Genetic dissection of disease resistance mechanisms hijacked by a necrotrophic pathogen of wheat

Justin D. Faris

USDA-ARS, Cereal Crops Research Unit,
Fargo, ND, USA

Outline

- Introduction to the wheat-*Parastagonospora nodorum* pathosystem
- The inverse gene-for-gene interactions
- The cloned genes
- Comparisons of structure, expression, function
- Molecular model
- Resistance breeding strategies and caveats
- Summary

Background

Plant Pathogens

Bacteria

Fungi

Nematodes

Viruses

Biotrophs
(feed on living tissue)

Necrotrophs
(feed on dead/dying tissue)

Stem rust

Powdery mildew

Generalists

Fusarium head blight

Specialists

Tan spot

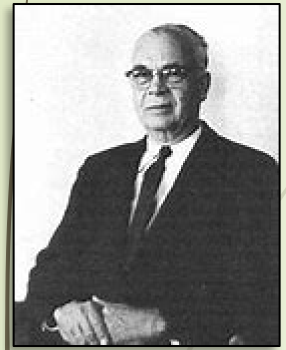
Septoria nodorum blotch

Septoria nodorum blotch

- *Parastagonospora nodorum*
- Class: Dothideomycetes
- Infect leaves and glumes
- Infection -> cell death (necrosis) -> loss in photosynthetic capacity -> yield loss
- >50% yield losses are possible
- Produce necrotrophic effectors (NEs) (host-selective toxins)
- Dominant host genes recognize NEs, leads to host-induced programmed cell death and disease
- Inverse gene-for-gene

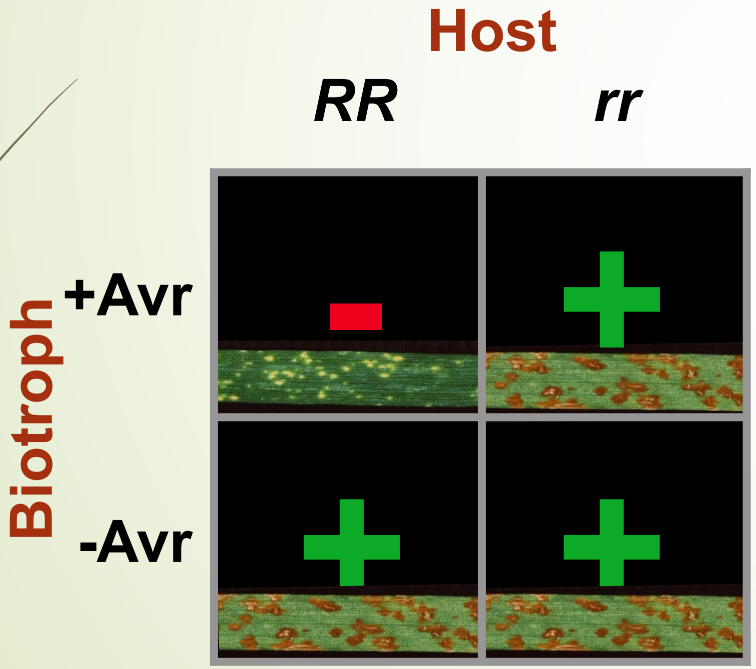
Inverse gene-for-gene

Harold H. Flor



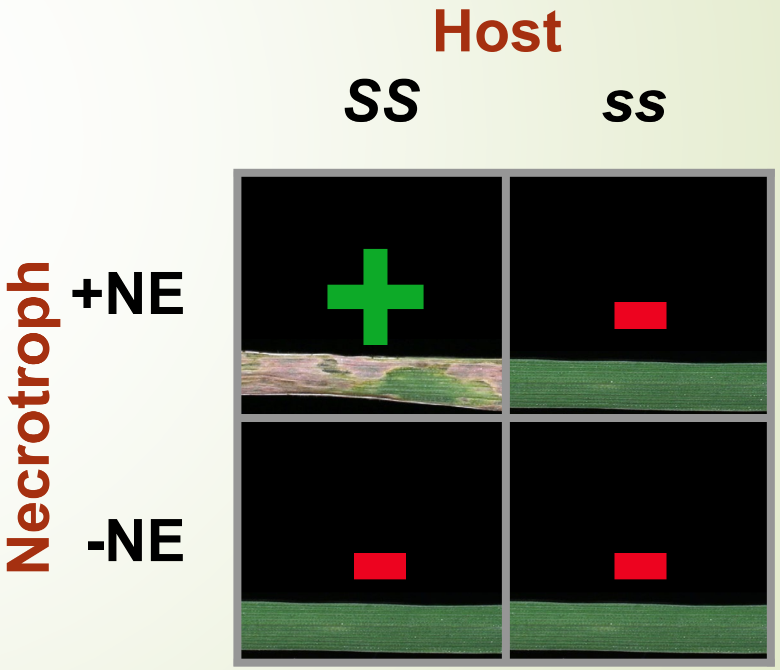
USDA Fargo, ND
1931-1969

Flor's Classic Gene-for-Gene Model



Biotroph	Host *RR*	Host *rr*
+Avr	−	+
-Avr	+	+

Host-NE Gene-for-Gene Model

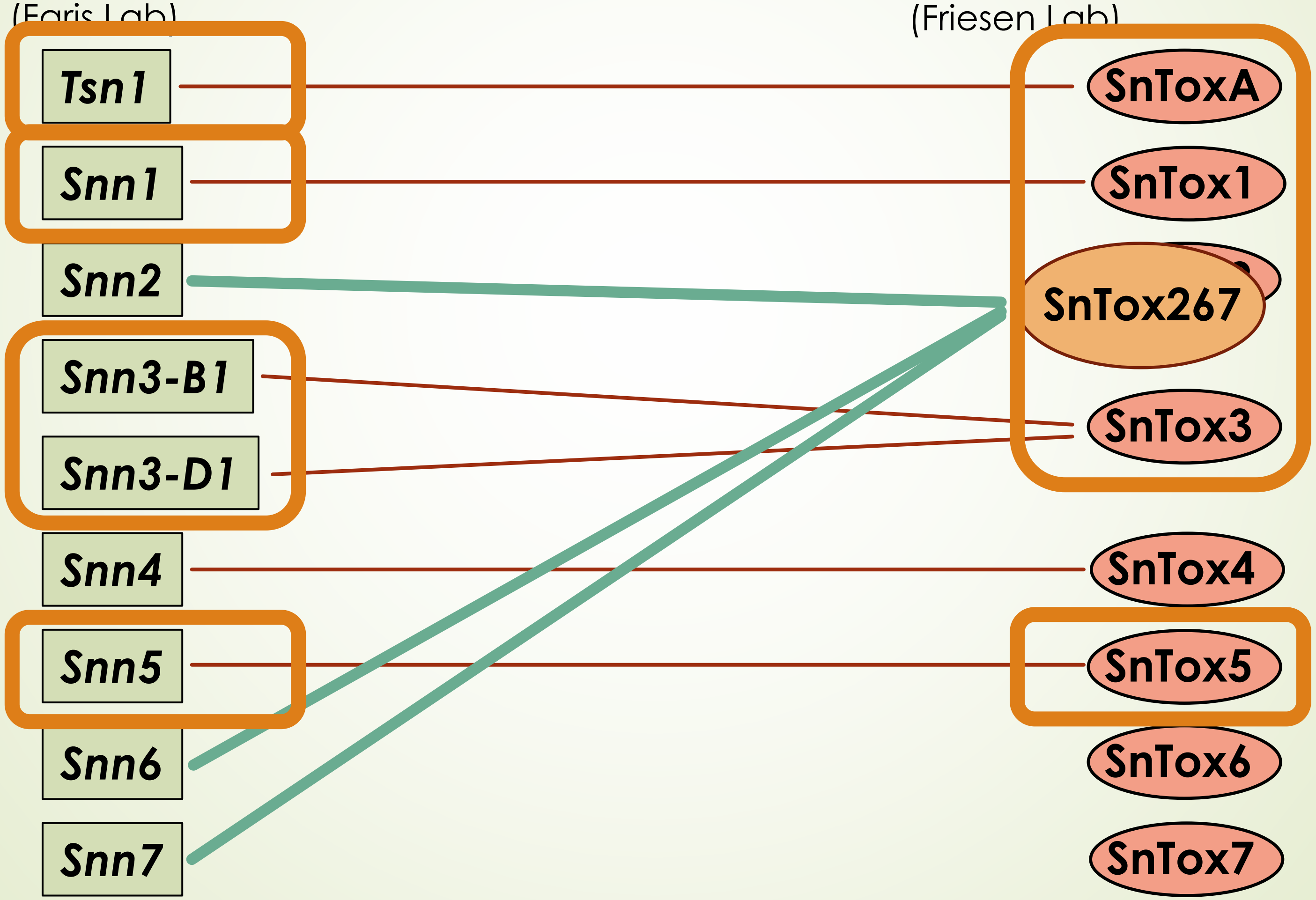


Necrotroph	Host *SS*	Host *ss*
+NE	+	−
-NE	−	−

The known interactions

Wheat gene
(Faris Lab)

