



GENOME 20+2

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

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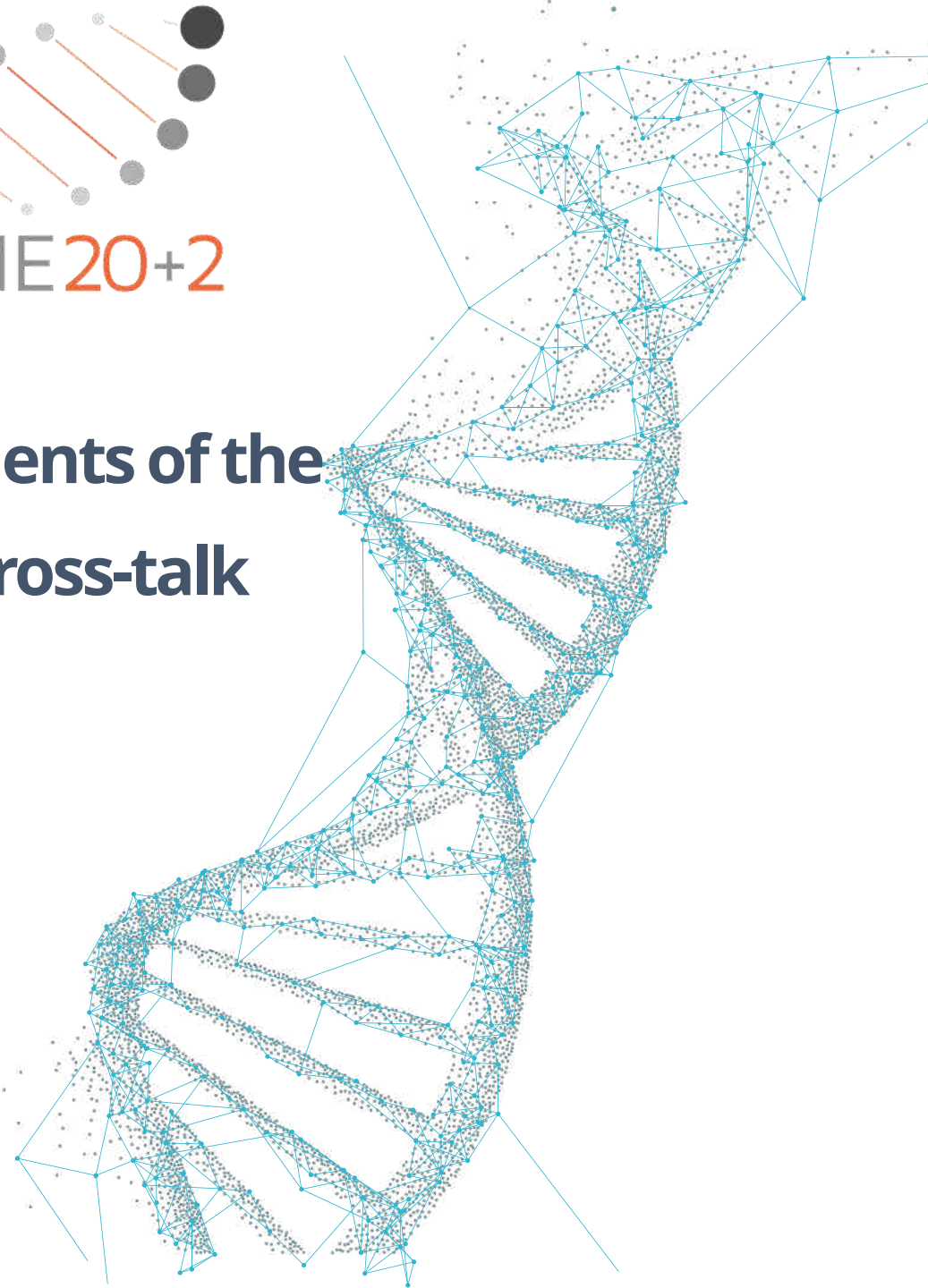
USP



ESALQ



GENOMICS
GROUP



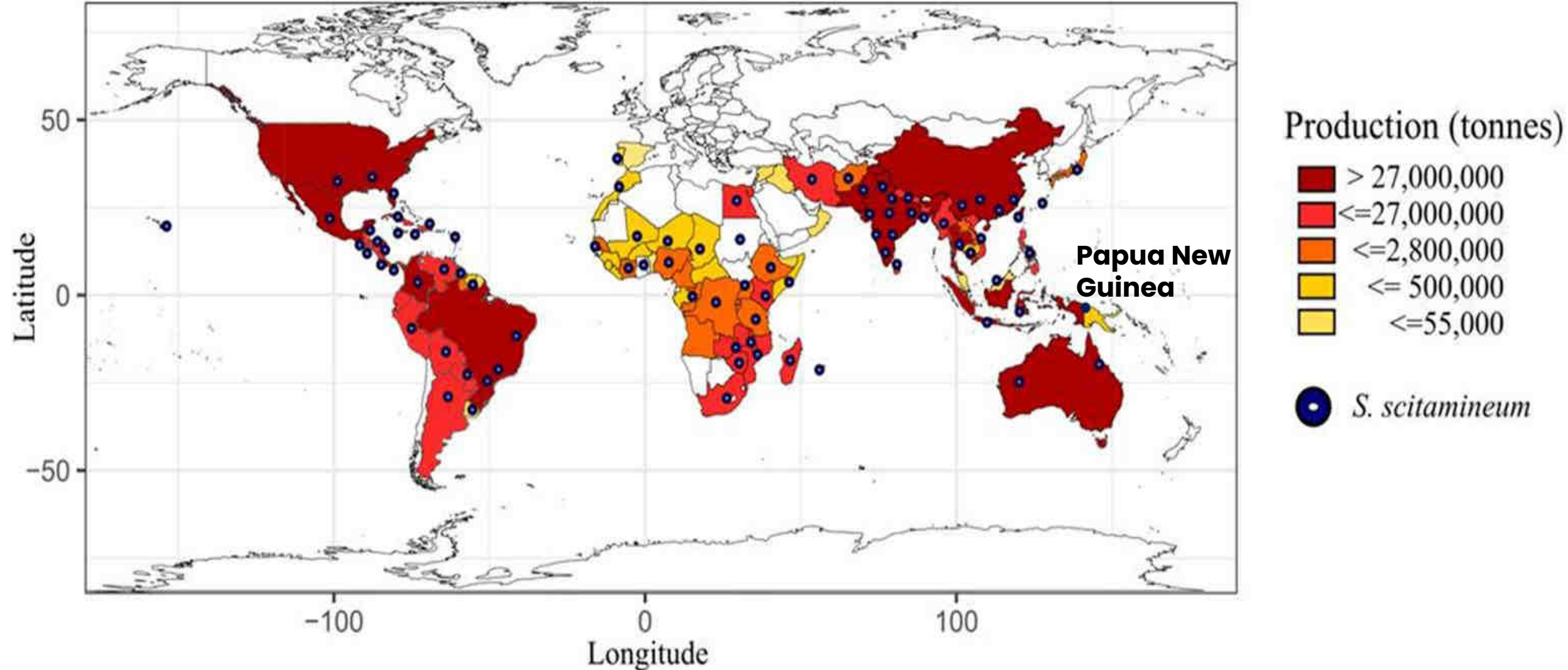
genética 2036

Um olhar para os 100 anos



Sugarcane smut disease

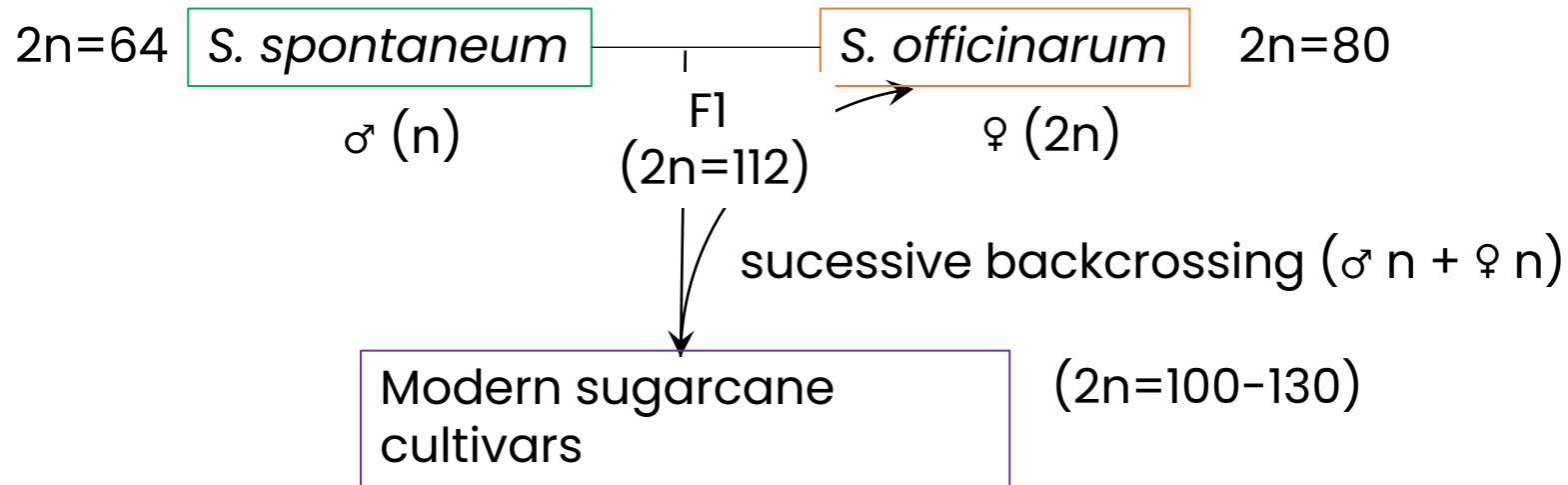
Sugarcane smut world distribution



Monteiro-Vitorello et al., 2018. Progress in understanding fungal diseases affecting sugarcane: smut. IN: Achieving sustainable cultivation of sugarcane Volume 2: Breeding, pests, and diseases (Burleigh Dodds Series in Agricultural Science) 1st Edition, Prof. Philippe Rott (Ed); 2018.

Complex *Saccharum* spp.

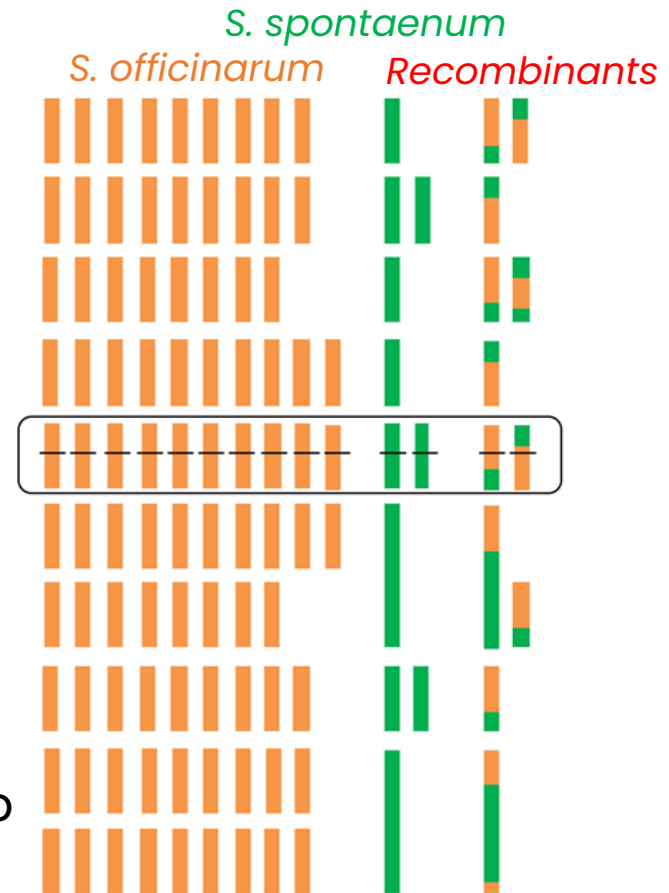
S. robustum *S. spontaneum* *S. officinarum* *S. sinense* *S. barbieri* *S. edule*
Narenga sp. *Sclerostachya* sp. *Erianthus* sp. *Miscanthus* sp.



