The *de novo* sequencing, assembly, annotation, gene expression, and public database housing genomic dataset sequences for the sugar beet root maggot *Tetanops myopaeformis*, TmSBRM_v1.0

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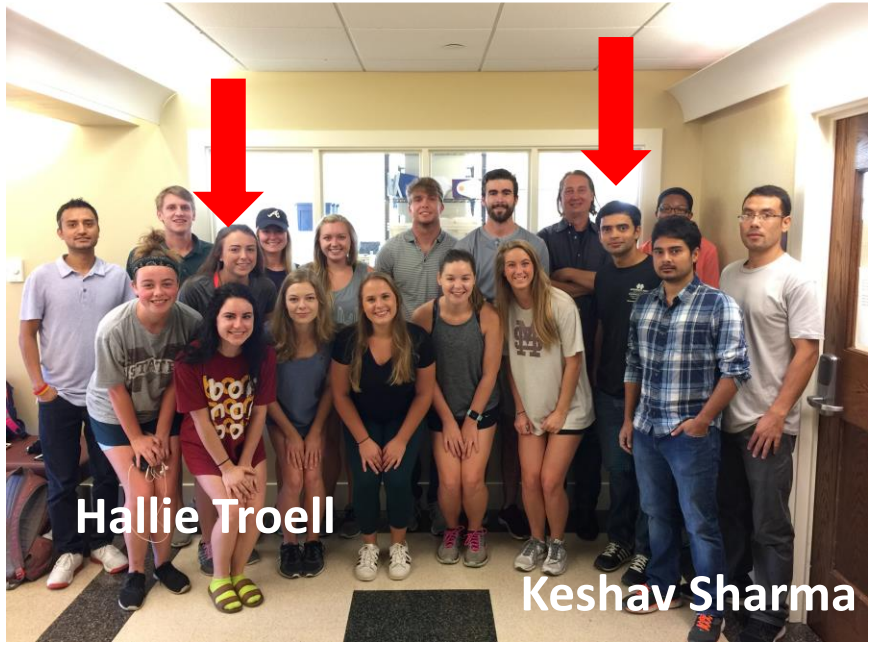
Vincent Klink: USDA-ARS-NEA-BARC, Molecular Plant Pathology Laboratory

The people doing the research:



Topics

1. Sugar beet root maggot (SBRM) genome:
    - Alkharouf *et al*. (2024)-genome assembly
    - Acharya et al. (2024a)-genome annotation
    - Acharya et al. (2024b)-transcriptome sample statistics
    - Acharya et al. (2024c)-web browser

2. Use of soybean and its pathogens to understand sugar beet diseases:
    - Acharya et al. 2024d
    - Acharya-dissertation in preparation

Urgency of the study

Sugar beet (SB) accounts for 55% of U.S. sugar and 35% of global raw sugar.

SB harvest estimates are about $1.0B U.S., $4.6B worldwide (ww).

SBRM can cause loss of about 42% (up to 100% yield loss, locally)

An associated pesticide cost of $57-$171M U.S.

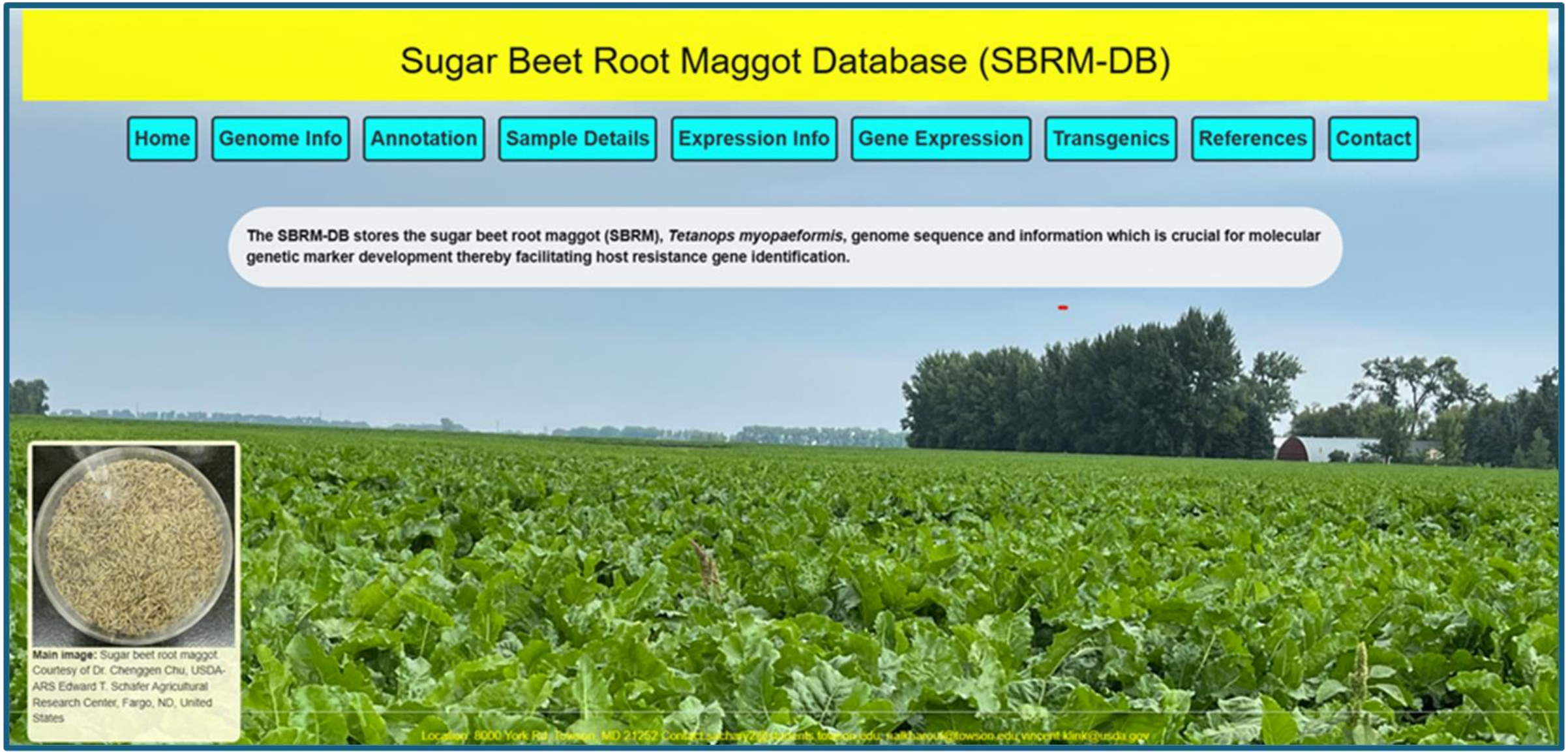
The pesticide problem, alone, includes the further indirect decimation of pollinators that pollinate 75% of U.S. fruits/nuts/vegetables ($15B value).

Pesticide bans and public opinion pressures make the science even more urgent.

The SBRM genome would aid in understanding the SBRM-SB interaction

Cooke DA. *et al*. The Sugar Beet Crop, 1993 Chapman & Hall, London [doi: 10.1007/978-94-009-0373-9]; Brewer MJ. *University of Wyoming Extension Bulletin*. 1995 B-1013.16 [https://www.wyoextension.org/agpubs/pubs/B1013.16.pdf]; Hein GL, *et al*. *Sugarbeet root maggot*. In: Harveson RM, Hanson LE, Hein GL (eds.). Compendium of Beet Diseases and Pests, 2nd edn. 2009. St. Paul, MN: APS Press, pp. 95–97. [doi:10.1017/S0021859609990311]; Hastings J. *American Crystal Sugar Company AgNotes Issue* 2022 622:1 [https://www.crystalsugar.com/media/1ybpccpu/622.pdf]; Fugate KK, et al. Plant Genetic Resources: Characterization and Utilization 2019 17:514; Boetel MA et al. North Dakota State Univ. Coop. Ext. Serv. 2009 39:164; Donley N. 2016. Center for Biological Diversity Newsletter. [https://www.biologicaldiversity.org/news/press_releases/2016/pesticides-02-24-2016.html]; Campbell L. PassbtVol29Agp179YieldLossassociatedwithsugarbeetrootmaggotdamage.pdf