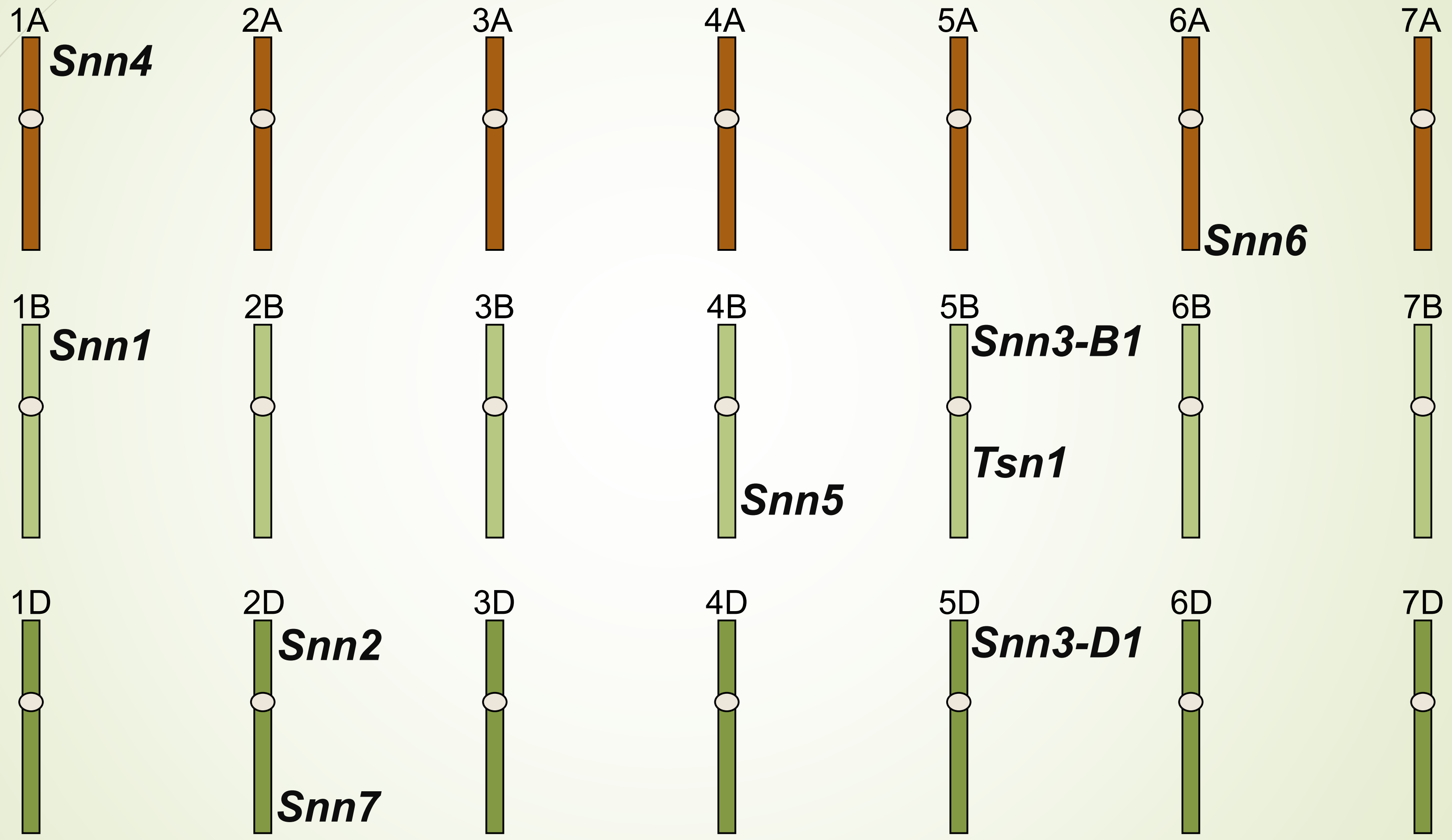
P. nodorum **NE** – small secreted proteins
(Friesen Lab)



Tsn1
Snn1
Snn2
Snn3-B1
Snn3-D1
Snn4
Snn5
Snn6
Snn7

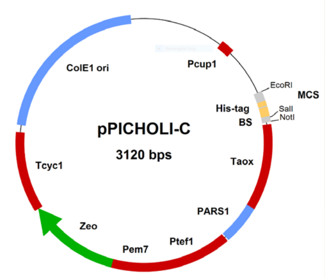
SnToxA
SnTox1
SnTox267
SnTox3
SnTox4
SnTox5
SnTox6
SnTox7

Genomic locations of SNB susceptibility genes

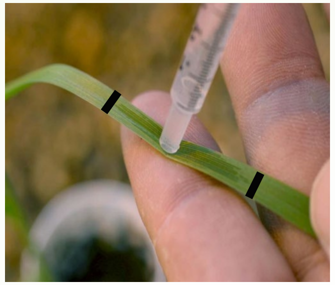


Genetic dissection of host gene-NE interactions

Pichia pastoris expression of NE

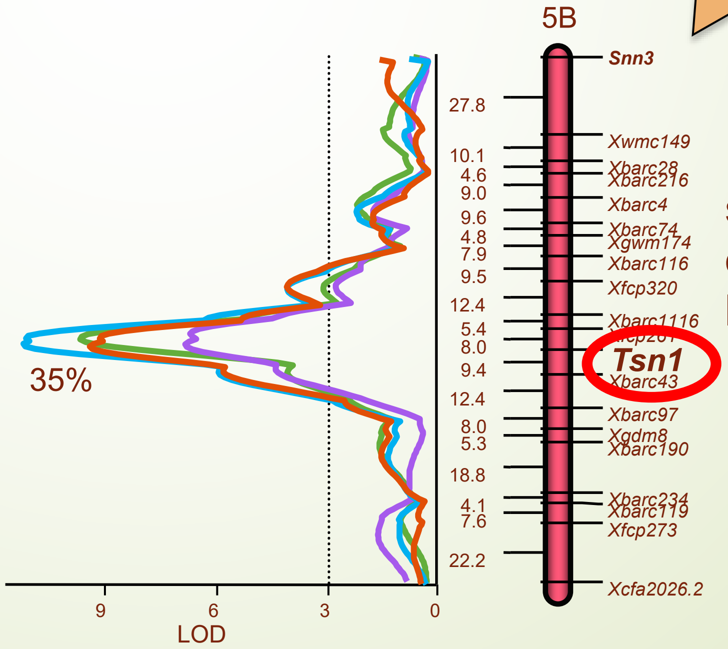


Infiltrate purified NE into wheat leaves



Score mapping population for reaction to the NE

Inoculate mapping population with spores of NE-producing isolate, score for disease reaction, and conduct QTL analysis to determine role of host-NE interaction in causing disease