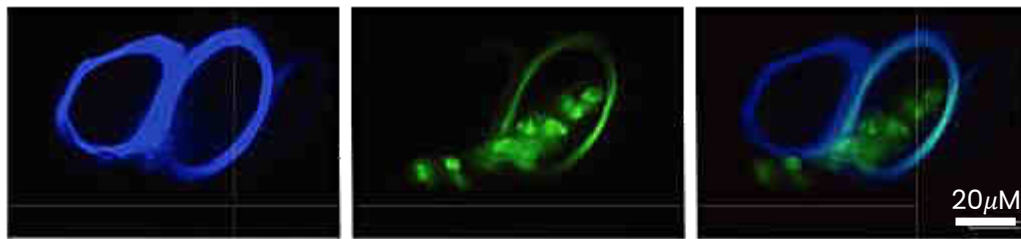
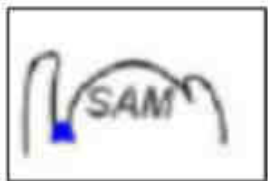
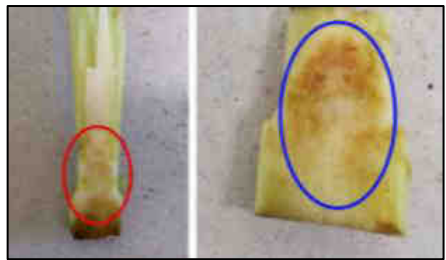
Genetic background of 20 *S. officinarum*, two *S. spontaneum* and a couple of *S. barbieri* and *S. sinense* clones

Narrow genetic base

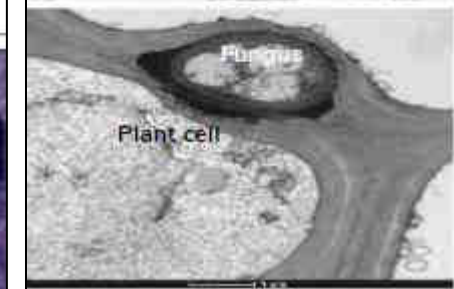
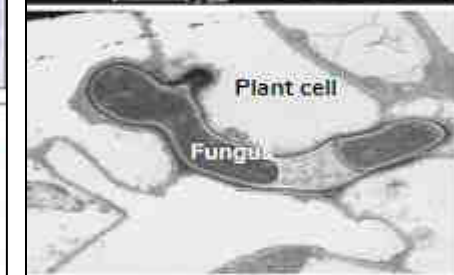
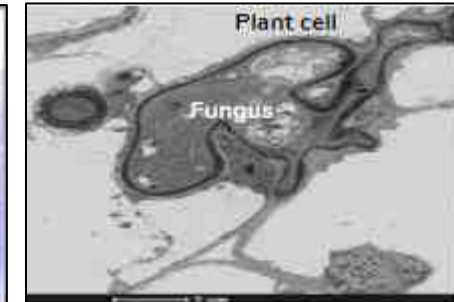
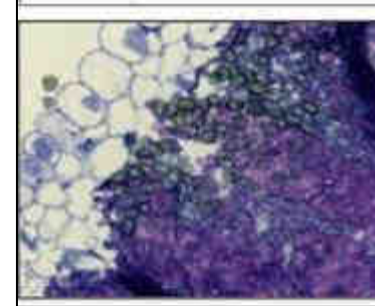
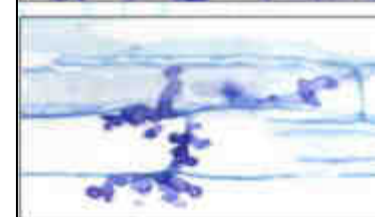
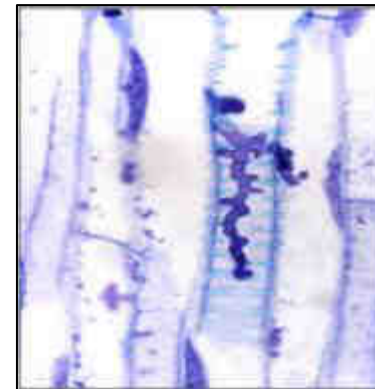
Cunff et al. Genetics. 2008, 180: 649-66



Smut fungi produce teliospores

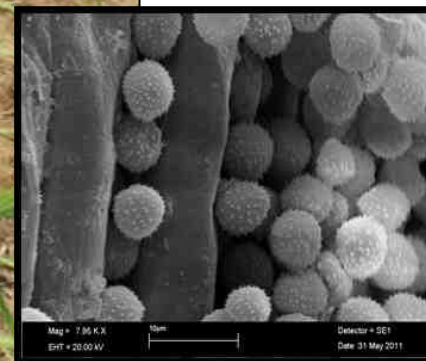
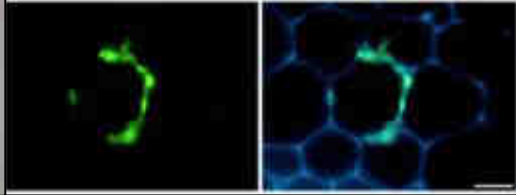
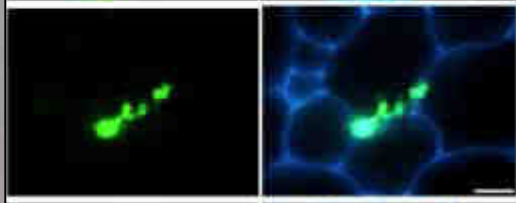
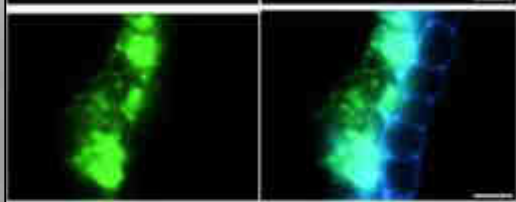
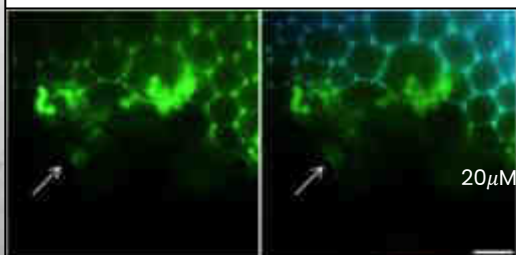


S. scitamineum gfp-expressing (GFP filter – 505 nm)



Biotrophic fungus

Smut Whip



Carvalho, et al.
Ann Appl Biol. 2016; 169: 329–341.

smut susceptible genotype

Ann Bot. 2016;119(5):815–827.



Marques et al.

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

Fungal biology 124 (12), 1039-1051 (2020);
Planta 245 (4), 749-764 (2017)

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 EFFECTOR PROTEINS**

Journal of Fungi 6 (4), 339 (2020)

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

S.scitamineum **GENOME** (chromosome scale)
PloS one 10 (6), e0129318 (2015)

S.scitamineum **BIOLOGY** and interaction with sugarcane plants

Annals of botany 119 (5), 815-827 (2017);
Annals of Applied Biology 169 (3), 329-341(2017)

DUAL TRASCRIPOMIC profile of susceptible sugarcane genotypes

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

2nd SET OF TRASCRIPOMIC profile of resistant and susceptible sugarcane genotypes

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

Genome survey of **RESISTANCE GENE ANALOGS** in sugarcane

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

Arabidopsis-Based Dual-Layered **SUGARCANE BIOLOGICAL NETWORK ANALYSIS**

Frontiers in Plant Science. Plant Systems and Synthetic Biology (2020)

PROTEOMIC profile of resistant and susceptible sugarcane genotypes

IS-MPMI XVIII Congress (2019)