Sugar beet root maggot (SBRM) genome/related work



https://bioinformatics.towson.edu/SBRM

Photos and samples courtesy of Chenggen Chu & Muhammad Massub Tehseen

Acharya *et al*. 2024b

SBRM genome (TmSBRM_v1.0) information

Field work

SBRM collection

Photos and samples courtesy of Chenggen Chu & Muhammad Massub Tehseen

Sequencing platform: PacBio Sequel Revio

Assembly: HiFiasm

Contigs: 396

Largest contig: 34,092,264 nt

Total genome length: 509,667,004 nt

N50: 14,933,876 nt

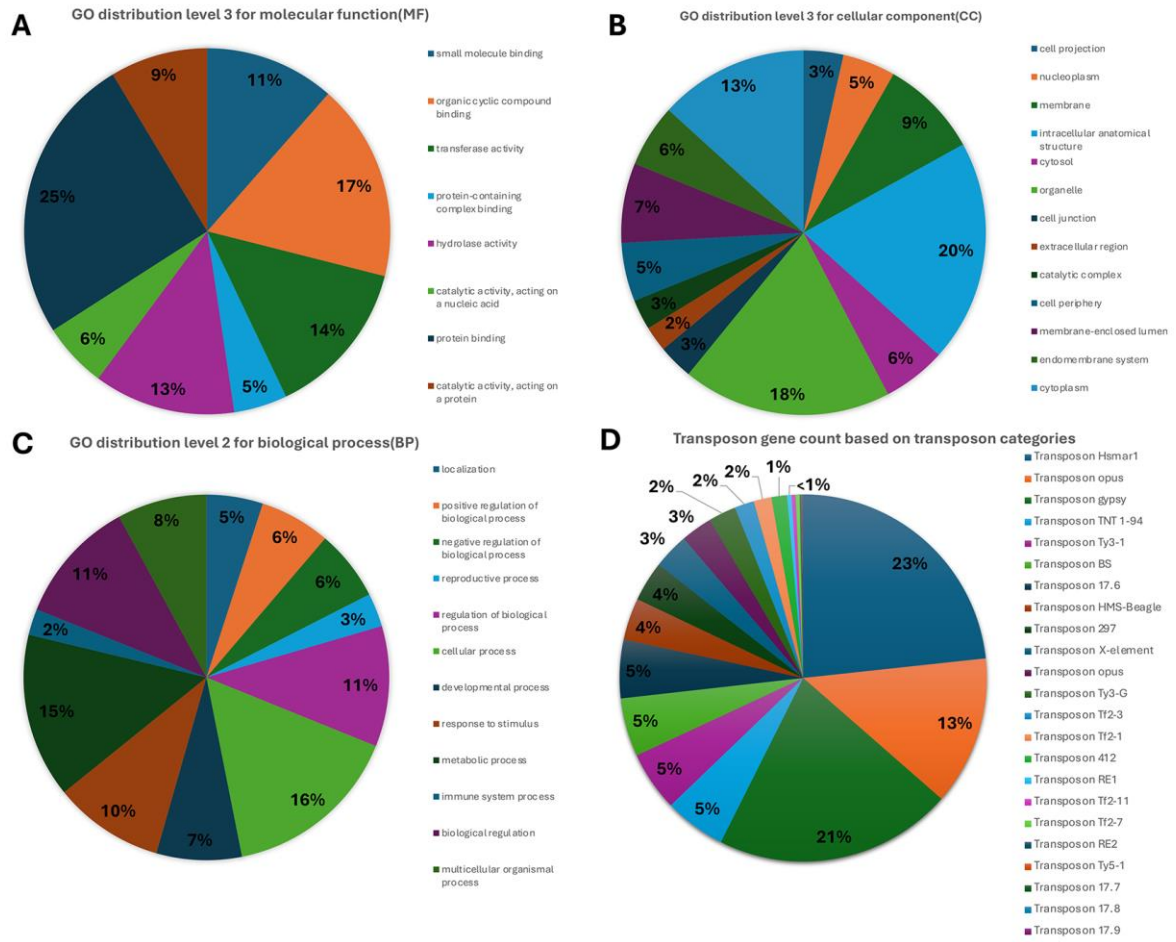
N90: 1,286,286 nt

G/C: 40%

Coverage: 94x

Alkhaouf *et al*. 2024
Grinstead *et al*. unpublished

SBRM genome annotation



<u>Gene count</u>:

SBRM:	28,276
Drosophila:	13,600
conopid fly:	25,472
lauxaniid fly:	25,606

A: molecular function
B: cellular component
C: biological process
D: transposon

Gene finding tool, AUGUSTUS 3.5.0
Functional annotation, Blast2GO 6.0
Protein domain finding, InterproScan 5.67-99.0
GO mapping and annotation, GeneOntology 2024-03-28

Acharya *et al*. 2024b

SBRM genes not found in the genome of *D. melanogaster* are identified

Rhagoletis zephyria (n = 576)

Ceratitis capitata, Mediterranean fruit fly (n = 174)

Rhagoletis pomonella, apple maggot (n = 119)

Anastrepha ludens, Mexcan fruit fly (n = 116)

Bactrocera latifrons, Solanum fruit fly (n = 90)

Anastrepha obliqua, West Indian fruit fly (n = 86)

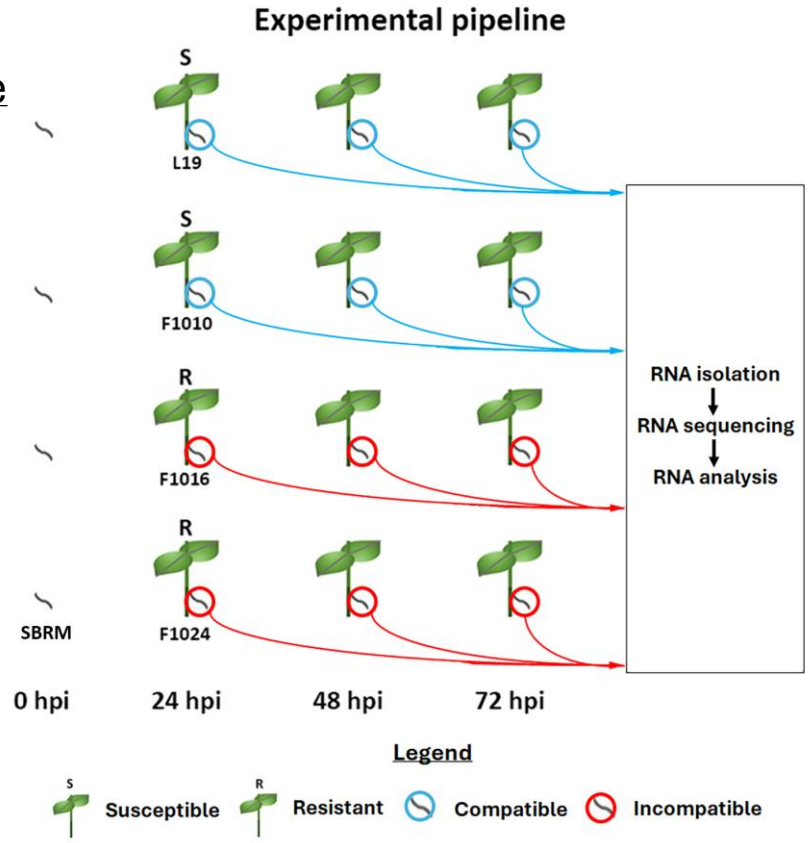
Sitodiplosis mosellana, wheat midge (n = 81)

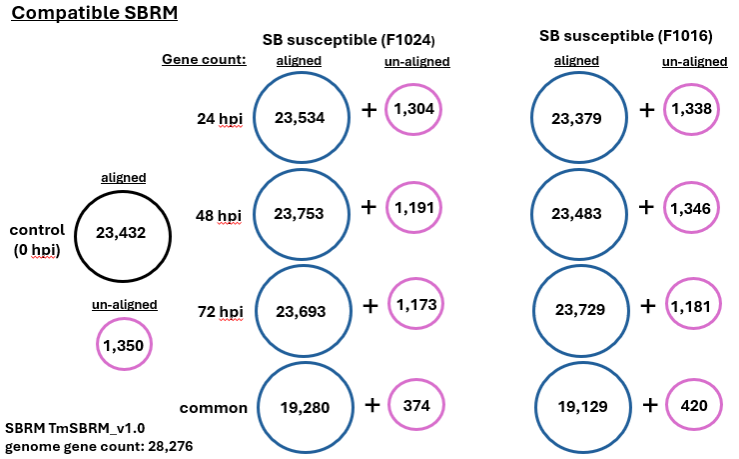
Genes encoding:
pathogen effectors
Immune suppressors
Transcription factors

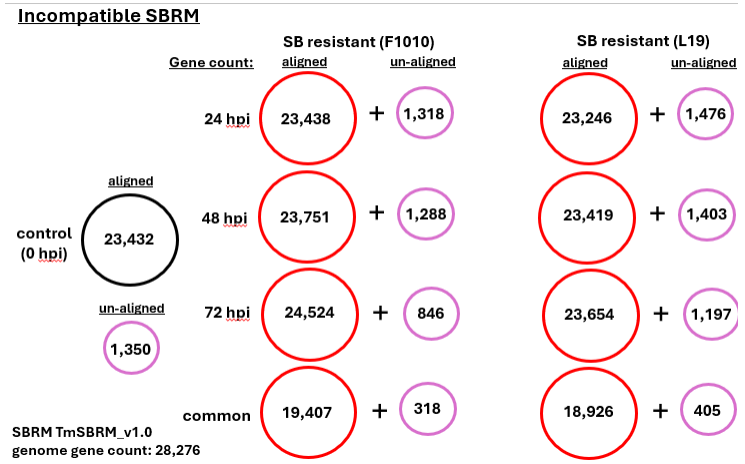
SBRM transcriptome work

<u>SBRM-susceptible</u>
F1010 (PI 535818)
L19 (PI 590690)