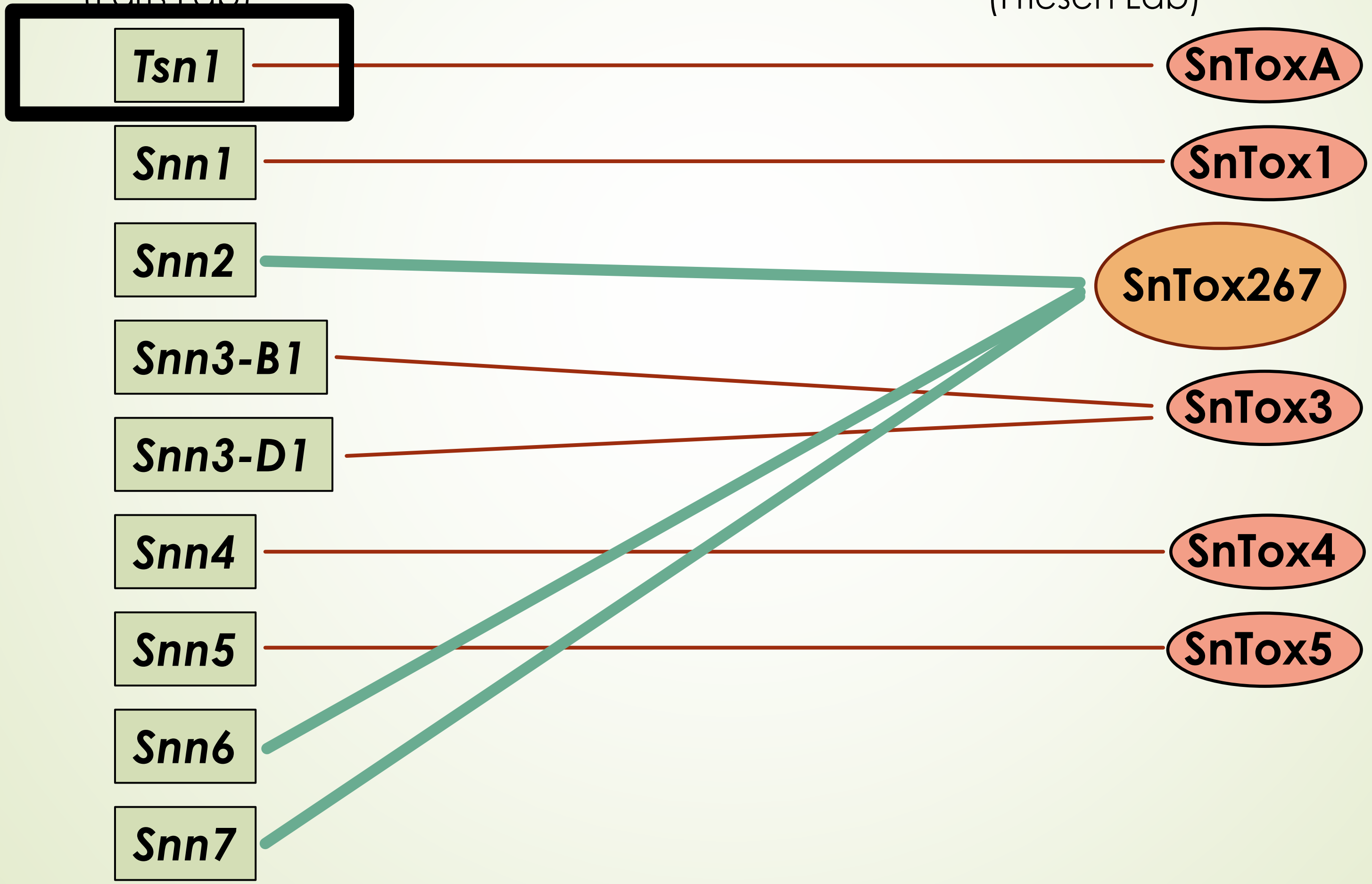
Place NE sensitivity locus on genetic linkage map

Cloning the Genes

The known interactions

<u>Wheat gene</u>
(Faris Lab)

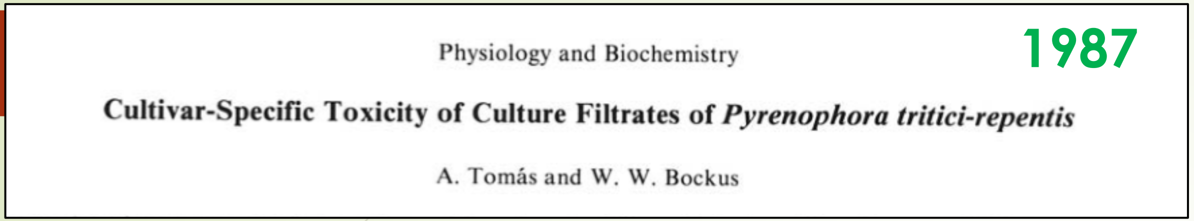
<u>*P. nodorum* NE</u> – small secreted proteins
(Friesen Lab)



Tsn1
Snn1
Snn2
Snn3-B1
Snn3-D1
Snn4
Snn5
Snn6
Snn7

SnToxA
SnTox1
SnTox267
SnTox3
SnTox4
SnTox5

Tsn1-ToxA

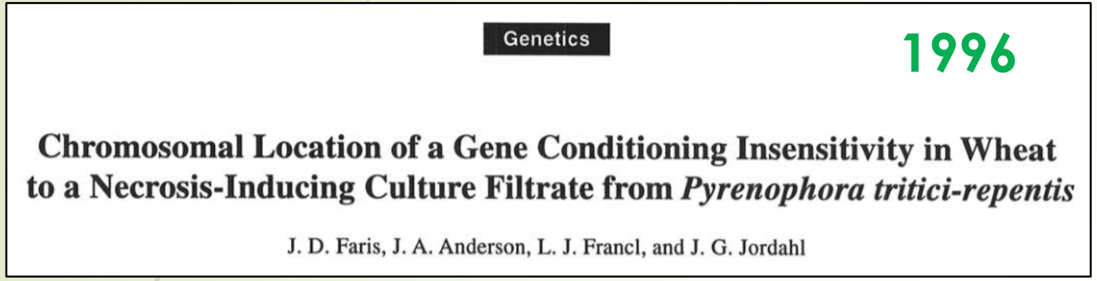


1987

Physiology and Biochemistry

Cultivar-Specific Toxicity of Culture Filtrates of *Pyrenophora tritici-repentis*

A. Tomás and W. W. Bockus



Genetics

1996

Chromosomal Location of a Gene Conditioning Insensitivity in Wheat to a Necrosis-Inducing Culture Filtrate from *Pyrenophora tritici-repentis*

J. D. Faris, J. A. Anderson, L. J. Francl, and J. G. Jordahl

Tsn1-ToxA in wheat-**tan spot** system

nature genetics

2006

Emergence of a new disease as a result of interspecific virulence gene transfer

Timothy L Friesen[1], Eva H Stukenbrock[2], Zhaohui Liu[3], Steven Meinhardt[3], Hua Ling[4], Justin D Faris[1], Jack B Rasmussen[3], Peter S Solomon[5], Bruce A McDonald[2] & Richard P Oliver[5]