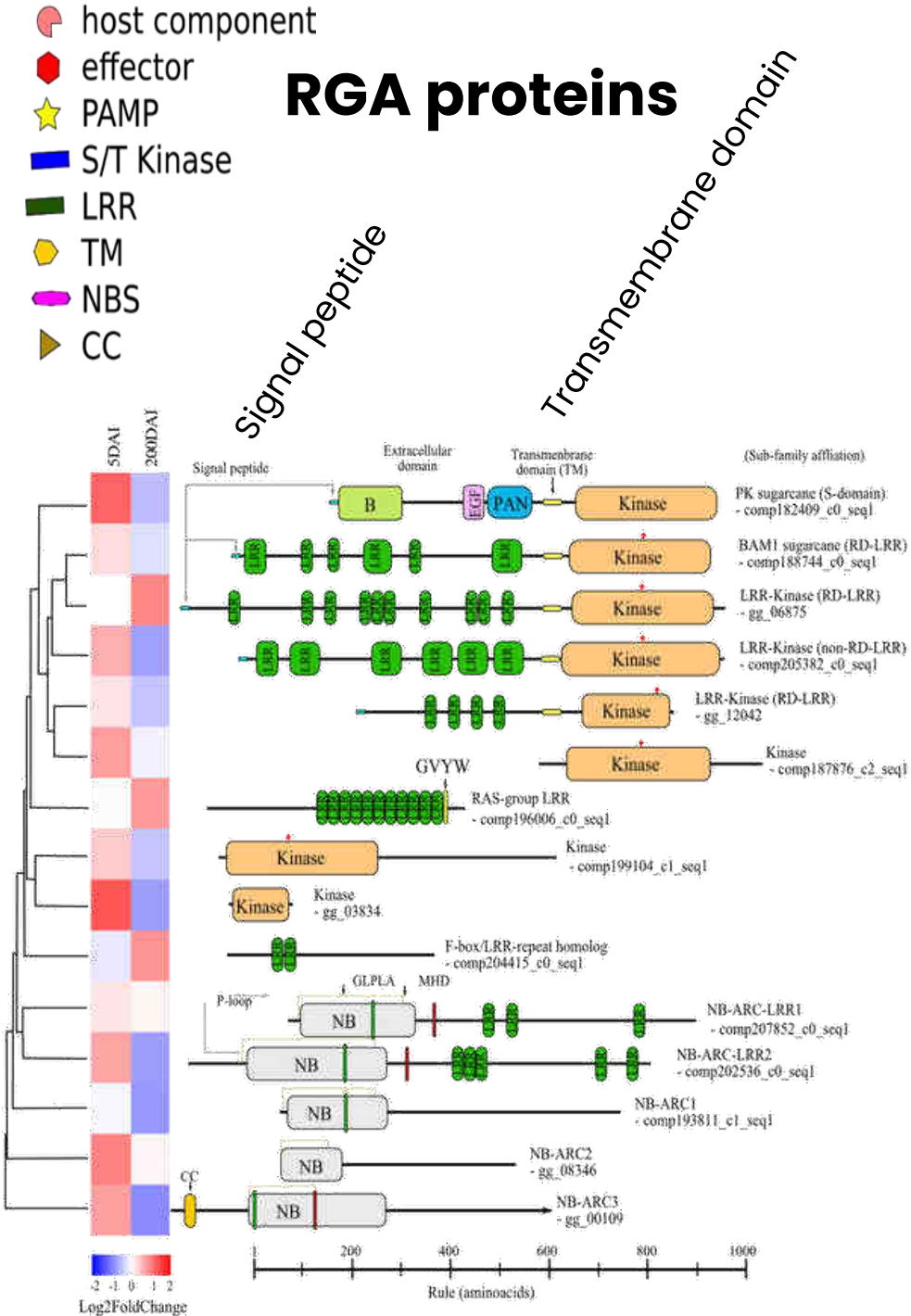
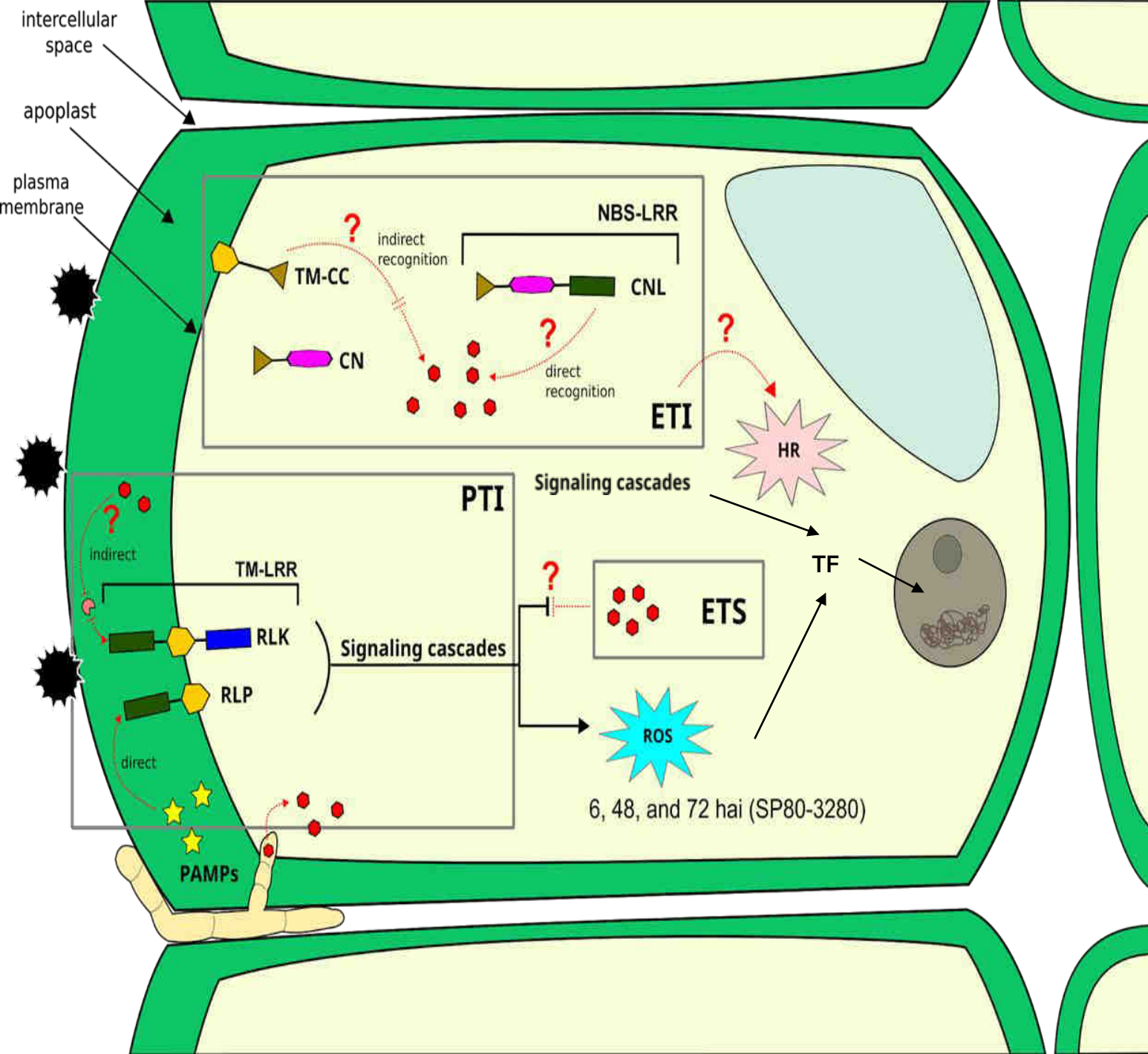
3rd SET OF TRASCRIPOMIC CANE ENERGY → smut disease

4th SET OF TRASCRIPOTOME → comparative analysis



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Schaker, et al.
Plos One. 2016, 8:882.

RESISTANT X SUSCEPTIBLE
1st transcriptomic data 2nd transcriptomic data

Data collection

Genomes

- R570,
- AP85-441,
- Sorghum.

Type of sequences: protein, coding-DNA
Annotation: of predicted genes, chromosome coordinates

Transcriptomes

- COMPGG: Cardoso-Silva et al. (2014) and Schaker et al. (2016).

Type of sequences: transcript, largest ORFs

RGA predictions

Input
Protein sequences, ORFs

Structural analysis

- InterproScan v5.33
- PfamScan v32
- Phobius
- TMHMM v2
- Coils v2

Orthology searches

- PRGdb
- BLASTp

Shared synteny

Input
Protein sequences, ORFs

DAIGCHAINER

- BLASTp all-against-all
- E-value cutoff 1e-05

RGA clusters

Input
Protein sequences, ORFs

DAIGCHAINER

- BLASTp all-against-all
- E-value cutoff 1e-05

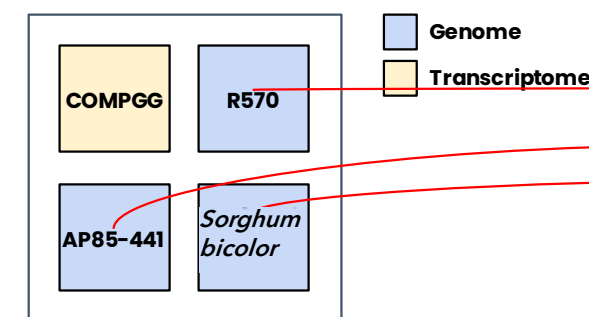
RGADE phylogeny

Input
RGAs differentially expressed
COMPGG ORFs

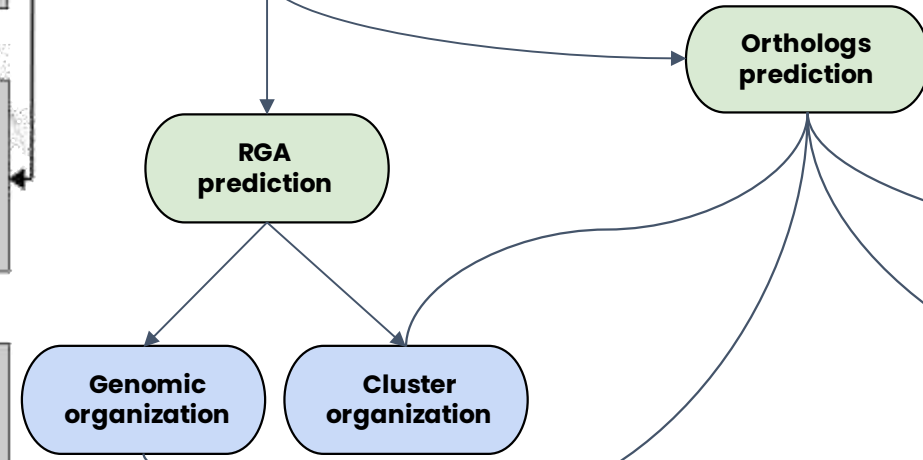
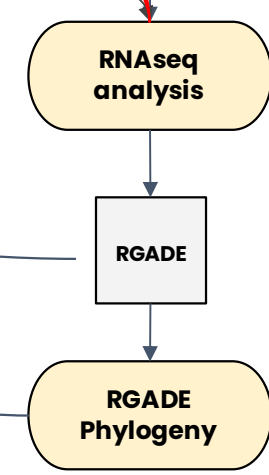
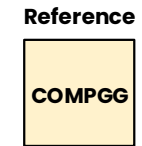
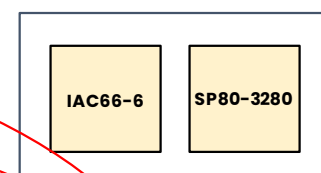
- Muscle
- FastTree v2.1.10
- iTOL v4.3.2