<u>SBRM-resistant</u>
F1016 (PI 608437)
F1024 (PI 658654)



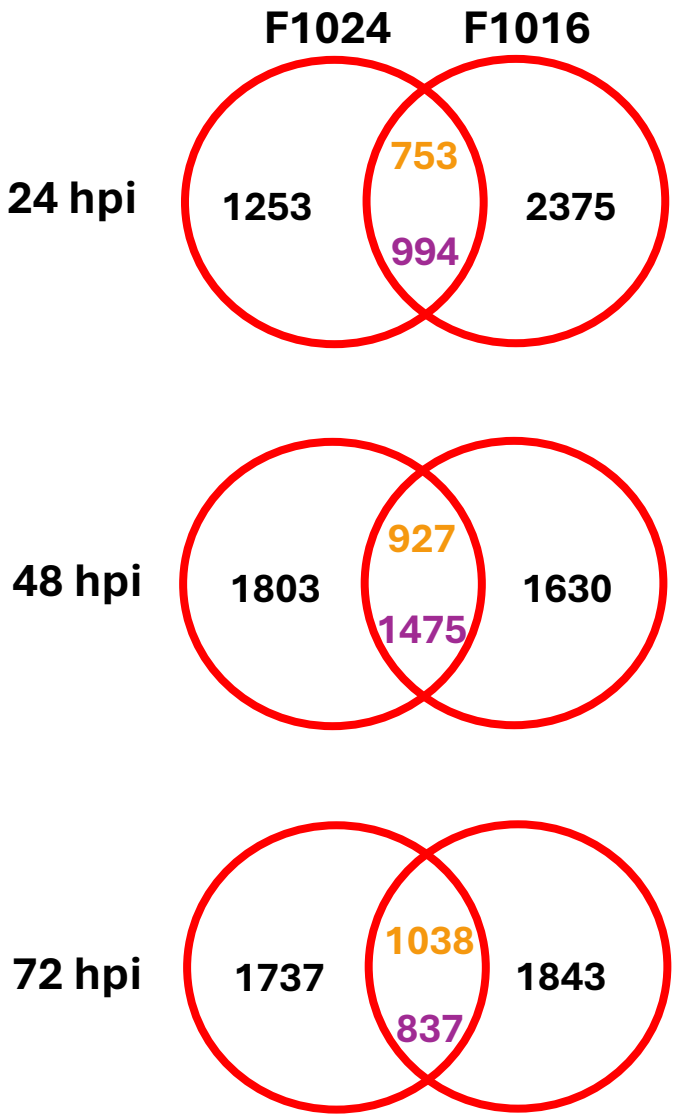




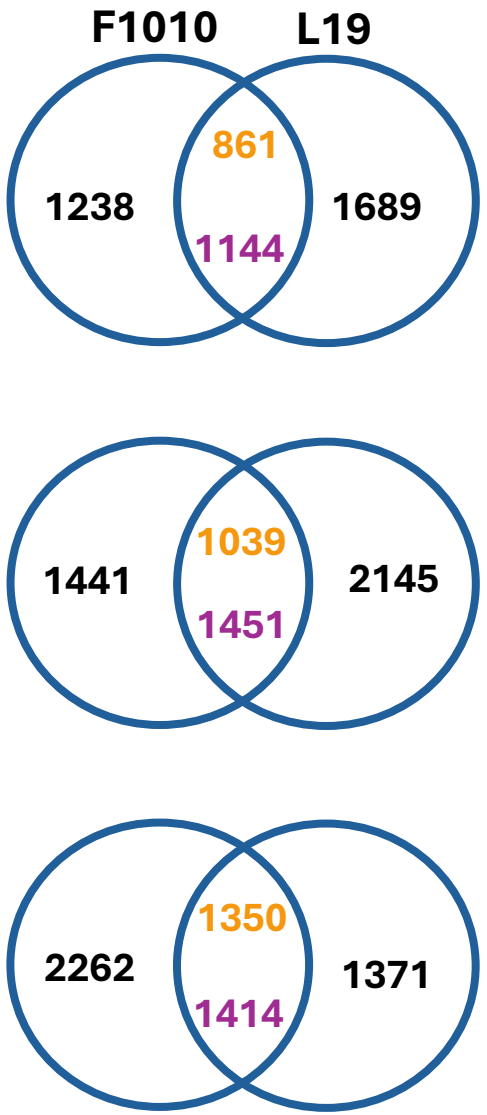
Theurer CJ. Crop Sci. 1978 18:1101; Campbell LG. Crop Sci. 1990. 30:429; Campbell LG. Journal of Plant Registrations 2015 9:382; Campbell L, et al. J. Plant Reg. 2011; Campbell LG, et al. Crop Science 2000 40:867; **Acharya *et al*. 2024b**

Comparative transcriptomic analysis of 2 different resistant and 2 different susceptible reactions

Res. 1 vs Res. 2



Susc. 1 vs Susc. 2



Legend:

Resistant 1: F1024

Resistant 2: F1016

Susceptible 1: F1010

Susceptible 2: L19

Induced

Suppressed

Unique

Note: The genes identified in the resistant F1016 & F1016 are compared; The genes identified in the susceptible F1010 & L19 are compared.

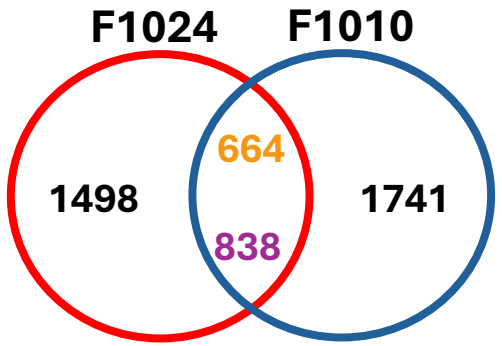
Outcome 1:

The 2 resistant reactions occurring on F1024 and F1016 are different from each other.

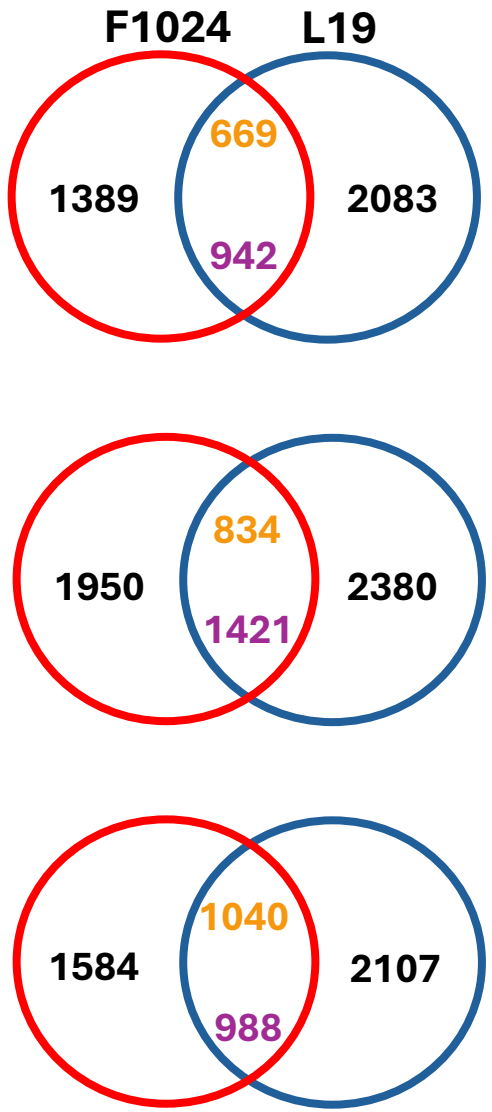
The 2 susceptible reactions occurring on F1010 and L19 are different from each other.