Tsn1-ToxA in wheat-**Septoria nodorum blotch** system

AMERICAN SOCIETY FOR MICROBIOLOGY mBio®

2019

RESEARCH ARTICLE
Ecological and Evolutionary Science

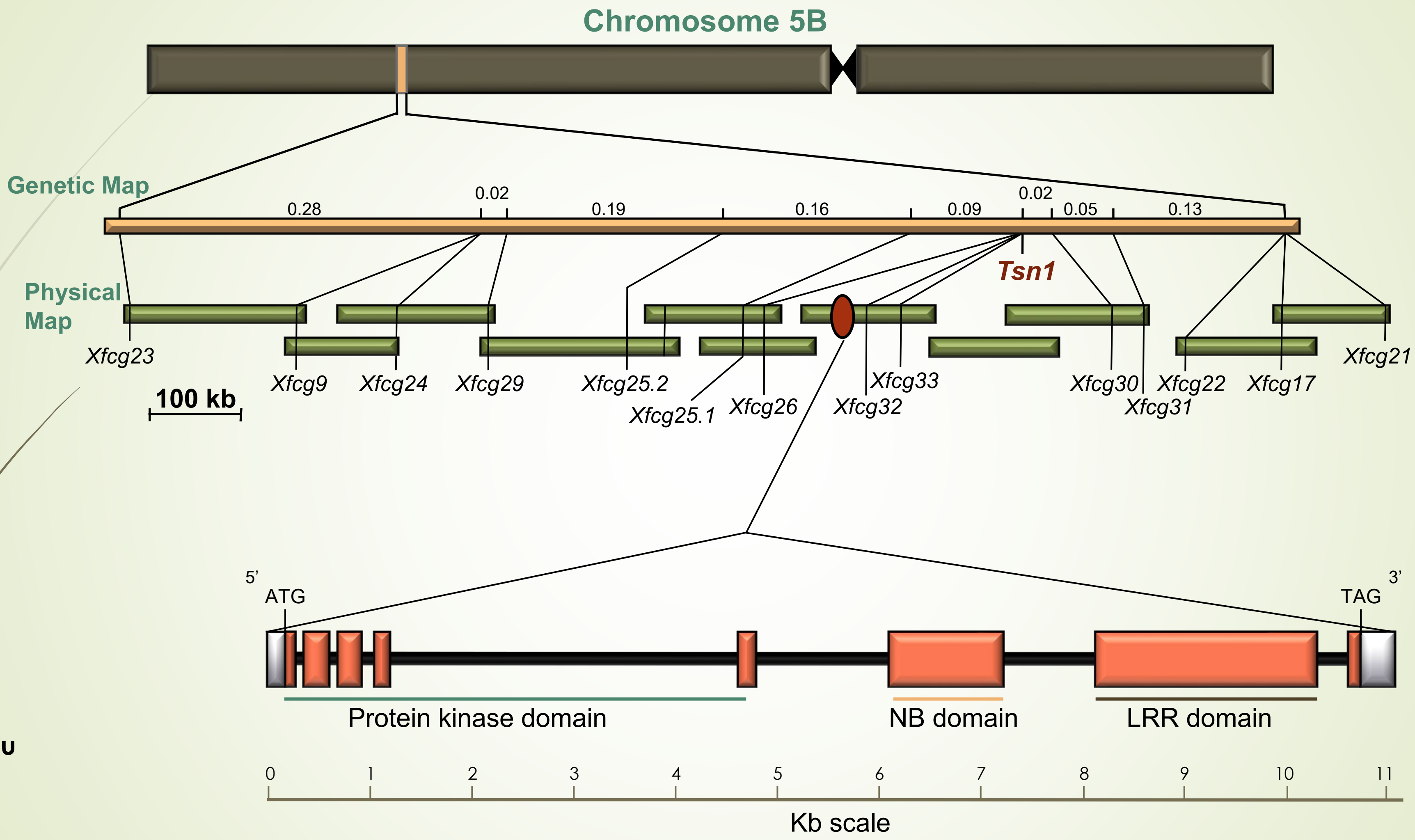
Check for updates

Transposon-Mediated Horizontal Transfer of the Host-Specific Virulence Protein ToxA between Three Fungal Wheat Pathogens

Megan C. McDonald,[a] Adam P. Taranto,[a] Erin Hill,[a] Benjamin Schwessinger,[a] Zhaohui Liu,[b] Steven Simpfendorfer,[c] Andrew Milgate,[d] Peter S. Solomon[a]

Tsn1-ToxA in wheat-**spot blotch** system

Map-based cloning of ***Tsn1***



Zengcui Zhang

Huangjun Lu

Effector sensitivity genes cloned

PNAS

A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens

Justin D. Faris[a,1], Zengcui Zhang[b], Huangjun Lu[b,2], Shunwen Lu[a], Leela Reddy[c,3], Sylvie Cloutier[d], John P. F
Steven W. Meinhardt[b], Jack B. Rasmussen[b], Steven S. Xu[a], Richard P. Oliver[f], Kristin J. Simons[a], and Timothy

[a]Cereal Crops Research U … Research Service, Fargo, ND 58
of [b]Plant Pathology and … Centre, Agriculture and Agri-

***Tsn1* = PK-NLR**

PNAS

Plant disease susceptibility conferred by a "resistance" gene

Jennifer M. Lorang, Teresa A. Sweat, and Thomas J. Wolpert*

*Department of Botany and Plant Pathology, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331

Edited by Steven P. Briggs, University of California at San Diego, La Jolla, CA, and approved July 12, 2007 (received for review March 20, 2007)

The molecular nature of many plant disease resistance (*R*) genes is known; the largest class encodes nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins that ar

confers gene-for-gene-specified disease susceptibility (7). Likewise, for Victoria blight of oats and a handful of other diseases … ens (pathogens that incite cell

***LOV1* = NLR**

Letter

Pathogen corruption and site-directed recombination at a plant disease resistance gene cluster

Ervin D. Nagy and Jeffrey L. Bennetzen[1]

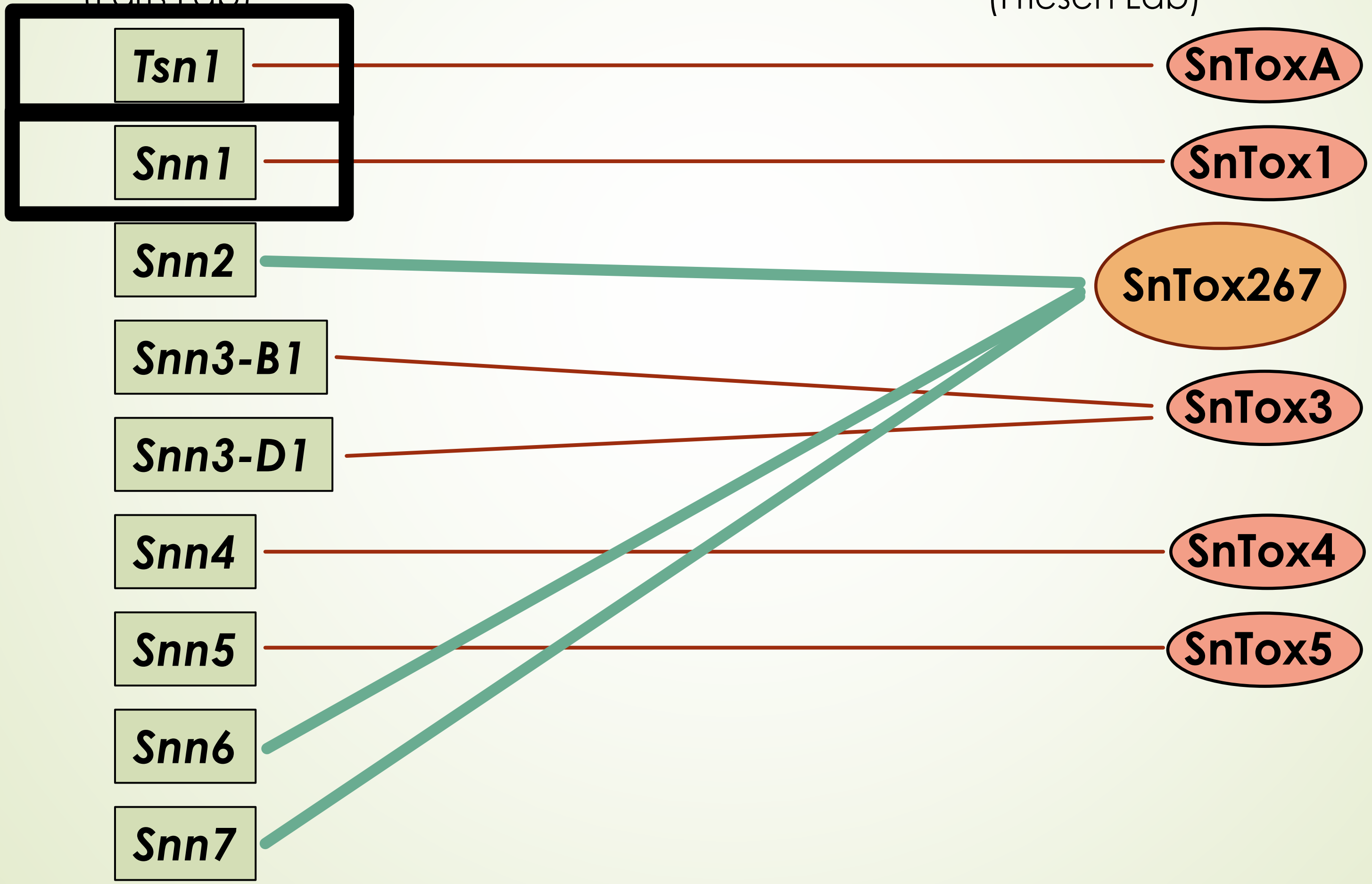
Department of Genetics, University of Georgia, Athens, Georgia 30602, USA

***Pc* = NLR**

The known interactions

<u>Wheat gene</u>
(Faris Lab)