1st transcriptomic data

Download data



Brand new data



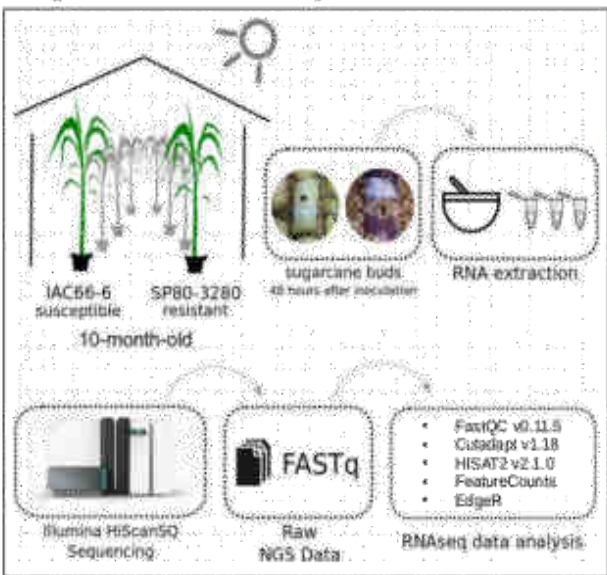
Orthology searches

Input
Protein sequences, ORFs

OrthoMCL

- BLASTp all-against-all
- E-value cutoff 1e-05

Sugarcane transcriptomes obtention



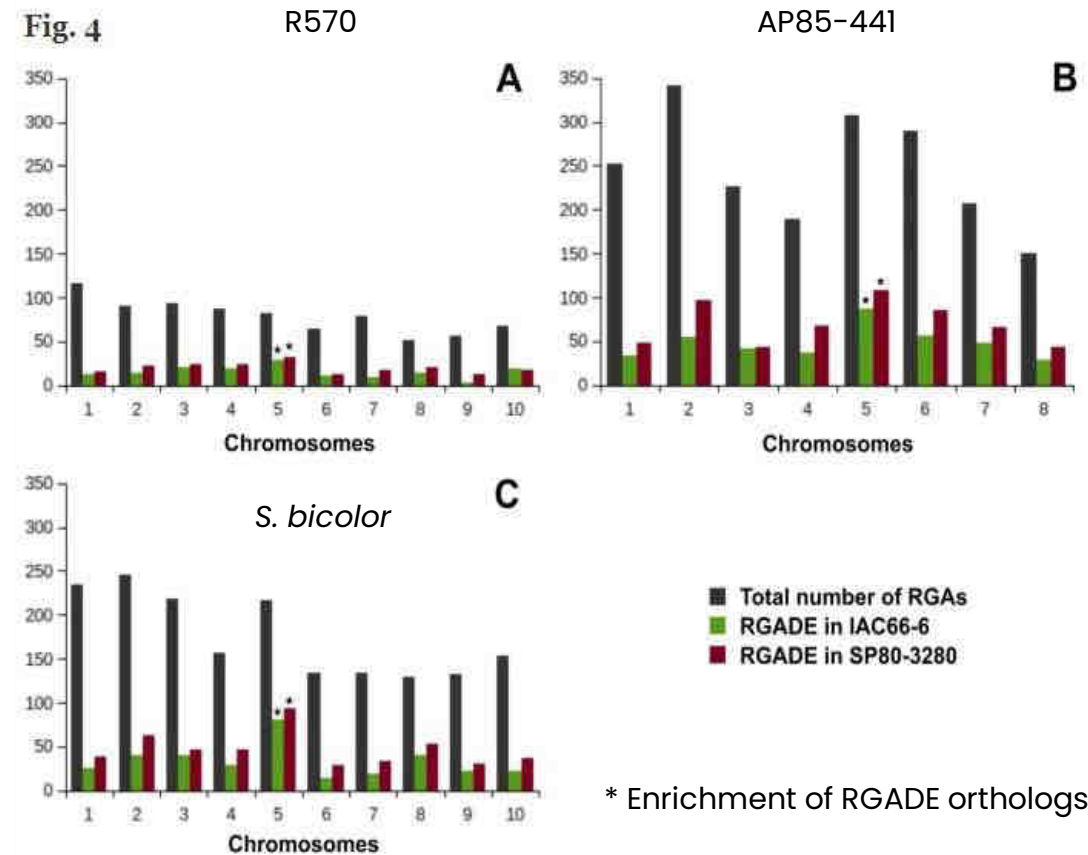
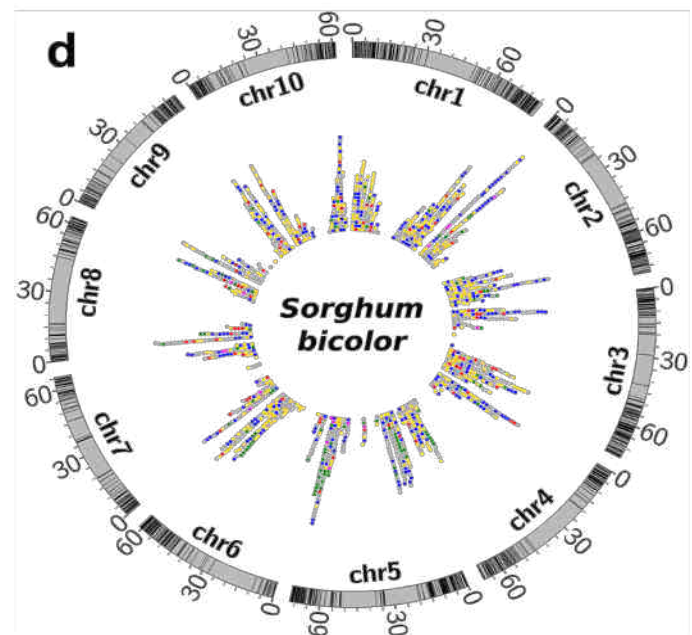
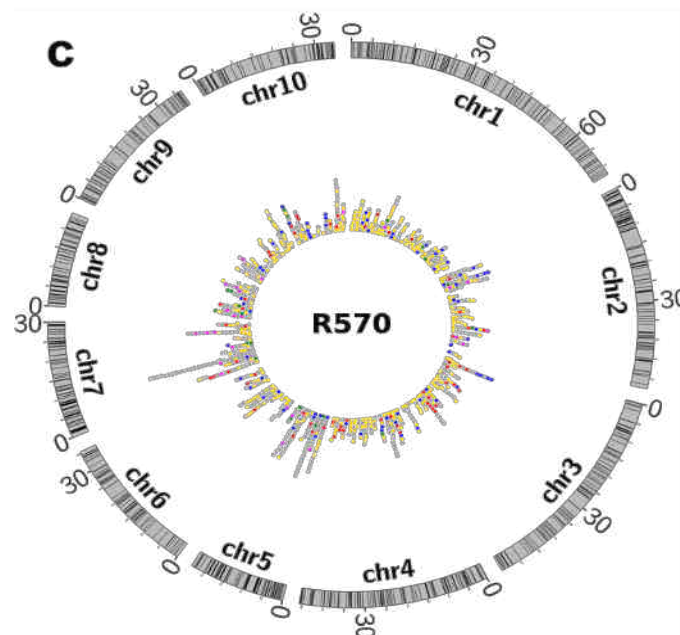
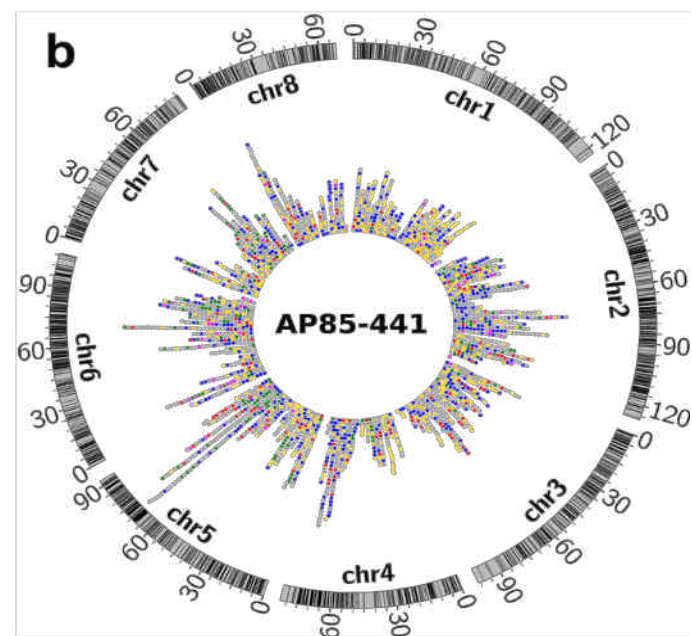
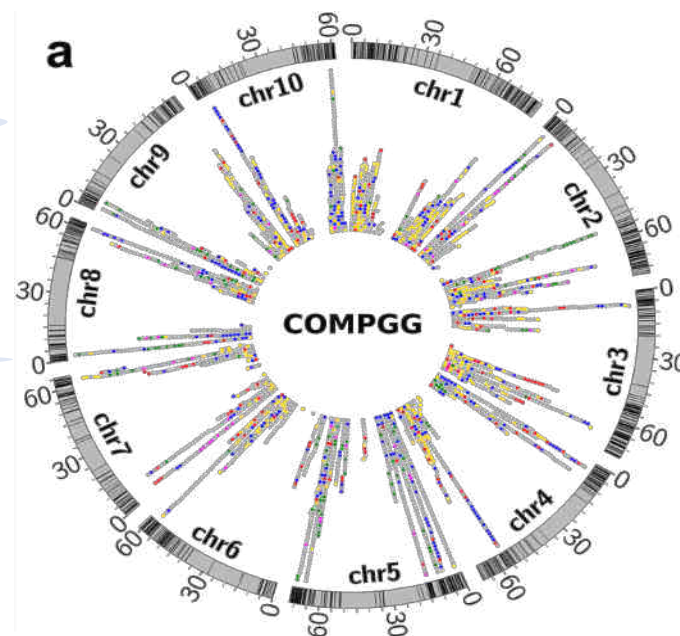
COMPGG: 72,269 (Cardoso-Silva, 2014) + 16,219 = 88,488

□ Data obtention/collection ◆ Data/analysis integration
■ Analysis



Table 2 Overview of clusters of RGAs predicted within three genome references of sugarcane

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





Model organism: *A. thaliana*

metabolic networks

PPI networks



Uniprot model organisms: Viridiplantae
Experimental and text mining interactions

Text mining: Immune system-enriched networks

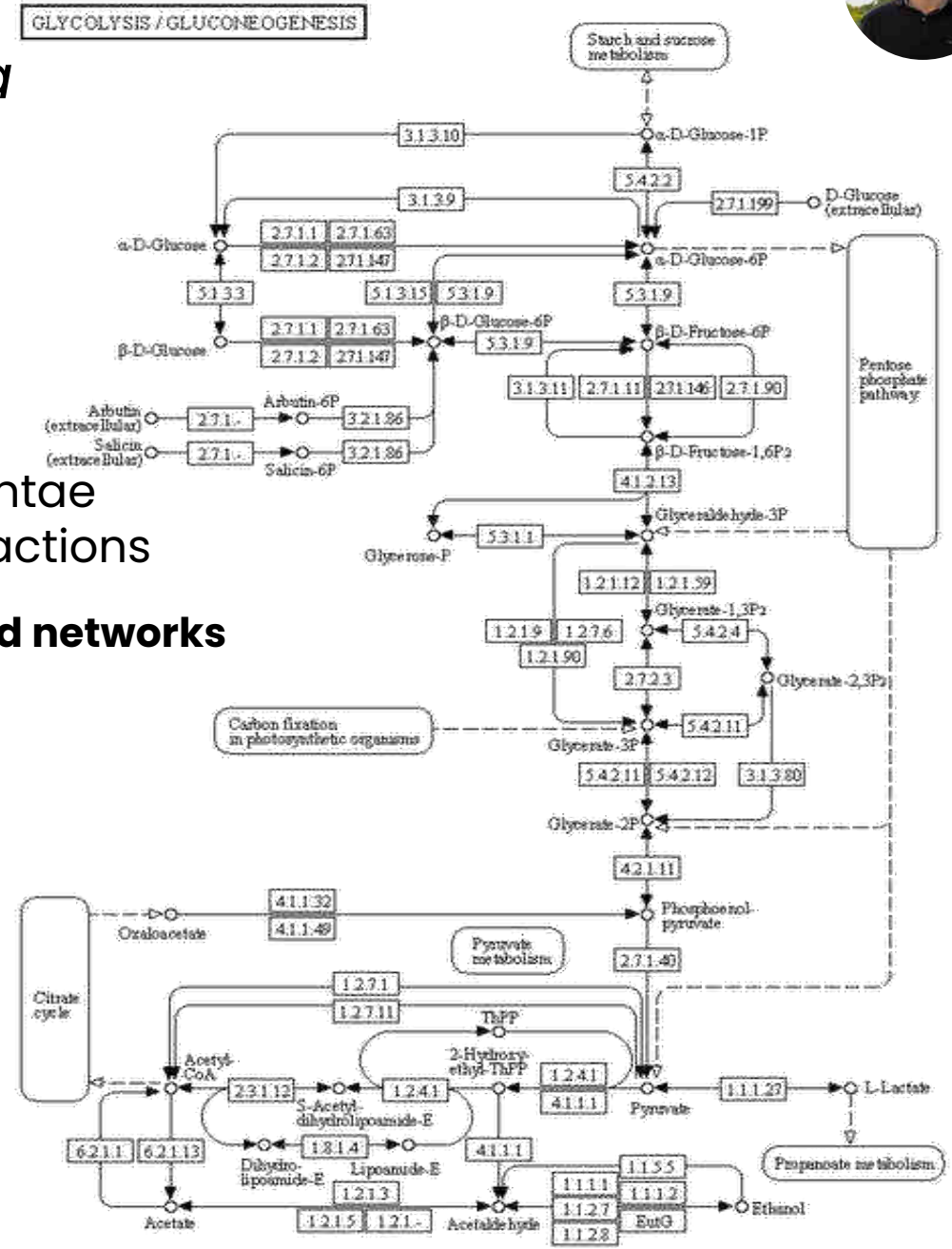
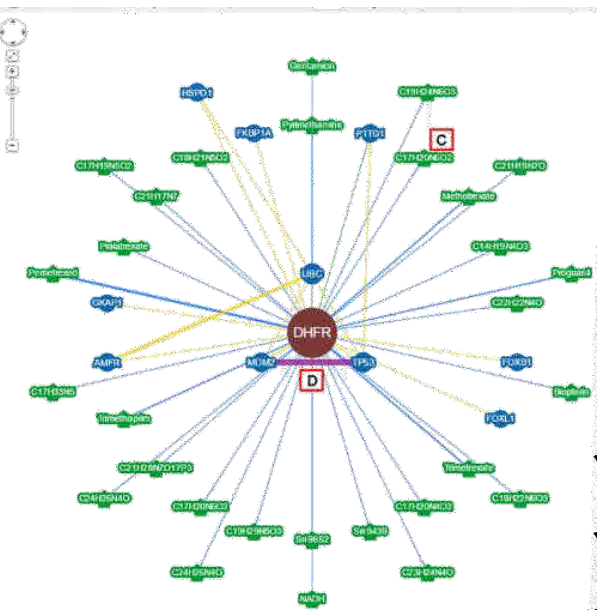