Comparative transcriptomic analysis of 2 different resistant and susceptible reactions

Res. 1. Vs Susc. 1



Res. 1. Vs Susc. 2



Legend:

Resistant 1: F1024

Resistant 2: F1016

Susceptible 1: F1010

Susceptible 2: L19

Induced

Suppressed

Unique

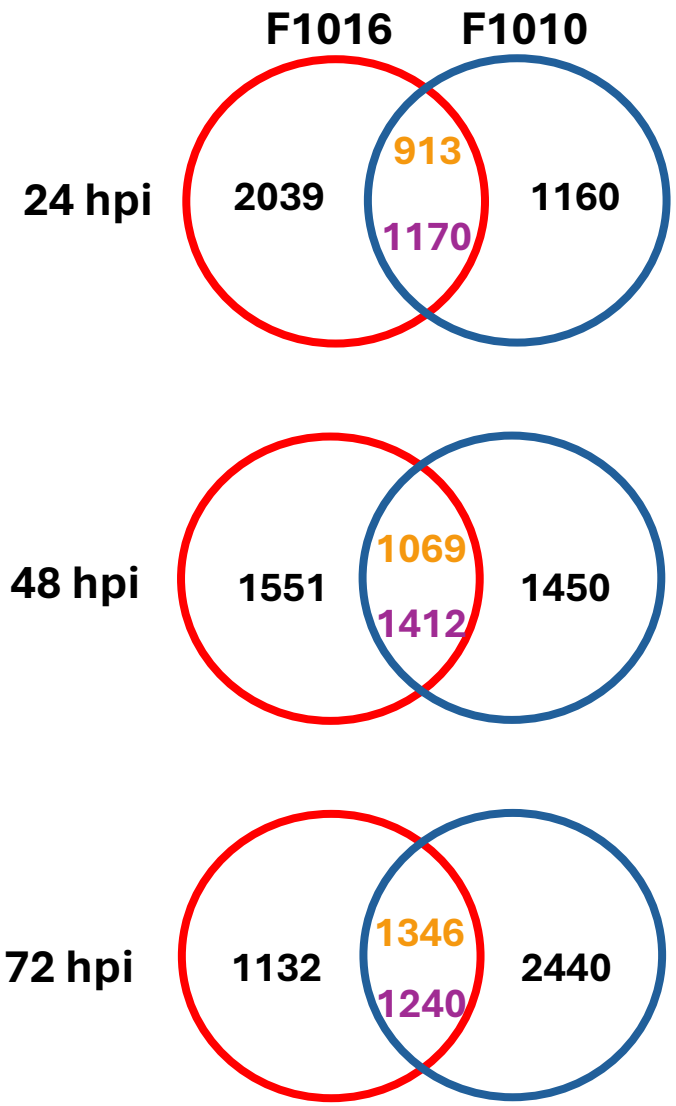
Note: The genes identified in the resistant F1024 are compared, individually to the genes in the susceptible F1010 & L19

Outcome 2:

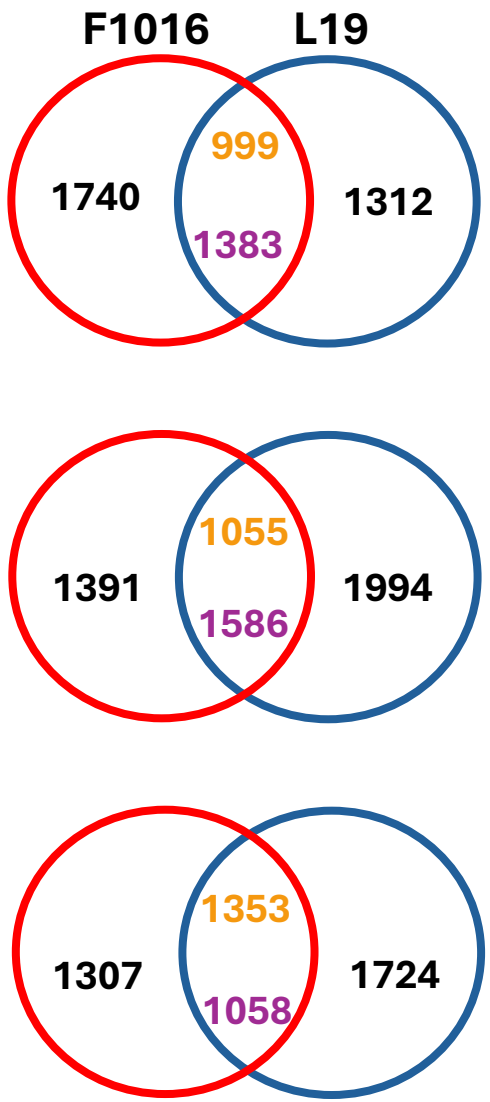
The 2 F1024 resistant reactions occurring in comparison to the 2 different susceptible reactions on F1010 and L19 are different from each other.

Comparative transcriptomic analysis of 2 different resistant and susceptible reactions

Res. 2. Vs Susc. 1



Res. 2. Vs Susc. 2



Legend:

Resistant 1: F1024

Resistant 2: F1016

Susceptible 1: F1010

Susceptible 2: L19

Induced

Suppressed

Unique

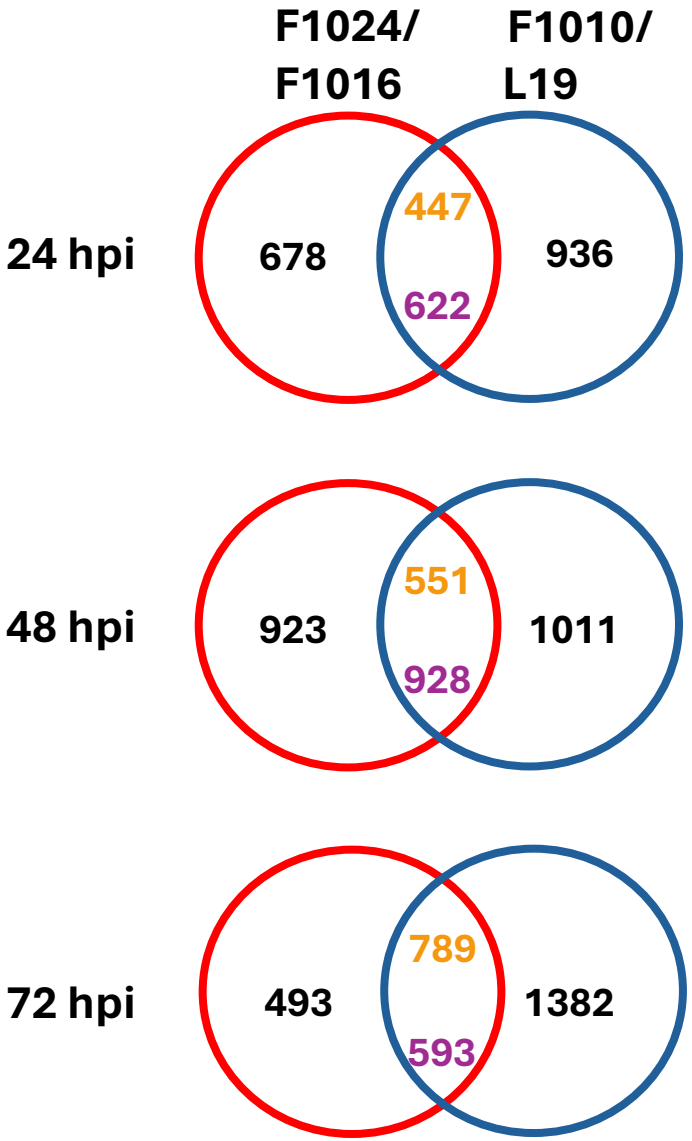
Note: The genes identified in the resistant F1016 are compared, individually to the genes in the susceptible F1010 & L19

Outcome 3:

The 2 F1016 resistant reactions occurring in comparison to the 2 different susceptible reactions on F1010 and L19 are different from each other.

Comparative transcriptomic analysis of 2 different combined resistant and susceptible reactions

Res. 1/2. Vs Susc. 1/2



Legend:

Resistant 1: F1024

Resistant 2: F1016

Susceptible 1: F1010

Susceptible 2: L19

Induced

Suppressed

Unique

Note: The genes that are common between F1024 & F1016 are compared to the genes common between F1010 & L19

Outcome 4:

There is a core set of genes differentially expressed that are common to the two resistant and 2 susceptible reactions.

Identification of lethal SBRM CRISPR/RNAi targets

Objective: identify genes that when mutated, silenced, or edited in related organisms (i.e. *Drosophila*, and mosquito) result in a lethal phenotype/phenocopy.