<u>P. nodorum</u> <u>NE</u> – small secreted proteins
(Friesen Lab)



Tsn1

Snn1

Snn2

Snn3-B1

Snn3-D1

Snn4

Snn5

Snn6

Snn7

SnToxA

SnTox1

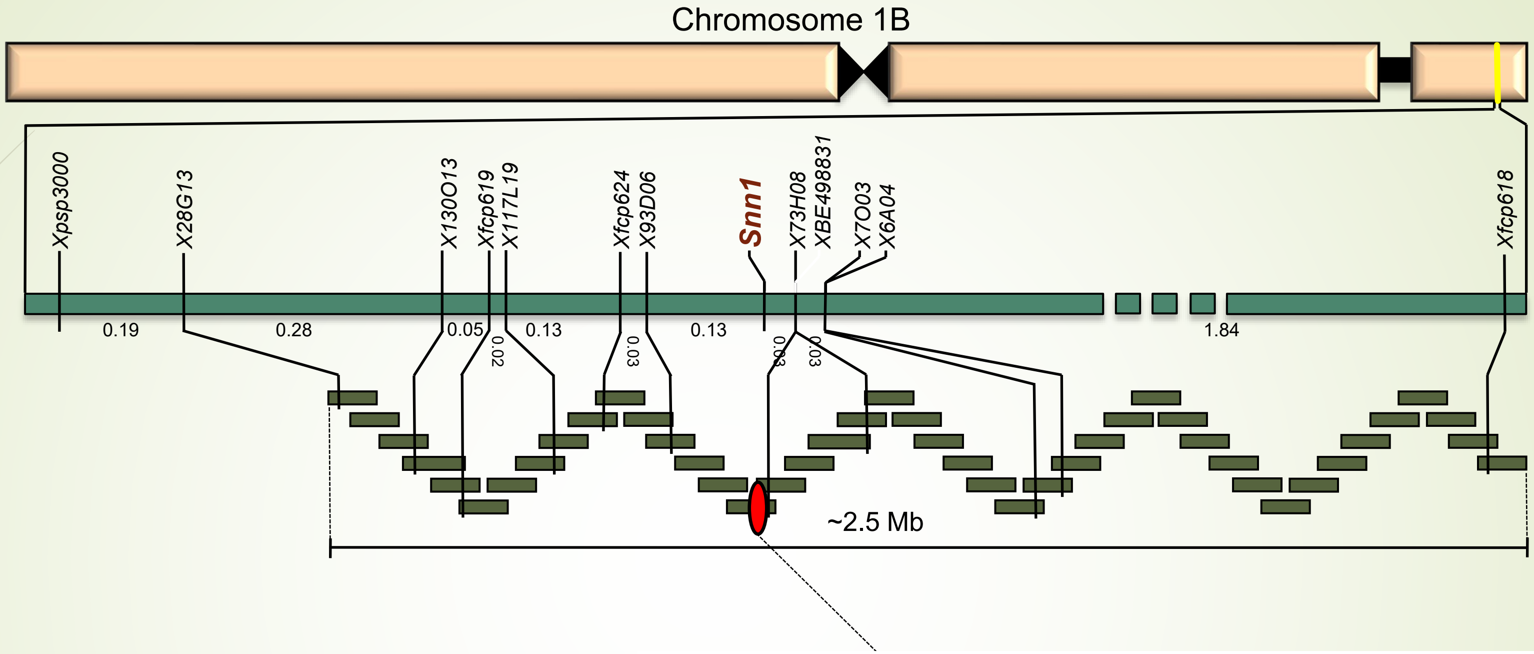
SnTox267

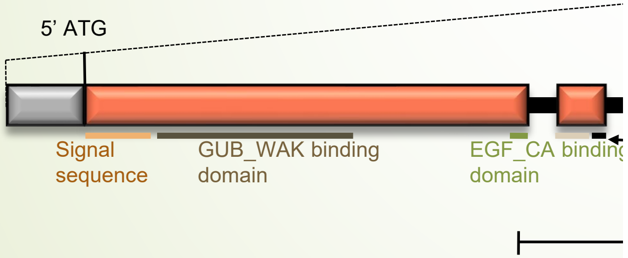
SnTox3

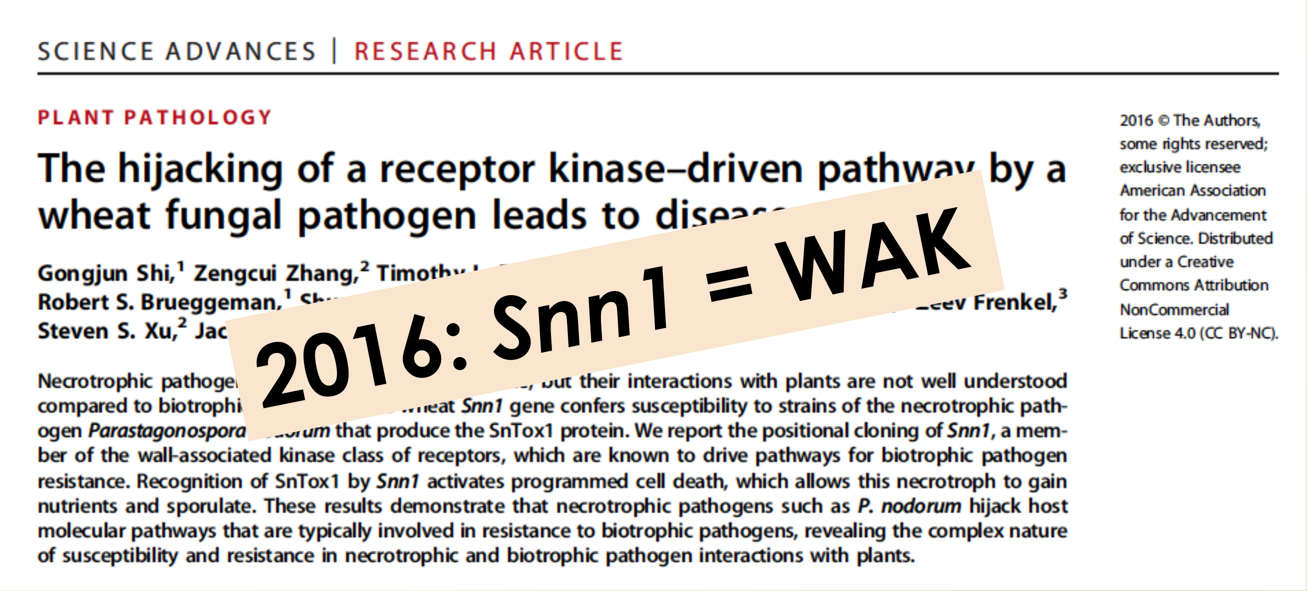
SnTox4

SnTox5

Map-based cloning of *Snn1*






SCIENCE ADVANCES | RESEARCH ARTICLE

PLANT PATHOLOGY

The hijacking of a receptor kinase–driven pathway by a wheat fungal pathogen leads to disea

Gongjun Shi,[1] Zengcui Zhang,[2] Timothy
Robert S. Brueggeman,[1] Sh
eev Frenkel,[3]
Steven S. Xu,[2] Jac

Necrotrophic pathoge , but their interactions with plants are not well understood compared to biotrophi heat *Snn1* gene confers susceptibility to strains of the necrotrophic pathogen *Parastagonospora* orum that produce the SnTox1 protein. We report the positional cloning of *Snn1*, a member of the wall-associated kinase class of receptors, which are known to drive pathways for biotrophic pathogen resistance. Recognition of SnTox1 by *Snn1* activates programmed cell death, which allows this necrotroph to gain nutrients and sporulate. These results demonstrate that necrotrophic pathogens such as *P. nodorum* hijack host molecular pathways that are typically involved in resistance to biotrophic pathogens, revealing the complex nature of susceptibility and resistance in necrotrophic and biotrophic pathogen interactions with plants.