Search terms

"Plant defence"

"Plant resistance"

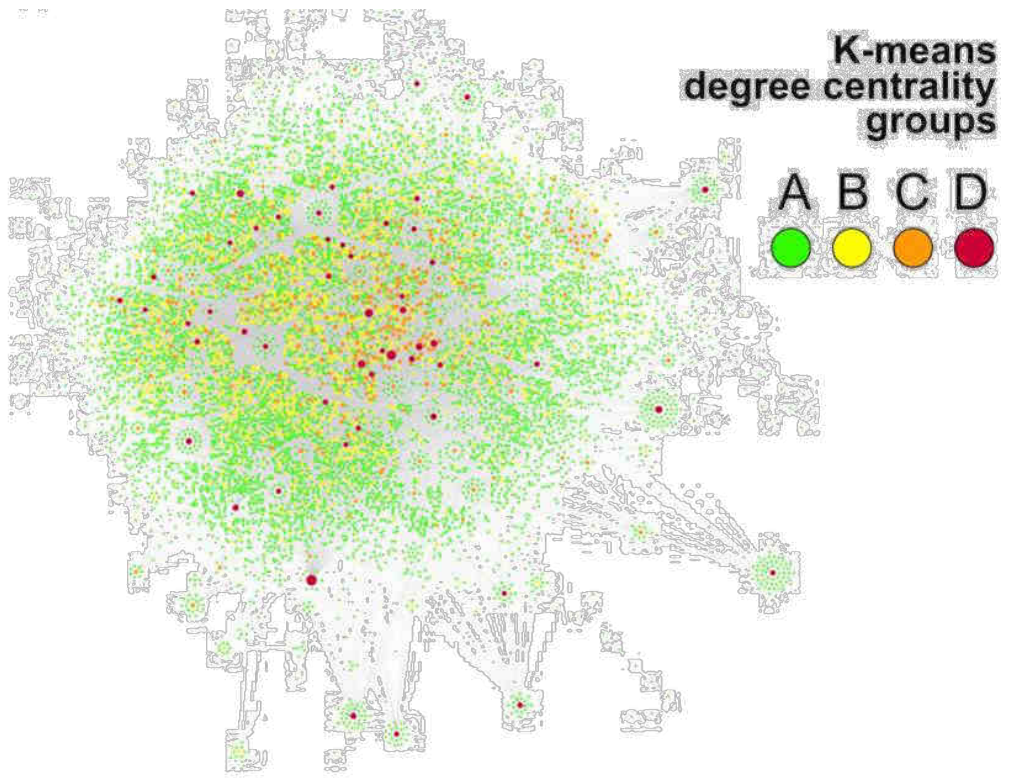
- ✓ Houses ~1.93 million manually curated protein and genetic interactions from 70 species;
- ✓ Includes low-throughput and high-throughput;
- ✓ Can be used to build complex networks;
- ✓ Free access



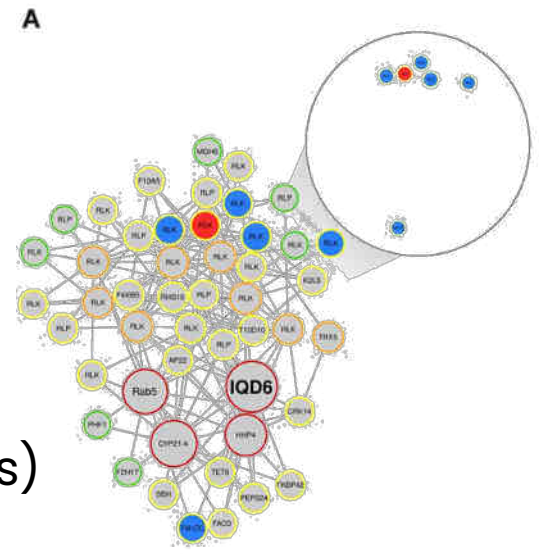
Sugarcane Biological Network (COMPGG)

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

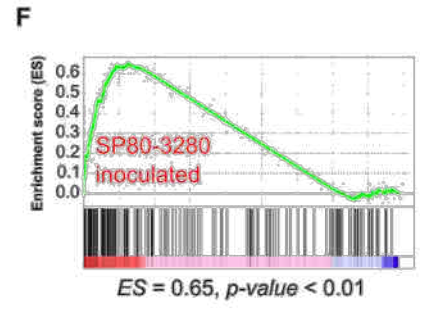
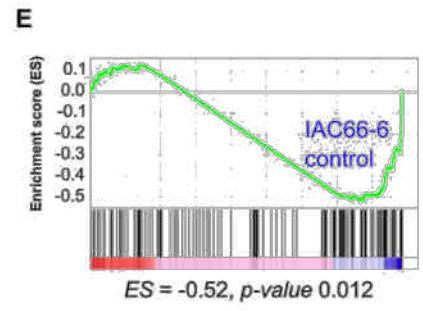
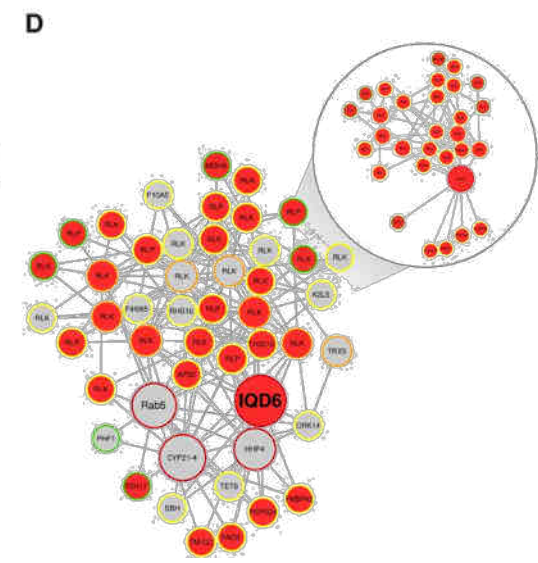
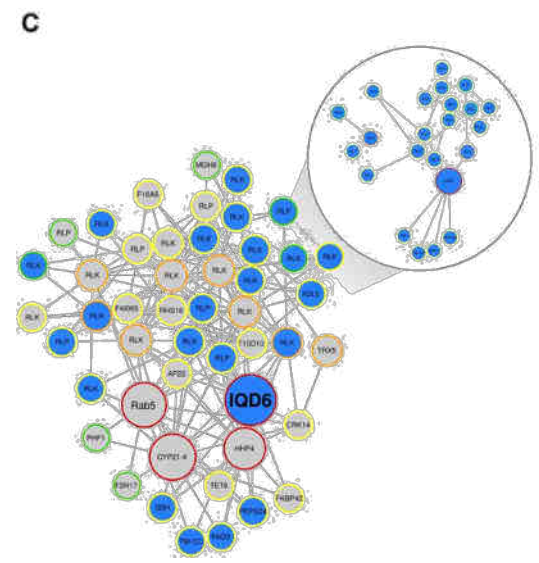
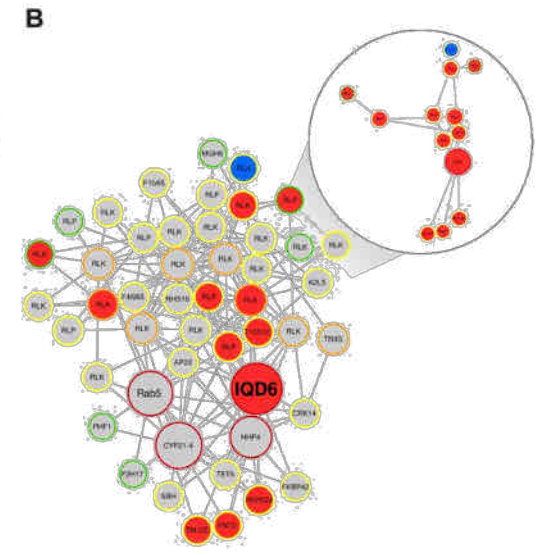
RGAs (Resistance Analog Genes)



Susceptible



Resistant



Gene expression

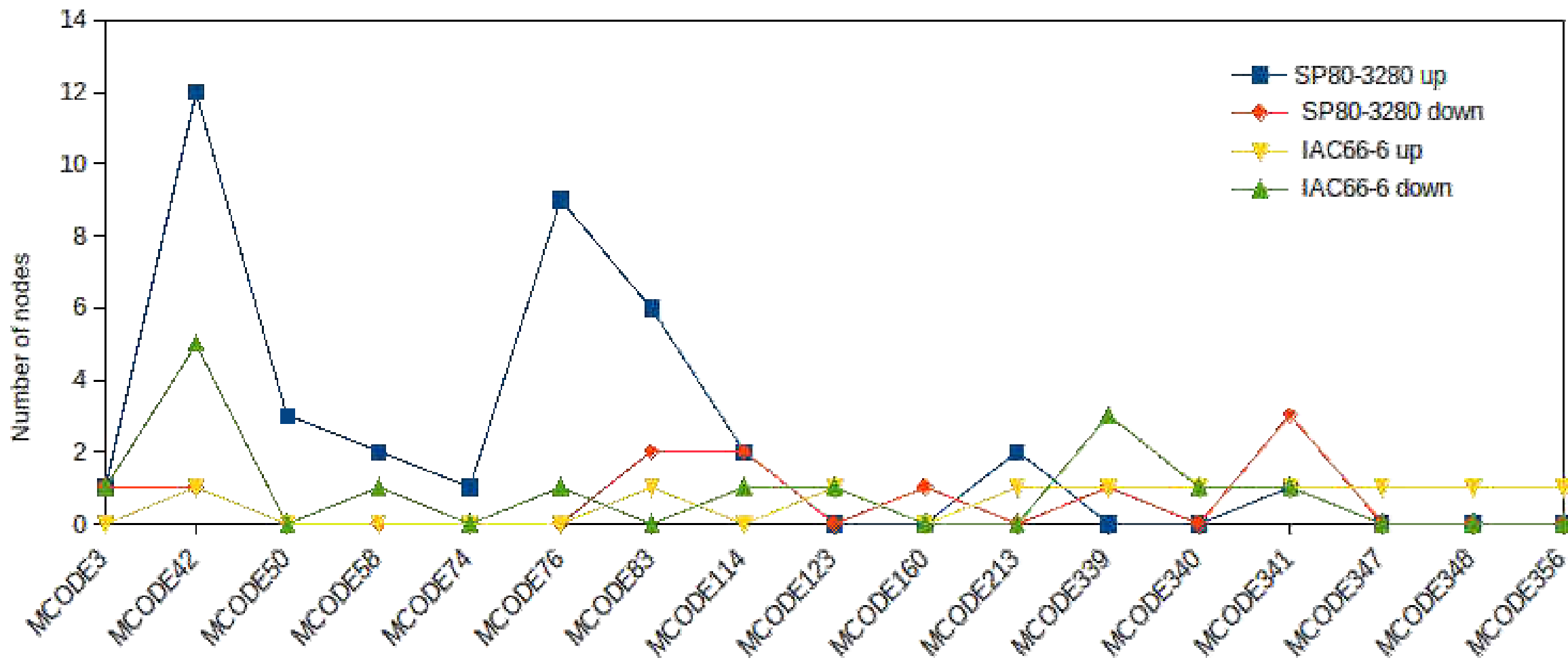
- up-regulated (red)
- down-regulated (blue)
- not DE (grey)