Whole genome RNAi and CRISPR analyses are available for *Drosophila* and mosquito

Transgenic RNAi Project (TRiP) at the Drosophila RNAi Screening Center Harvard University

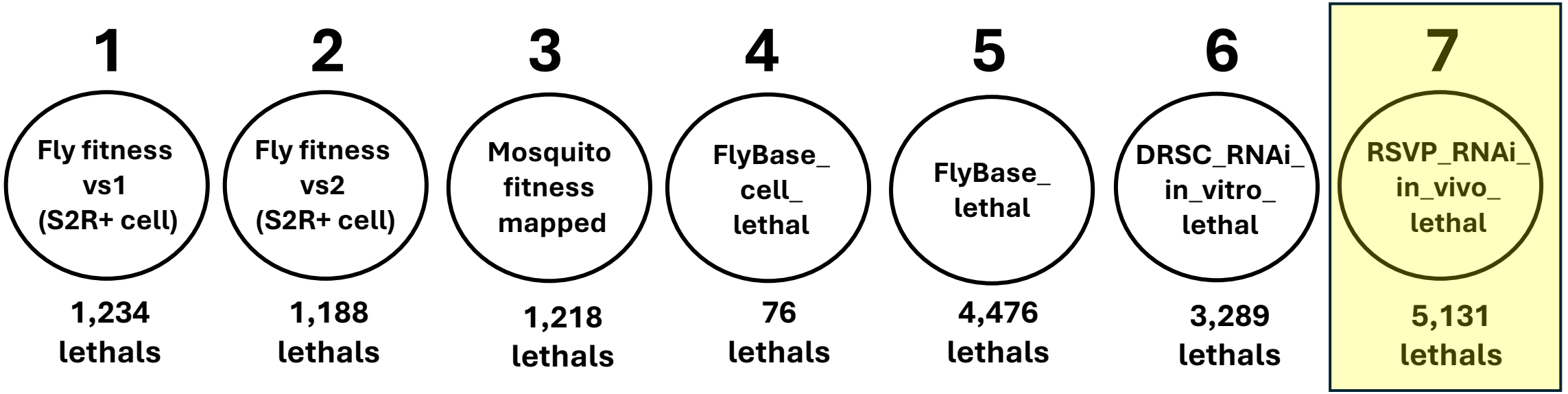
Stephanie Mohr-Harvard University
Claire Yanhui Hu-Harvard University

Vienna Drosophila Resource Center, Vienna Biocenter

National Institute of Genetics, Japan

Caplen et al. 2000; Kiger et al. 2003; Flockhart et al. 2006; Viswanatha et al. 2018, 2024; Hu et al. 2025

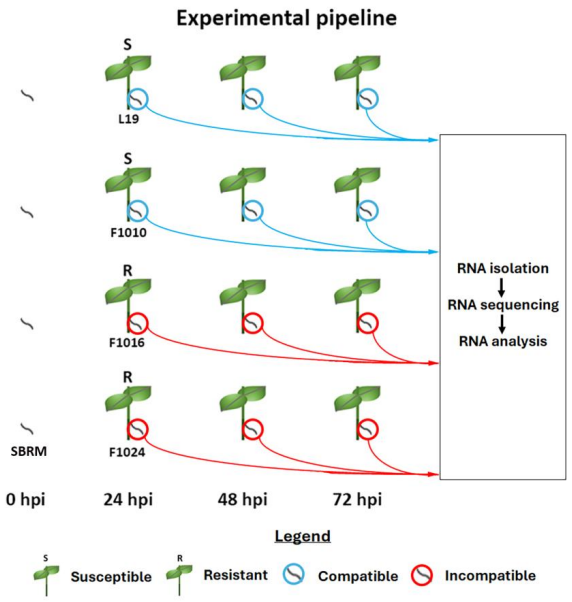
Identification of SBRM CRISPR/RNAi targets



	Comparison	***Drosophila***	**SBRM**
a.	Find the SBRM lethals in 1:	1,234	930
b.	Find the SBRM lethals in 2:	1,188	923
c.	Find the SBRM lethals common to 1 & 2	682	530
d.	Find the SBRM lethals in 3	1,218	942
e.	Find the SBRM lethals in 4	76	56
f.	Find the SBRM lethals in 5	4,476	3,424
g.	Find the SBRM lethals in 6	3,289	2,498
h.	Find the SBRM lethals in 7	5,131	3,719
i.	Find the SBRM lethals common to 1, 2, 5, 6, 7	422	342
j.	Find the SBRM lethals common to 1, 2, 3, 5, 6, 7	223	223

Acharya et al. Ph.D. dissertation

Identification of expressed SBRM CRISPR/RNAi targets



RSVP_RNAi_in_*vivo*_lethal

	F1024	F1016	F1010	L19
24 hpi	2495	2471	2488	2463
48 hpi	2495	2478	2483	2490
72 hpi	2528	2532	2563	2528

2499

control (0 hpi)

Legend:

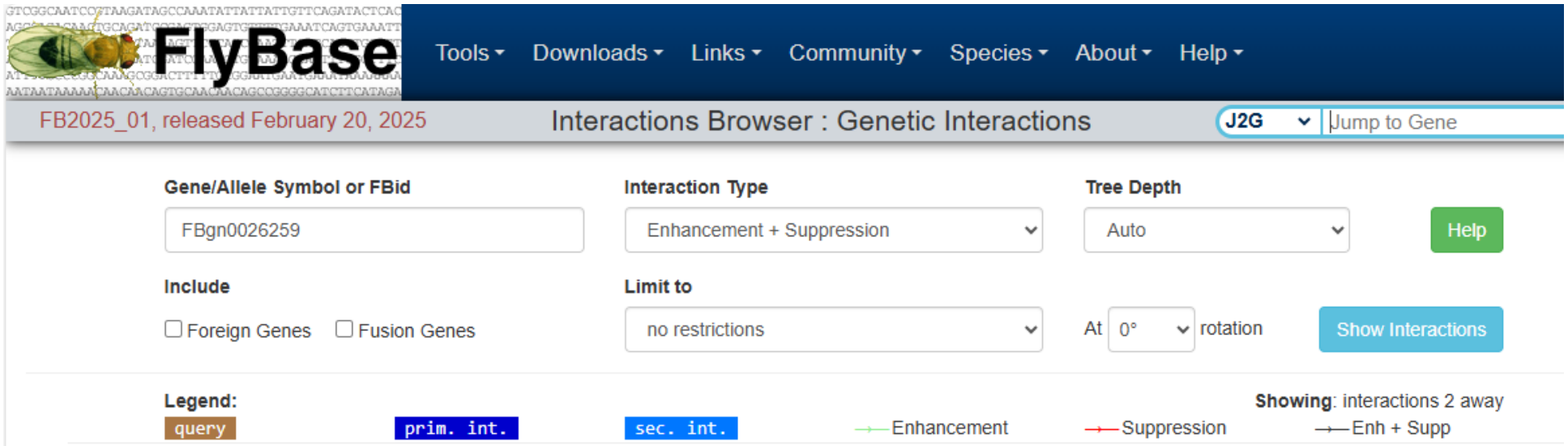
Resistant 1: F1024

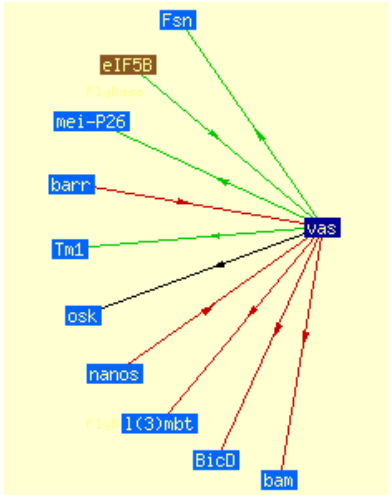
Resistant 2: F1016

Susceptible 1: F1010

Susceptible 2: L19

SBRM homologs are identified that in *Drosophila* interact with other proteins to accomplish essential functions





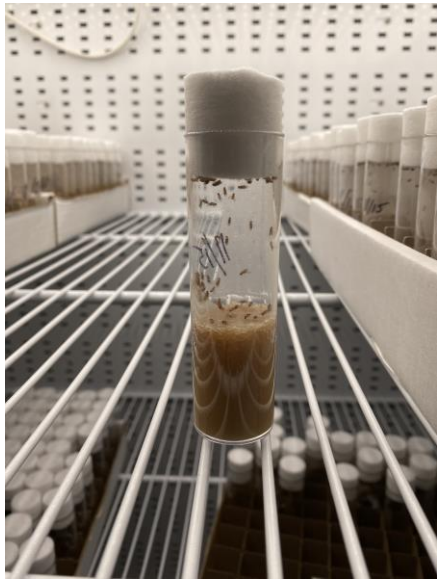
CRISPR and RNAi analyses of SBRM are in the process of being set up through collaborations with:

USDA-ARS-Invasive Insect Biocontrol & Behavior Laboratory

University of Maryland

USDA-ARS-NA- Northern Crop Science Laboratory

Department of Plant Sciences, North Dakota State University



Alkharouf et al. 2007; Klink et al. 2009

Topics

1. Sugar beet root maggot (SBRM) genome:
   Alkharouf *et al*. (2024)

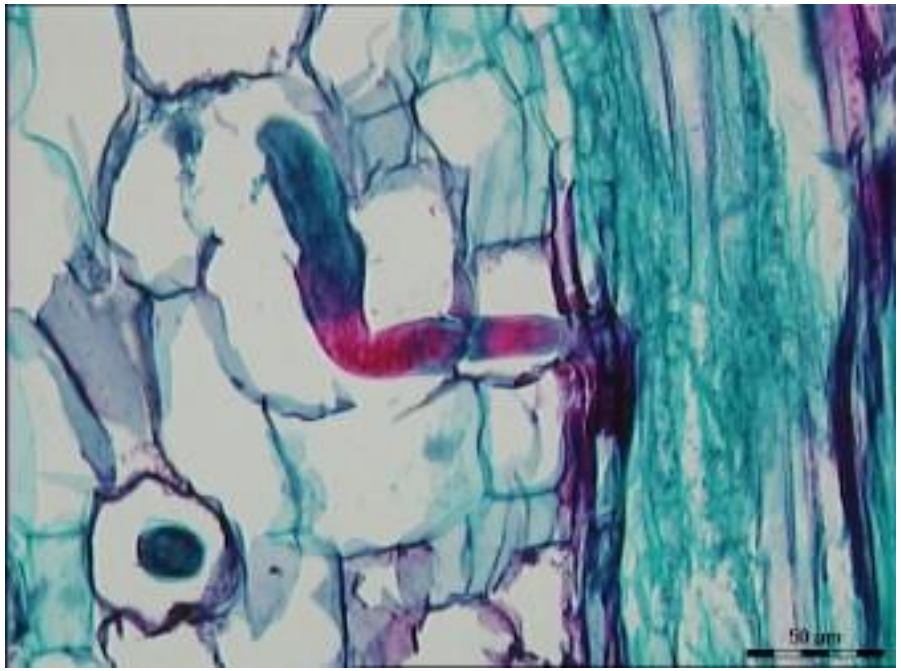
2. Use of soybean and its pathogens to understand sugar beet diseases:
   Acharya et al. 2024a
   Troell et al. revision under review