2016: Snn1 = WAK

Gongjun Shi

Tsn1 vs *Snn1*

Tsn1 = NLR

- In plant-**biotroph** systems, recognition of effectors by NLRs activates effector-triggered immunity (ETI)
- *P. nodorum* uses ToxA to subvert *Tsn1* to activate **ETI** pathway

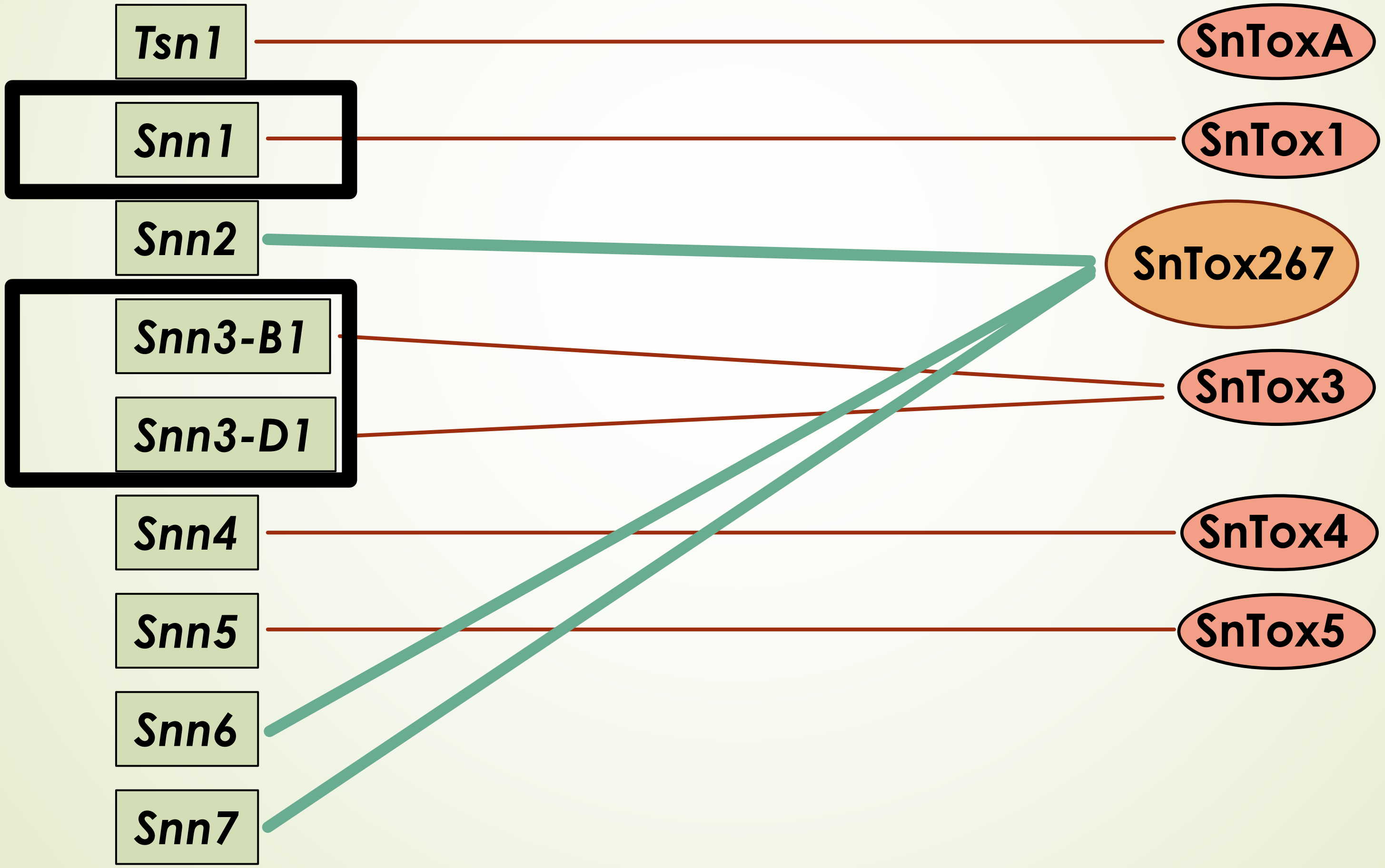
Snn1 = RLK (WAK)

- In plant-**biotroph** systems, recognition of PAMPs by RLKs activates PAMP-triggered immunity (PTI)
- *P. nodorum* uses SnTox1 to subvert *Snn1* to activate **PTI** pathway

The known interactions

Wheat gene
(Faris Lab)

P. nodorum NE – small secreted proteins
(Friesen Lab)

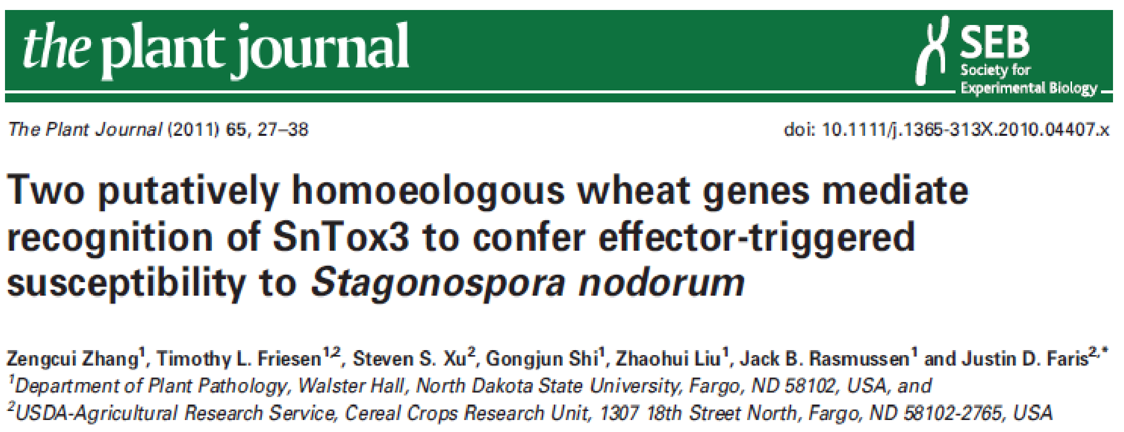


Tsn1
Snn1
Snn2
Snn3-B1
Snn3-D1
Snn4
Snn5
Snn6
Snn7

SnToxA
SnTox1
SnTox267
SnTox3
SnTox4
SnTox5

Genetic mapping of *Snn3-B1* and *Snn3-D1*

- **Sensitivity to SnTox3 mapped to 5BS in hexaploid wheat and 5DS in *Ae. tauschii***



the plant journal SEB Society for Experimental Biology

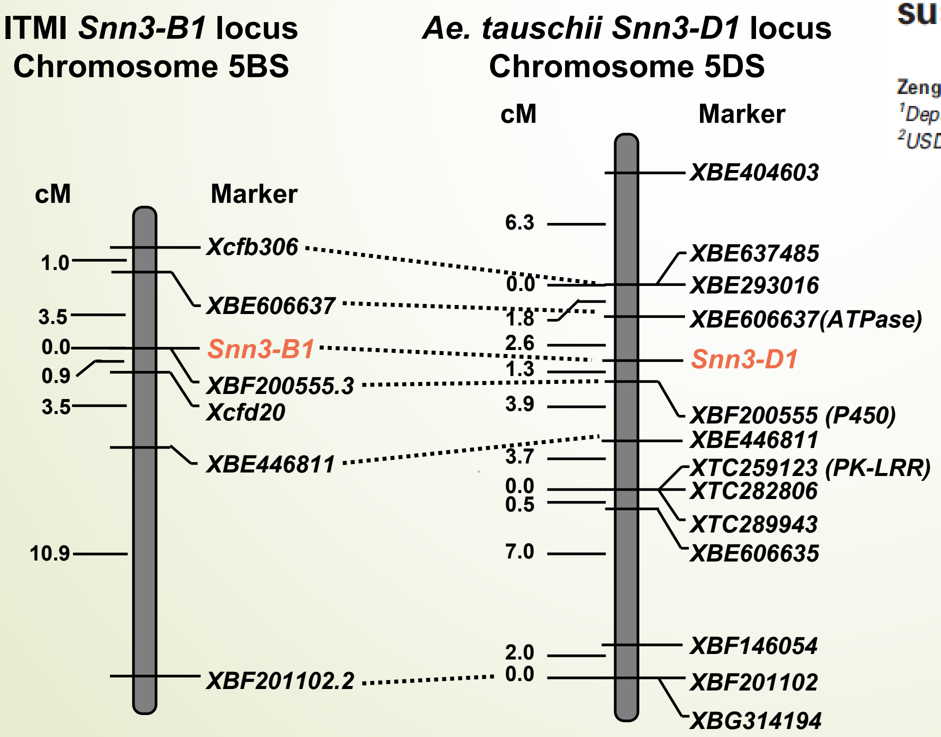
The Plant Journal (2011) 65, 27–38 doi: 10.1111/j.1365-313X.2010.04407.x

Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*

Zengcui Zhang[1], Timothy L. Friesen[1,2], Steven S. Xu[2], Gongjun Shi[1], Zhaohui Liu[1], Jack B. Rasmussen[1] and Justin D. Faris[2,*]

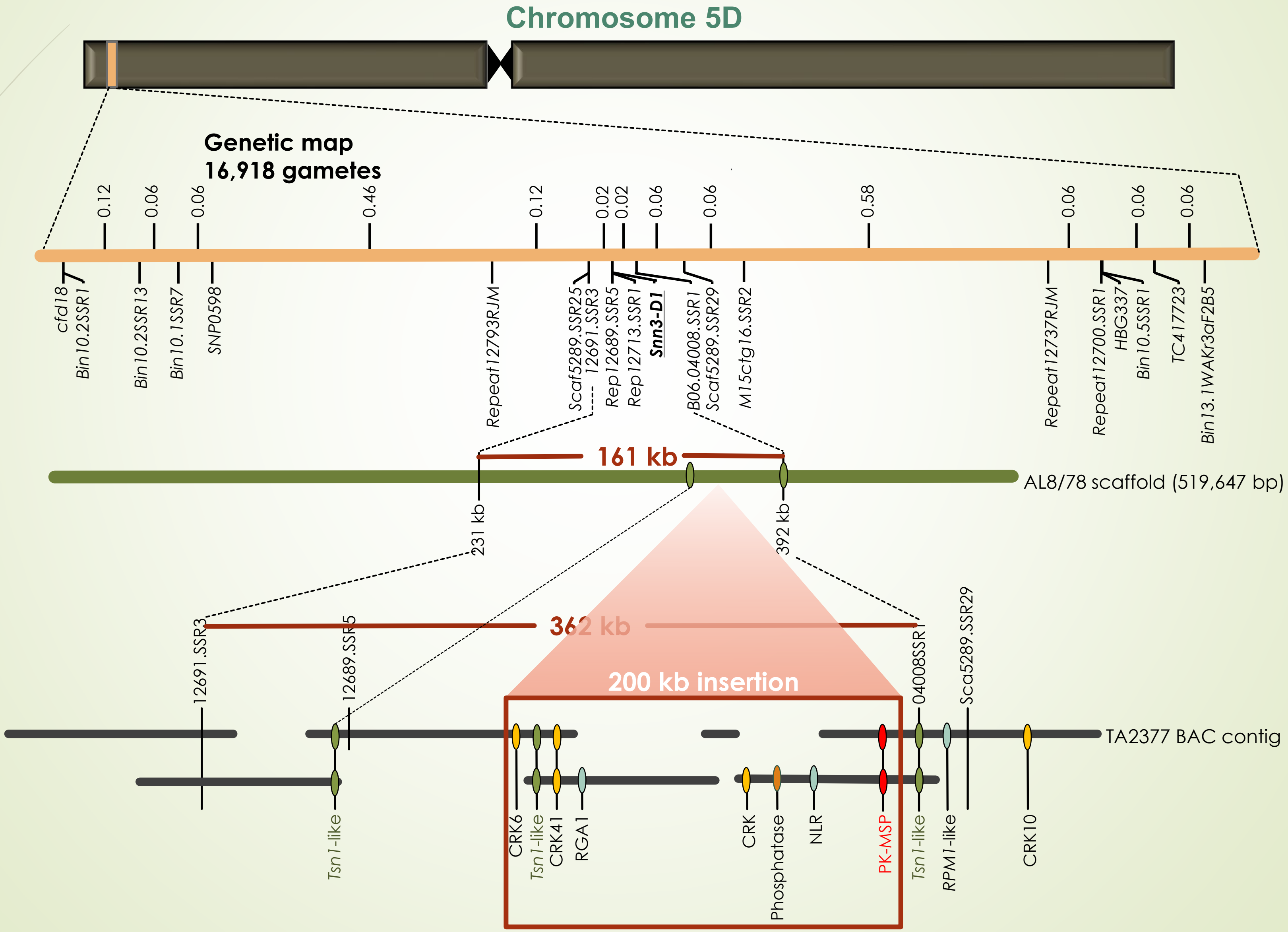
[1]*Department of Plant Pathology, Walster Hall, North Dakota State University, Fargo, ND 58102, USA, and*

[2]*USDA-Agricultural Research Service, Cereal Crops Research Unit, 1307 18th Street North, Fargo, ND 58102-2765, USA*



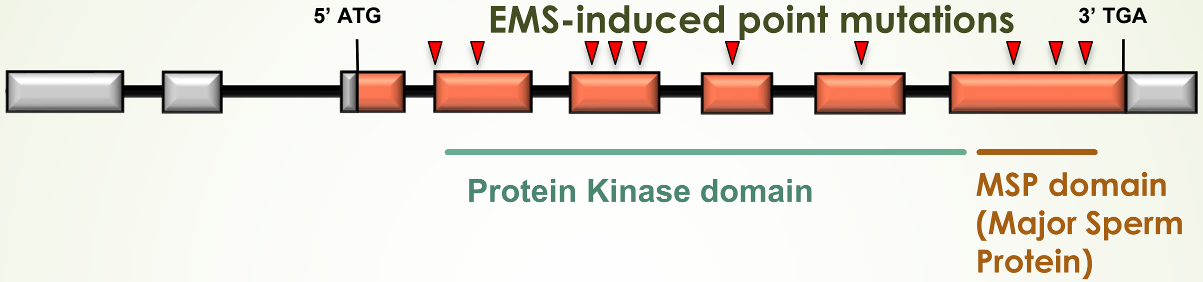
Zengcui Zhang

Map-based cloning of *Snn3-D1* in *Ae. tauschii*



Zengcui Zhang

Snn3-D1 structure and validation



5' ATG

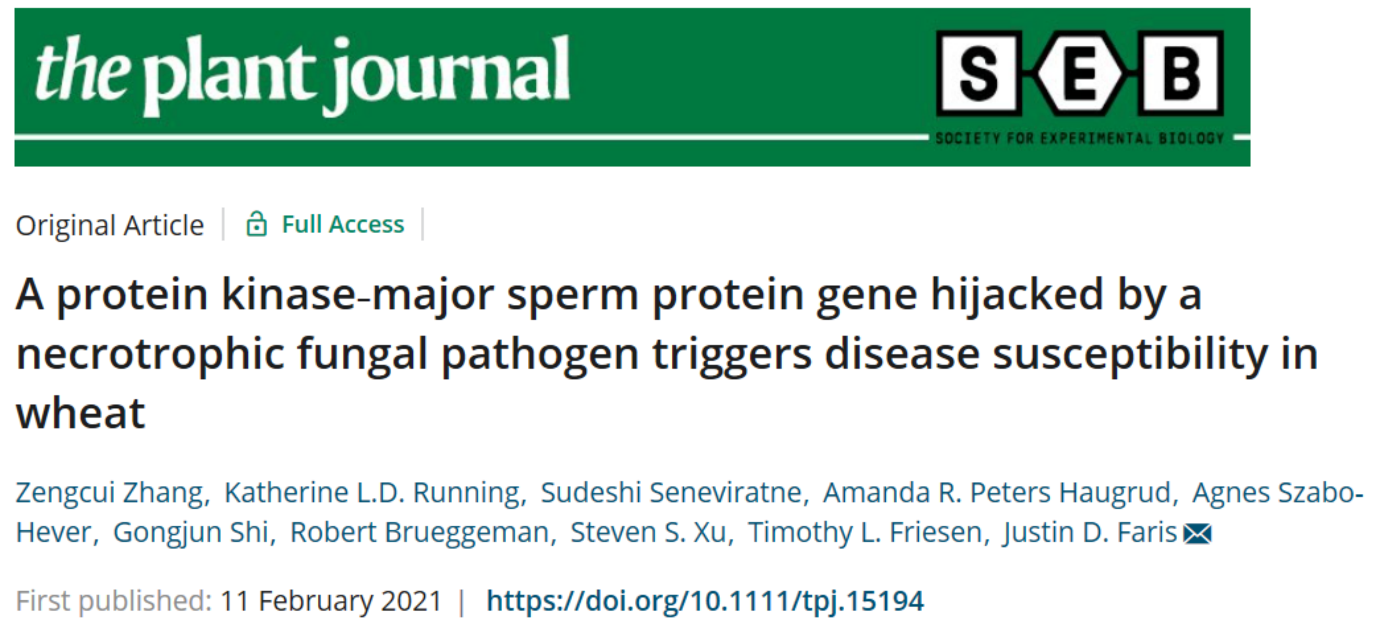
EMS-induced point mutations

3' TGA

Protein Kinase domain

MSP domain (Major Sperm Protein)