K-means degree centrality groups

- A (green)
- B (yellow)
- C (orange)
- D (red)

(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)

RGA Densely connected regions comparison

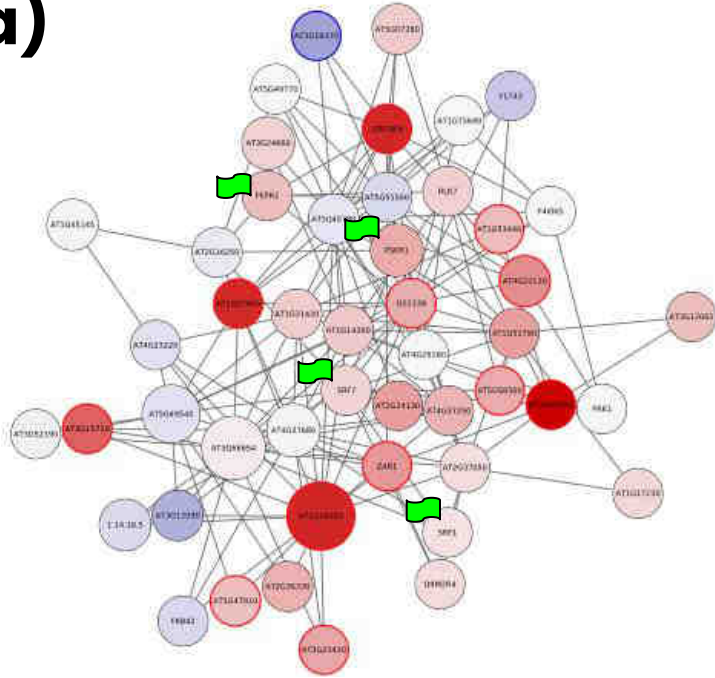


Number of nodes having RGADEs within 18 subgraphs predicted as densely connected regions by MCODE algorithm wrapped in Cytoscape v3.8. Nodes harboring RGADE are depicted according to legend.

RGA Densely connected regions comparison

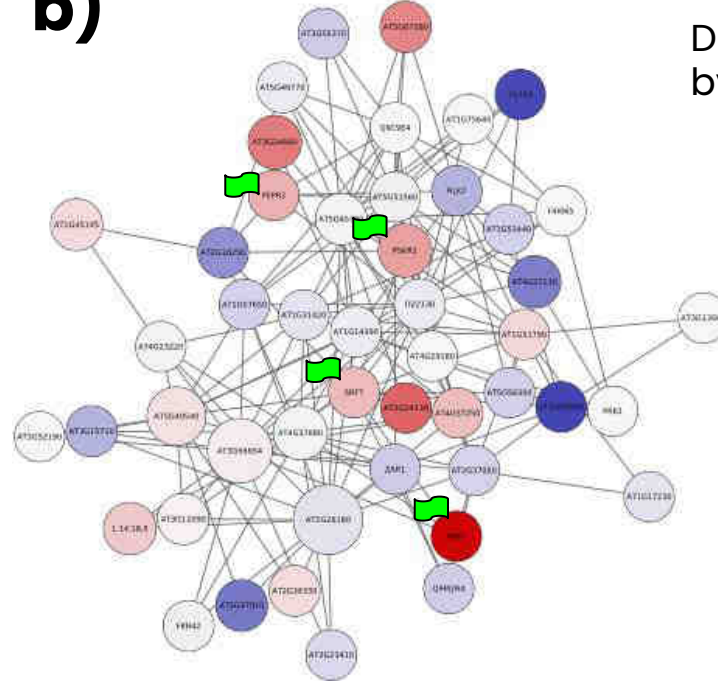
 Interacts with BAK1

a)



SP80-3280

b)

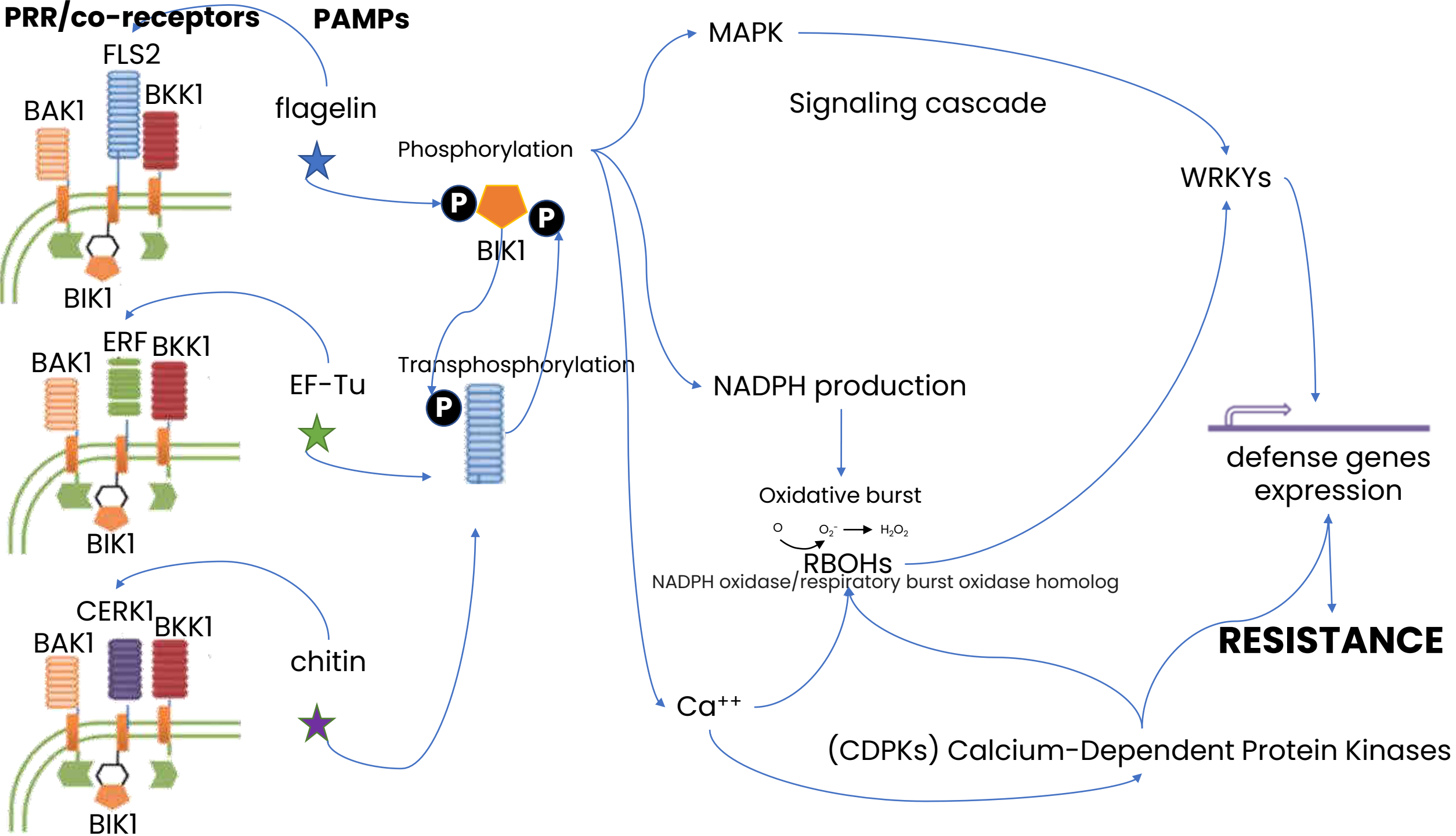


IAC66-6

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

Centrality group	Number of RGA CV nodes	Number of sugarcane orthologs	Nodes
A	7	45	AHK3, CESA6, AT4G21380, AT2G42620, AT2G45140, AT4G23180, AT1G04960
B	22	126	EFR, COI1, AB36G, AHK4, CESA3, CEPR2, CLV1, AHK2, MIK1, PXL1, 2.4.1.43, AT3G56370, AT5G56750, AT5G64560, AT3G05710, AT3G24660, AT5G47910, AT4G34220, AT5G67280, AT4G04570, AT5G49770, AT4G23270
C	8	61	BAK1, FLS2, BRI1, Y5838, SUVH2, BRL2, AT5G46860, AT1G45145

CV, cutting vertices; RGA, resistance gene analogs.