   NSF-funded collaboration with:
   Bao-Hua Song-UNC-Charlotte

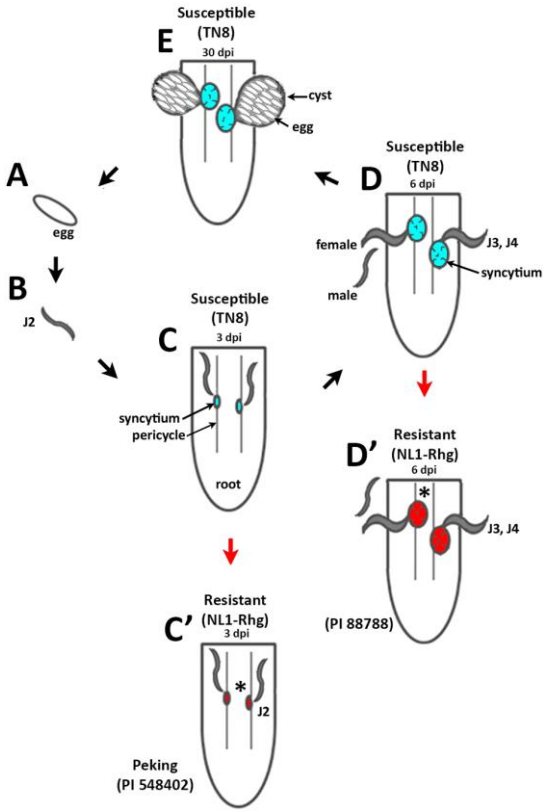
Use of soybean and its pathogens to understand sugar beet diseases

Soybean, *Glycine max* ($31.2B U.S., $155B ww value), the #1 U.S. export crop

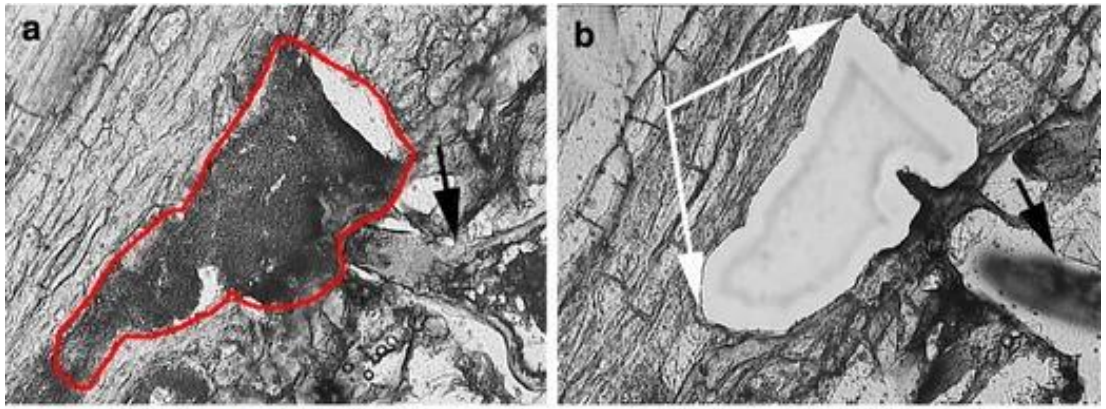
Soybean cyst nematode, *Heterodera glycines* $1.5B loss U.S., $23B loss worldwide [ww]



Life cycle and laser microdissection: *Heterodera glycines*, the soybean-soybean cyst nematode (SCN) pathosystem

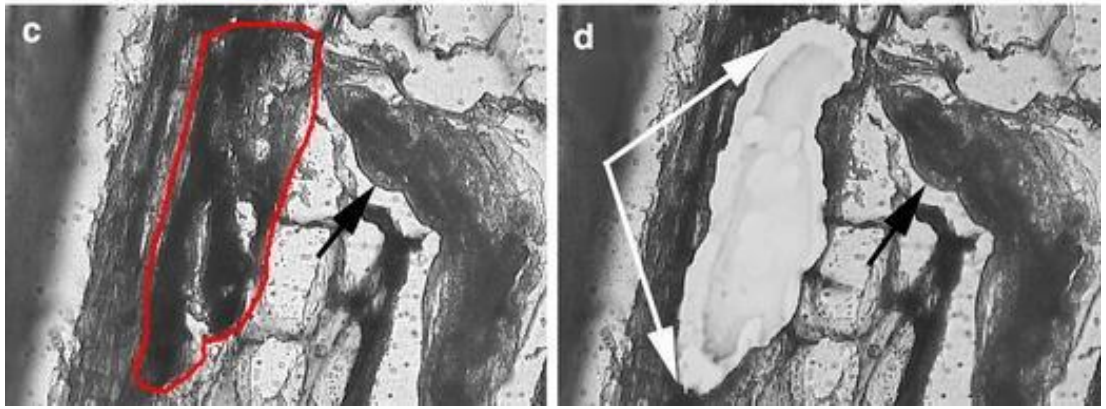


Before LCM 3 days post infection



After LCM 3 days post infection

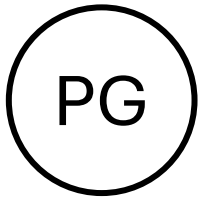
Before LCM 8 days post infection



After LCM 8 days post infection

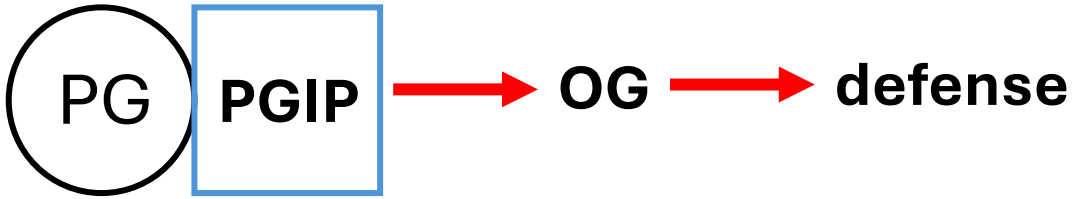
In a continuation of work done by Li Smigocki (2018), we compared sugar beet polygalacturonase inhibiting protein (PGIP) with expression and function in the soybean-soybean cyst nematode pathosystem

Polygalacturonase inhibiting protein (PGIP) and plant defense



Pathogen polygalacturonases (PGs) facilitate plant infection by cleaving the α-(1→4) linkages occurring between D-galacturonic acid residues in homogalacturonan (HG), causing cell wall separation, leading to the maceration of plant host tissue.

Plants and plant parasitic nematodes have active PGs



PGIPs directly interfere with the activity of the PGs leading to the accumulation of oligogalacturonides (OGs) that then elicit defense responses.

