD candidate
S. Scitamineum biological network (U.maydis-based)

Renato Bombardelli, PhD candidate

6696 nodes principal component
57003 edges
62 nodes: DEGs

K-means degree centrality groups
A  B  C  D

RNAseq data analysis
Cutadapt
Hisat2
FeatureCounts
EdgeR
Expressed genes: at least one cpm in all six samples

Dual-layered network modeling

Plant model
U. maydis
Orthologs
S. scitamineum
S. cerevisiae
S. reliantum
S. scitamineum

OrthoFinder:

Metabolic layer

NetworkX
Network Analysis in Python

Cytoscape

Based on Rody et al. 2021
Sugarcane duallayered network (Rody et al., 2020)

Metabolic networks

S. scitamineum network
(Bombardelli, ongoing)

GWAS

Transgenic Plants
Assisted-breeding
Genotype selection

Co-expression

Protein-Protein Interactions

Sugarcane RGAs
(Rody et al., 2019)

RGAs - SwissProt

Microbiome (Metabolic Reconstruction)

Functional Data

16S RNA (Microbiome)

Transcriptome
(Schaker et al., 2016;
Tanguti et al., 2015;
Rody et al., 2019)

Metabolome
(Schaker et al., 2017; Peters, unpublished)

Proteome
(Calderan-Rodrigues, ongoing)
Ph.D. candidates:
Gustavo S. Crestana (Genetics and Plant Breeding)
Gustavo L. Rodrigues (Genetics and Plant Breeding)
Jessica F. Mendes (Genetics and Plant Breeding)
Renato G.H. Bombardelli (Genetics and Plant Breeding)
Gustavo Husein (Genetics and Plant Breeding)
Tiarla G. Souto (Phytopathology)
Joyce D. Ferretti (Phytopathology)

Master’s students:
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Enrico D.R. Batista (Genetics and Plant Breeding)
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Ana Julia C. Franceschi
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