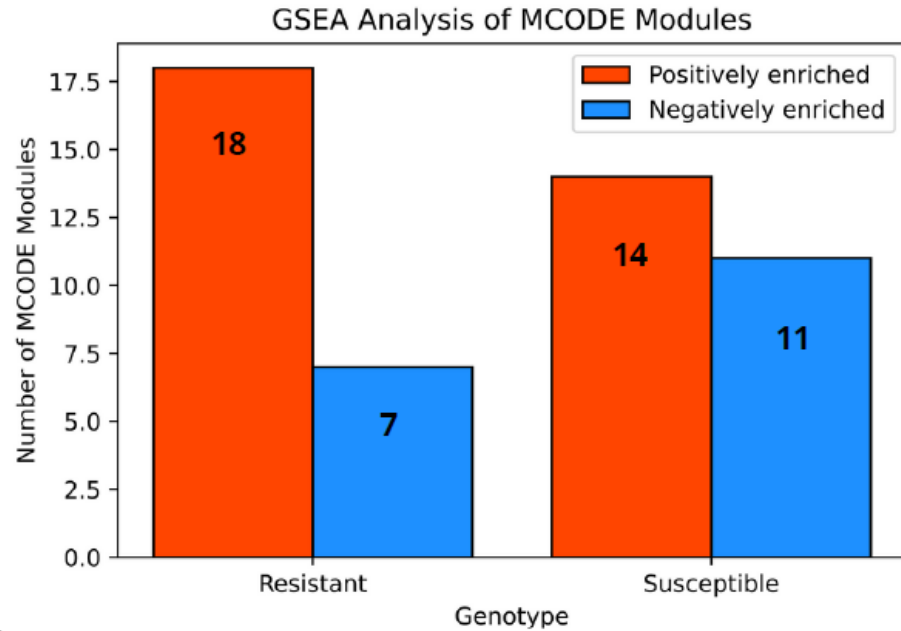


3rd transcriptomic data: Energy cane - smut

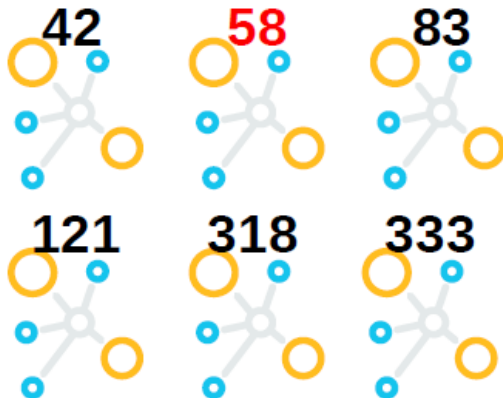
Gustavo Crestana,
PhD candidate



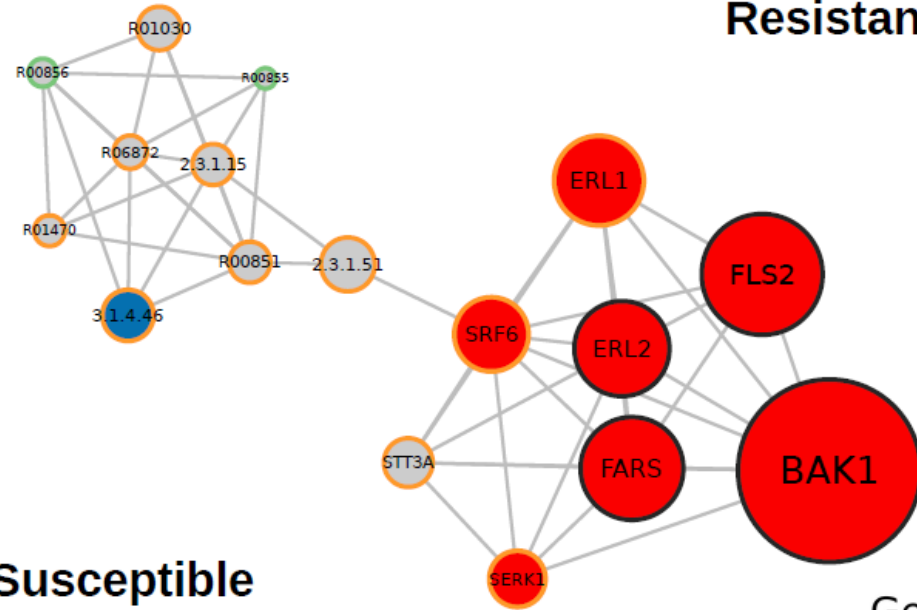
RGA-related modules



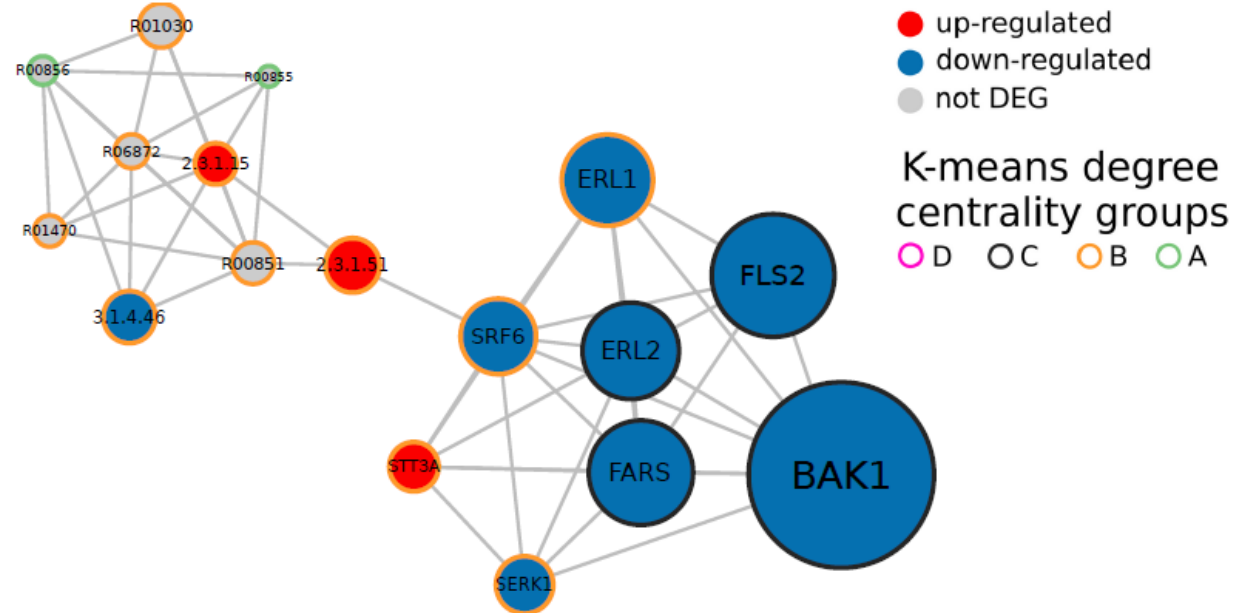
6 RGA-related modules



Resistant



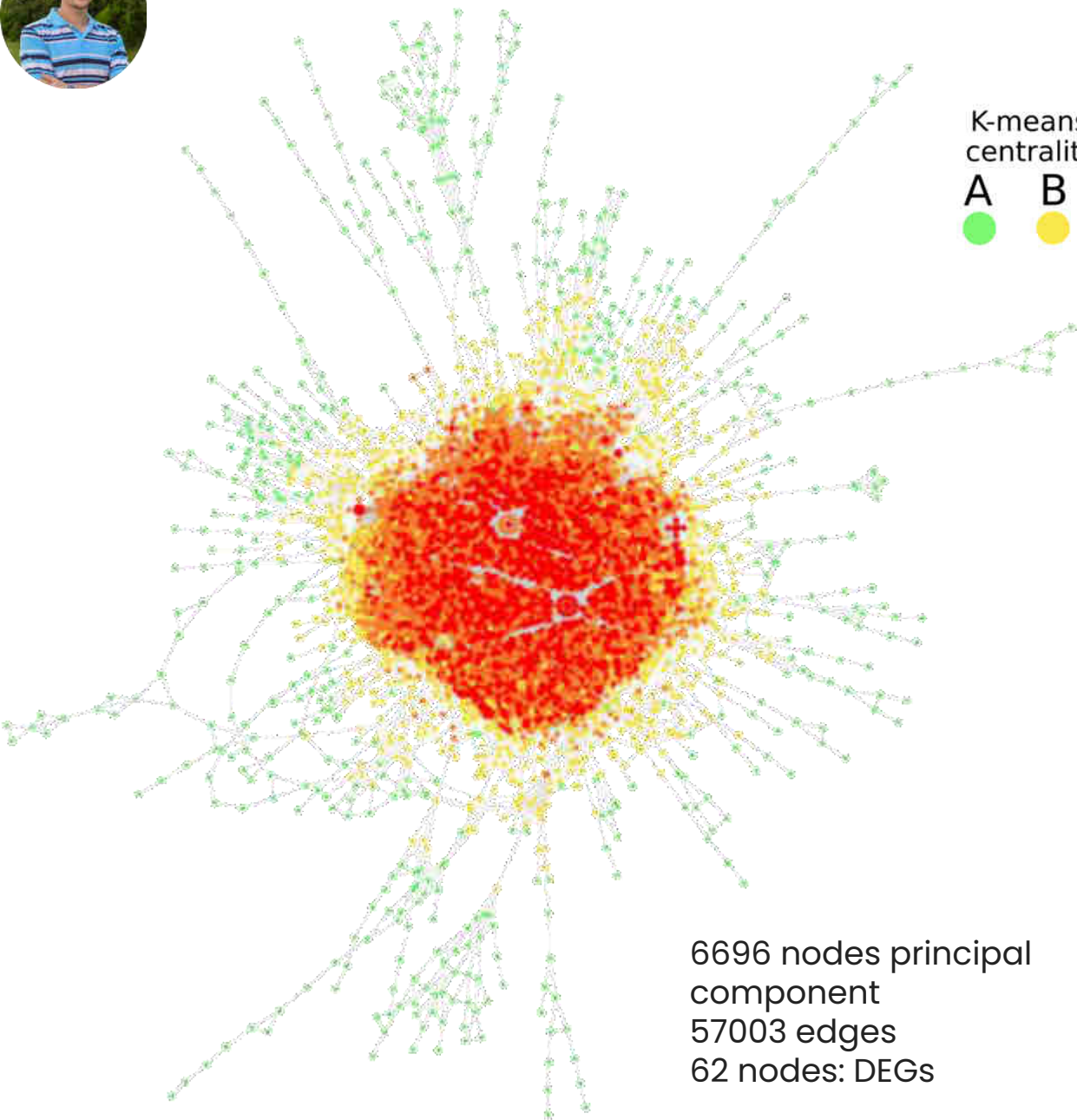
Susceptible





Renato Bombardelli,
PhD candidate

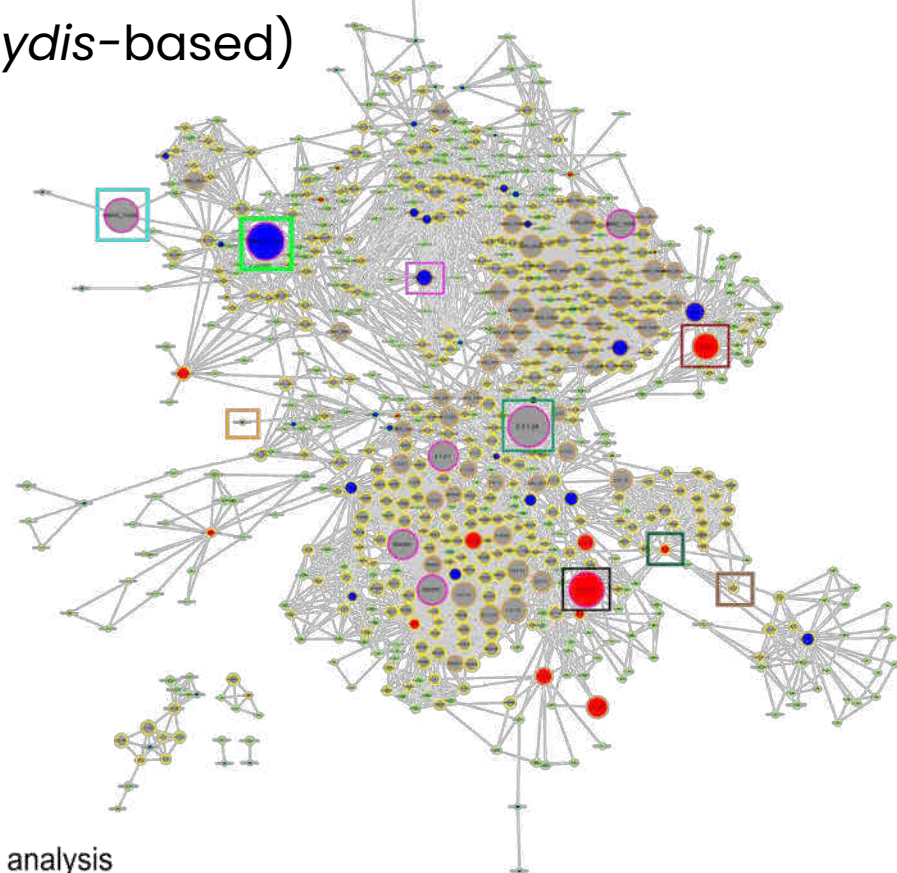
S. Scitamineum biological network (*U.maydis*-based)



K-means degree centrality groups

A B C D

● ● ● ●



RNAseq data analysis

Cutadapt
Hisat2
FeatureCounts



Expressed genes: at least one *cpm* in all six samples

Dual-layered network modeling



Metabolic layer

Plant model
U. maydis



Orthologs
S. scitamineum

OrthoFinder:
U. maydis
S. cerevisiae
S. reilianum
S. scitamineum



PPI layer



NetworkX
Network Analysis in Python

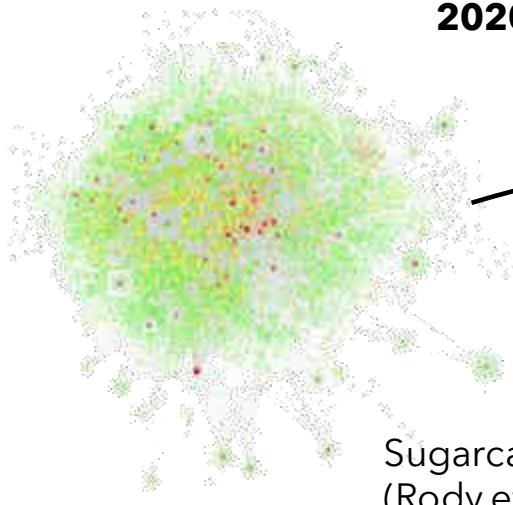


Cytoscape



Based on Rody *et al.* 2021

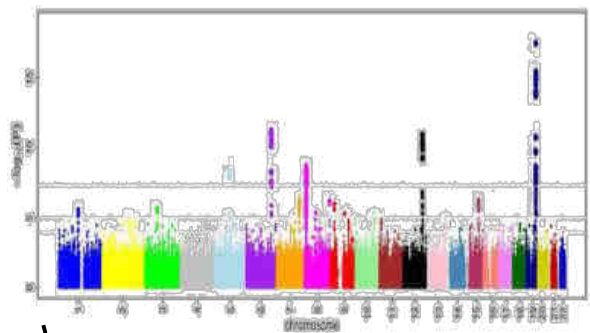
Sugarcane duallayered network (Rody et al., 2020)