Therefore, the OGs serve as damage associated molecular patterns (DAMPs)

(Phaff 1947; Muse et al. 1970; Chitwood and Krusberg, 1977; Jaubert et al. 2002; Bird et al. 2009; Danchin et al. 2010Cervone et al. 1997; Ridley et al. 2001; Matzinger 1994; Seong and Matzinger, 2004)

Soybean *PGIP11* gene expression was identified specifically in the root cells undergoing a resistant reaction

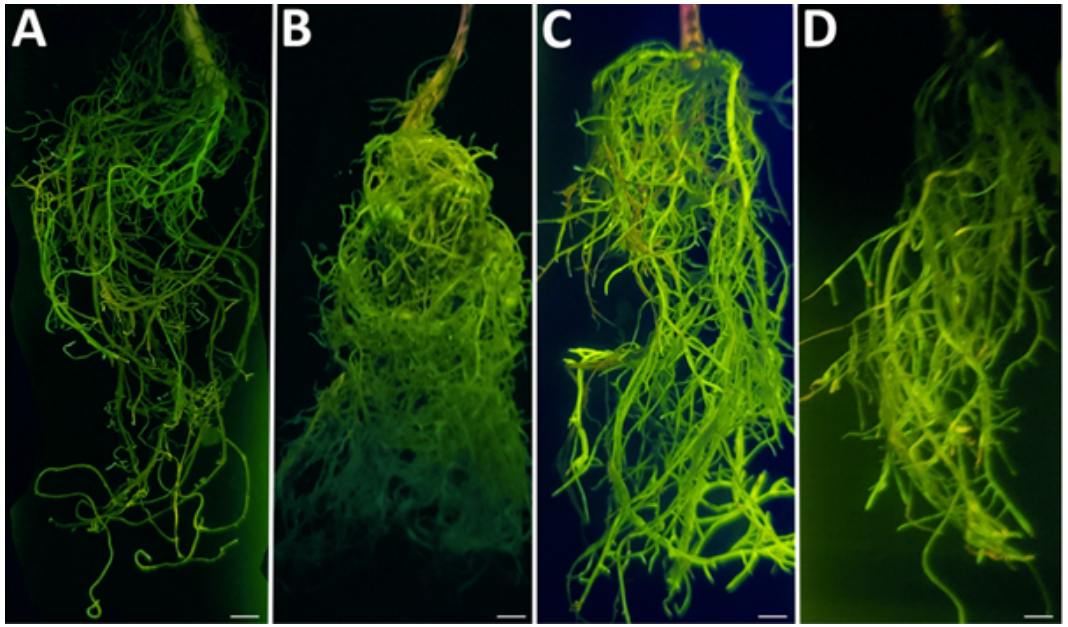
		Time point (days post infection)			
Gene	**Accession**	**0**	**3**	**6**	**9**
GmPGIP1	*Glyma.05G123700.1*	NM	NM	NM	NM
GmPGIP2	*Glyma.05G123800.1*	n/a	n/a	n/a	n/a
GmPGIP3	*Glyma.05G123900.1*	NM	NM	NM	NM
GmPGIP4	*Glyma.05G124000.1*	NM	NM	NM	NM
GmPGIP5	*Glyma.08G078800.1*	n/a	n/a	n/a	n/a
GmPGIP6	*Glyma.08G078900.1*	NM	NM	NM	NM
GmPGIP7	*Glyma.08G079100.1*	n/a	n/a	n/a	n/a
GmPGIP8	*Glyma.08G079200.1*	n/a	n/a	n/a	n/a
GmPGIP9	*Glyma.15G209200.1*	n/a	n/a	n/a	n/a
GmPGIP10	*Glyma.15G209300.1*	NM	NM	NM	NM
GmPGIP11	*Glyma.19G145200.1*	NM	M	M	N/M*

NM = gene expression not measured during the course of the experiments
n/a = not applicable because gene expression could not be measured due to how the analysis was performed

Acharya *et al*. revision under review

GmPGIP11 functions in resistance to SCN parasitism

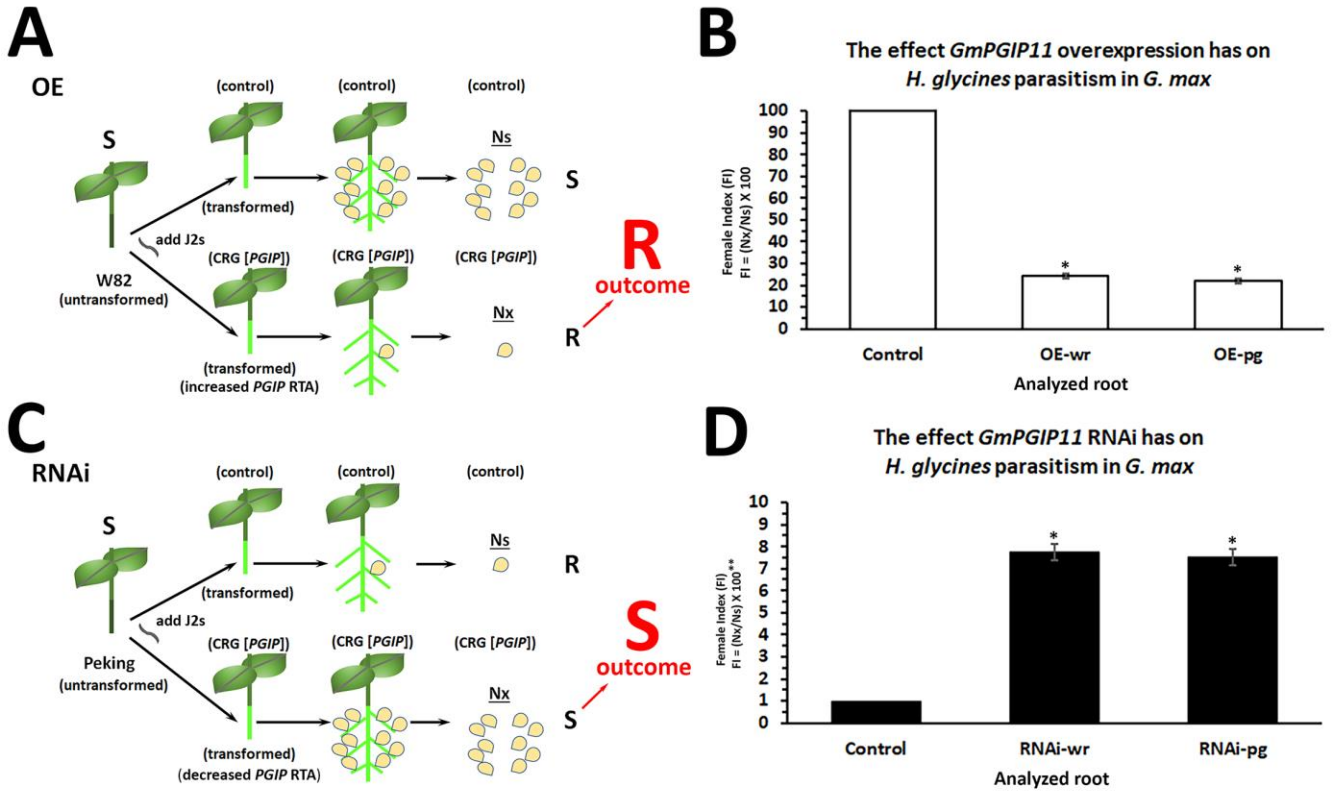
Transgenics



PGIP11-OE control | *PGIP11*-OE | *PGIP11*-RNAi control | *PGIP11*-RNAi

OE-overexpression
RNAi-RNA interference

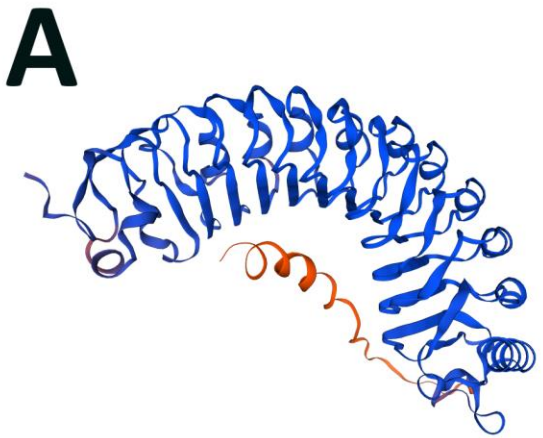
Strategy of the study

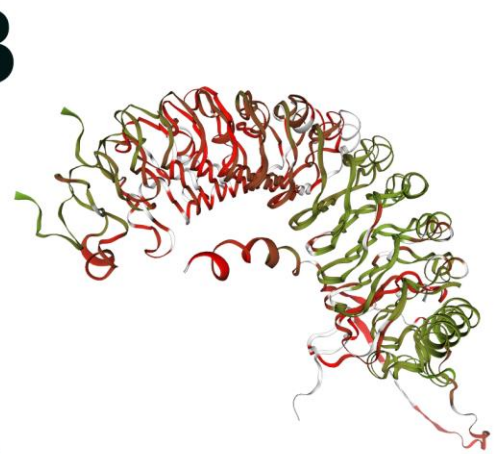


Acharya *et al*. revision under review

Comparison of sugar beet and soybean PGIPs shown to function in different types of defense responses

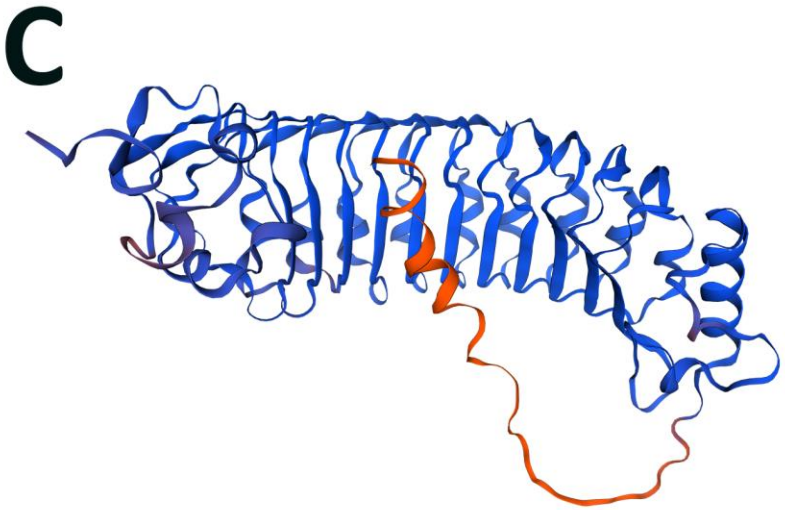
*Bv*PGIP1

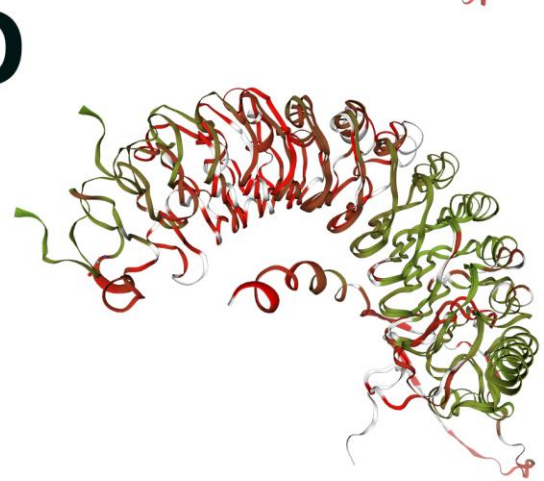




*Gm*PGIP11 overlain on *Bv*PGIP1

*Bv*PGIP2





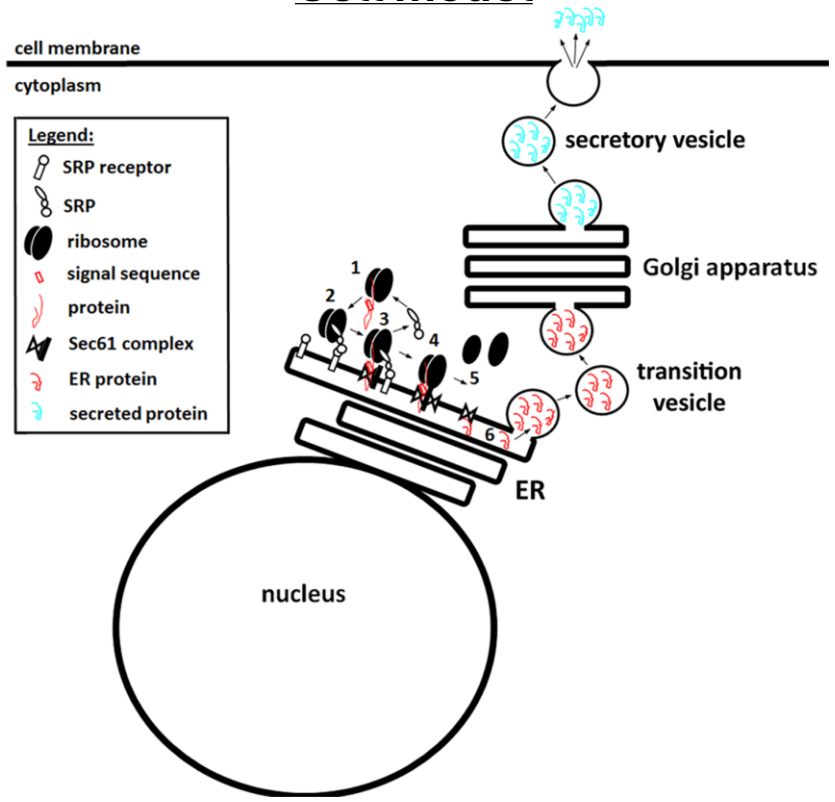
*Gm*PGIP11 overlain on *Bv*PGIP2

Goal: identify amino acid signatures that relate to the resistant reaction

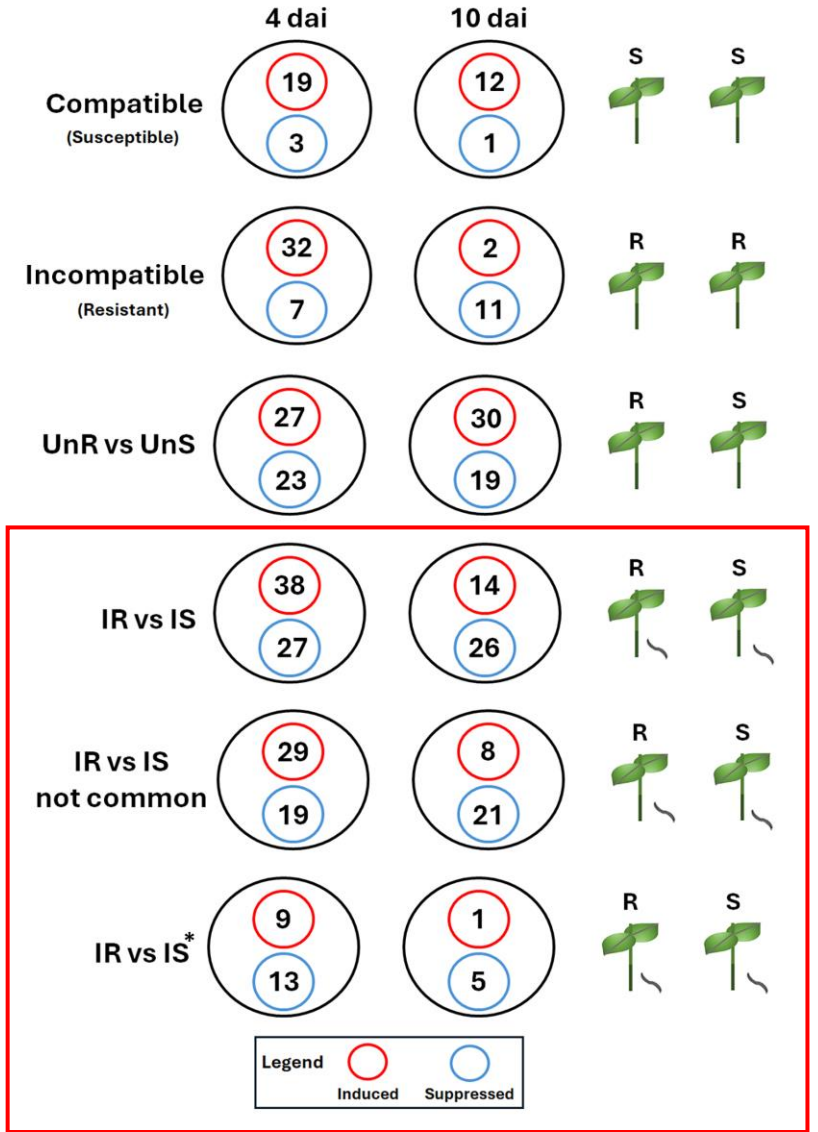
Acharya *et al*. 2024a

Linking work done in the soybean-SCN pathosystem back to sugar beet

Cell model



Comparative transcriptomics (in sugar beet)



SB candidate gene identification

Accession	Description
4 dai-Induced	
Bv_42650_ntwt	uncharacterized protein
Bv_42020_fuah	peroxidase 60
Bv_39260_wgyc	uncharacterized protein
Bv_06500_pdgi	factor Xa inhibitor BuXI
Bv1_006220_mxyn	hypothetical protein
Bv5_095600_hrph	thaumatin-like protein 1
Bv7_179640_gcjh	hypothetical protein
Bv9_228950_xiuj	pathogenesis-related protein PR-1
Bv4_083680_emej	defensin Ec-AMP-D1-like
Bv3_062940_dwio	hypothetical protein
Bv_43680_yiaj	hypothetical protein
Bv3_066580_ipuq	Protocadherin Fat 1
Bv_53930_enhx	hypothetical protein
Bv_54760_roze	hypothetical protein
Bv8_196530_giyo	EG45-like domain containing
Bv1_006210_hjep	hypothetical protein
Bv5_101160_hmgn	hypothetical protein
Bv_39220_meji	hypothetical protein
Bv_15660_xqjx	lipid-binding protein AIR1

Infected resistant (IR) *B. vulgaris*$_{[Nemakill]}$

Infected compatible (susceptible) *B. vulgaris*$_{[7112*SB36]}$

dai: days after infection with the beet cyst nematode

Troell *et al*. revision submitted

Conclusions

The sequencing of the SBRM led to the identification of its gene content and generation of a publicly-available resource for other types of experiments.

Many SBRM genes that relate to immune suppression and other strategies to circumvent resistance have been identified.

SBRM gene expression experiments have demonstrated each type of susceptible and resistant reaction is different but have a core set of utilized expressed genes.

Utilizing cell-type specific gene expression experiments performed in other pathosystems is a relevant way to identify new sources of resistance.

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