Metabolic networks

***S. scitamineum* network**
(Bombardelli, ongoing)

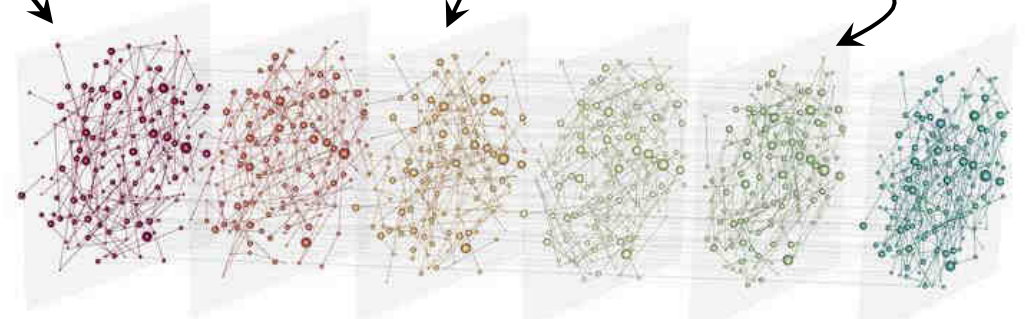
Microbiome (Metabolic Reconstruction)



GWAS

Sugarcane RGAs
(Rody et al., 2019)

RGAs - SwissProt



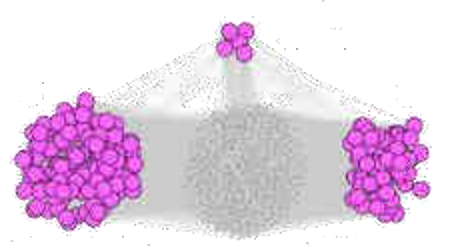
Transgenic Plants

Assisted-breeding

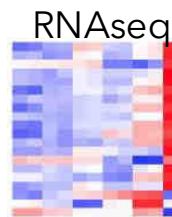
Genotype selection

Sugarcane genotypes
(genealogy and features)

Protein-Protein Interactions



Co-expression



RNAseq

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

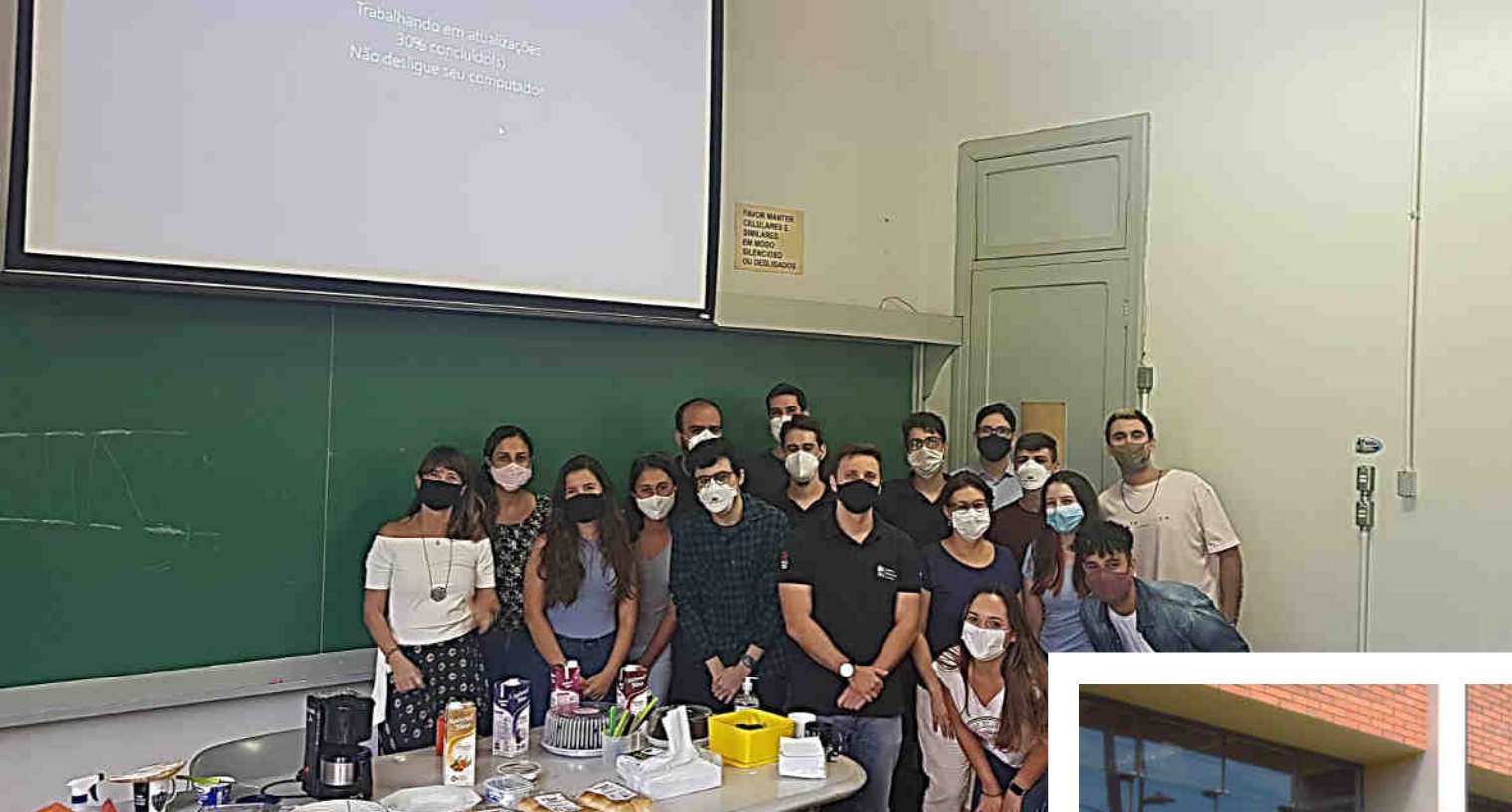
Metabolome
(Schaker et al., 2017; Peters, unpublished)

Proteome
(Calderan-Rodrigues, ongoing)

Functional Data

16S RNA (Microbiome)





Ph.D. candidates:

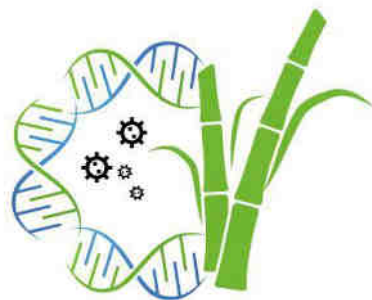
- Gustavo S. Crestana (Genetics and Plant Breeding)
- Gustavo L. Rodrigues (Genetics and Plant Breeding)
- Jessica F. Mendes (Genetics and Plant Breeding)
- Renato G.H. Bombardelli (Genetics and Plant Breeding)
- Gustavo Husein (Genetics and Plant Breeding)
- Tiarla G. Souto (Phytopathology)
- Joyce D. Ferretti (Phytopathology)

Master's students:

- Marcella Ferreira (Phytopathology)
- Enrico D.R. Batista (Genetics and Plant Breeding)
- Gabriela R. Campos (Genetics and Plant Breeding)

Post-Docs:

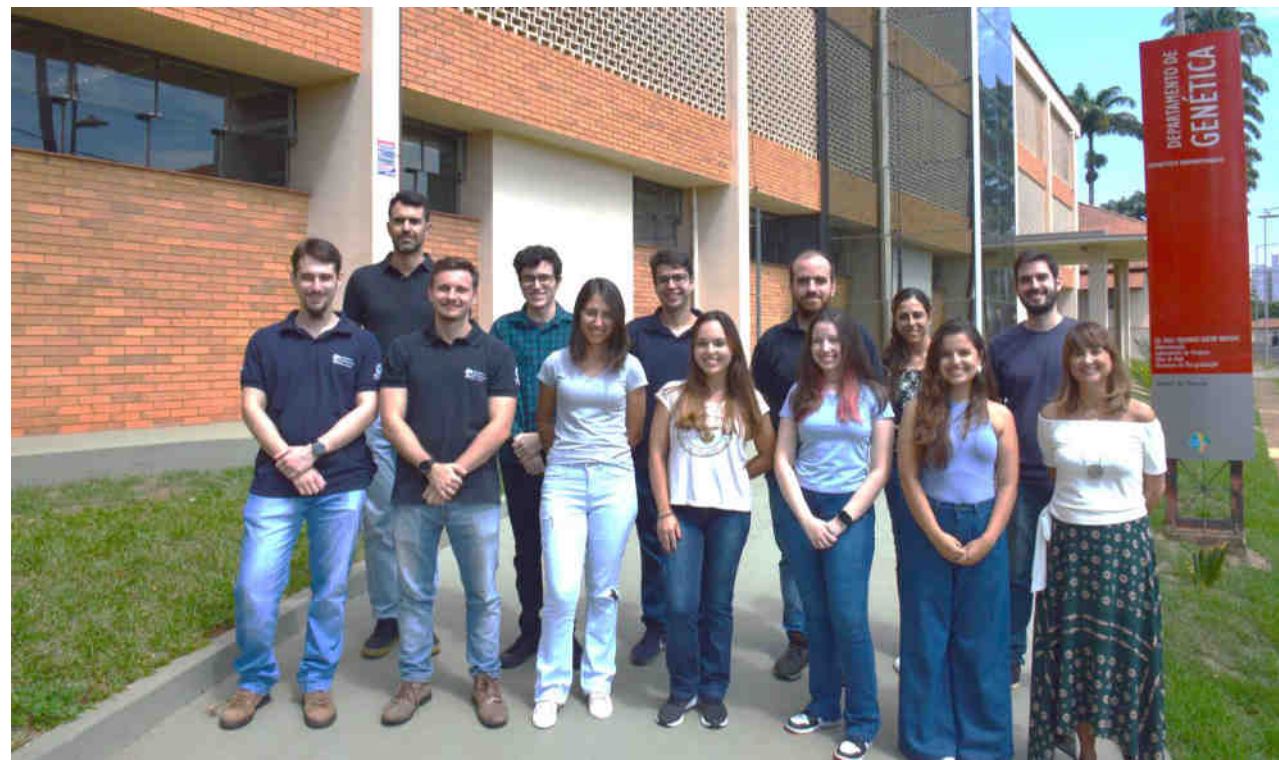
- Thaís C.S. Dal'Sasso
- Thiago Maia



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Undergraduate students:

- Pedro F. V. Ferreira
- Mauricio J. Souza
- Jonathan F. Macedo
- Ana Julia C. Franceschi
- João Vitor F. Mezzei



Technician:

- Elaine Vidotto Batista

Silvana Creste – Centro de Cana



Walter Gassmann



Maeli Melotto



Daniel Croll



Kranthi K. Mandadi



Luis Eduardo Aranha Camargo – ESALQ/USP



Marie-Anne Van Sluys – IB/USP



João Paulo Kitajima Mendelics

José Bressiani



Hugo Rody



gênética
2036

Maria Lúcia Carneiro Vieira – ESALQ/USP



Um olhar para os 100 anos