Genomic sequence: 1,977 bp
Protein: 492 amino acids



the plant journal

Original Article | Full Access |

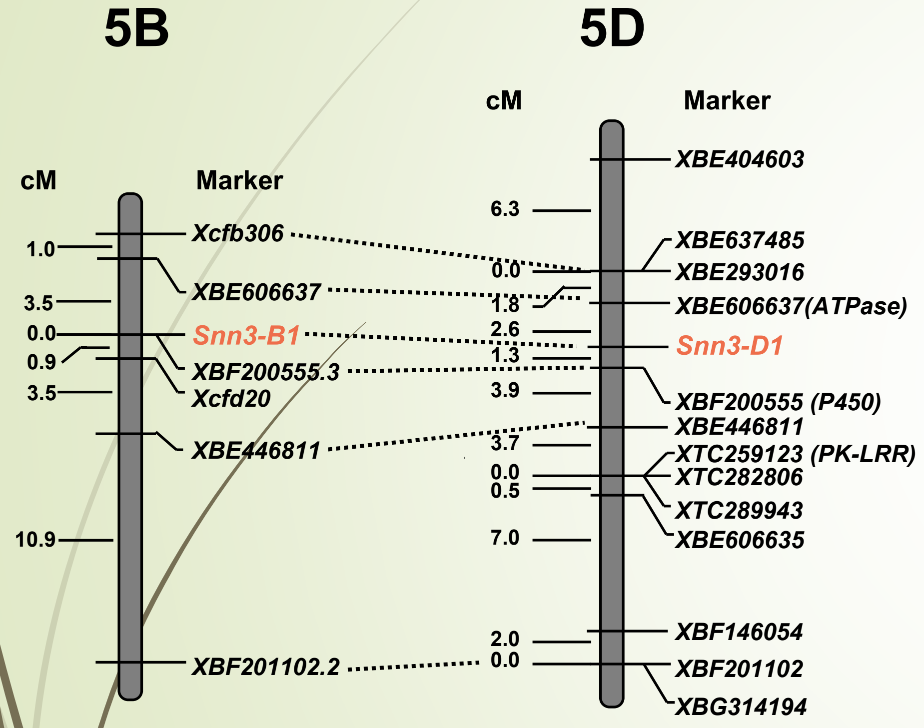
A protein kinase-major sperm protein gene hijacked by a necrotrophic fungal pathogen triggers disease susceptibility in wheat

Zengcui Zhang, Katherine L.D. Running, Sudeshi Seneviratne, Amanda R. Peters Haugrud, Agnes Szabo-Hever, Gongjun Shi, Robert Brueggeman, Steven S. Xu, Timothy L. Friesen, Justin D. Faris

First published: 11 February 2021 | https://doi.org/10.1111/tpj.15194

Zengcui Zhang

Cloning *Snn3-**B1***



Snn3-D1 alignment to best hit on chromosome 5B


```
Snn3-D1         ATGCAGTACCTGTCAGATAAAATGTCCTCGTCCGATCCACAACATATCAAATTATGTTTG    60
Snn3-B1         ATGCAGTACTTGTCAGATAAAATGTCCTCGTCTGATCCACAATATATCAAATTATGTTTG    60
                ********* ********************** ********* *****************

Snn3-D1         TTAGAAGCAATCACAGAGGAGTTTTCAGAGAAGATGAAAATTGGTACCGGTGGCTATGGA    120
Snn3-B1         TTACAAGCAATCACAGAGGAGTTTTCAGAGAAGATGAAAATTGGTACTGGTGGCTATGGG    120
                *** ******************************************* ***********

Snn3-D1         GAAGTTTACAAGGTAGGT--ATGGTCAATCATTTATGTTAGTTATATTAACTAATCGTAT    178
Snn3-B1         GAAGTTTACAAGGTAGGTATATGGTCAATCATTTATGTTTGTTATGCTAACTAGTCGTAT    180
                ******************  ******************* *****  ****** ******

Snn3-D1         TATGTTTTCTGTAATTATCATATGGTCAAAATGAACAGGGAGAGCTTAATGGGGACGAAA    238
Snn3-B1         TATGTTTTCTGTAATGATCATATGGTTAATATGAACAGGGTGAGCTTAATGGGGACGAAA    240
                *************** ********** ** ********** *******************

Snn3-D1         TTGCTGTCAAGAAGCTTTTTCCTGTCCAAGCAGTTAATGACGAGTCATTTGATAATGAAT    298
Snn3-B1         TTGCTGTCAAGAAGCTCTTTCCCATCCAAGGAGTTAATGATGAGTCATTTGATAATGAAT    300
                **************** *****  ****** ********* *******************
```

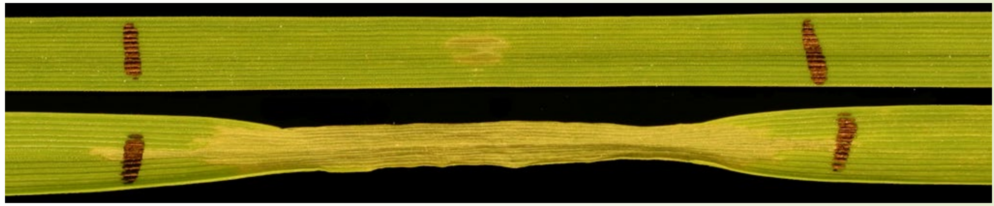
Zengcui Zhang

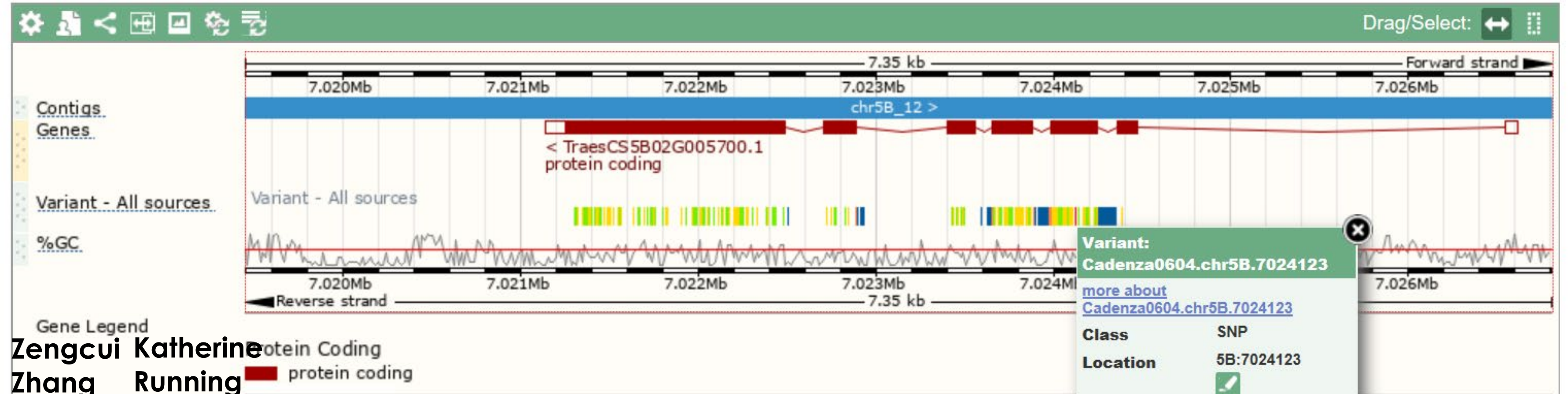
Katherine Running

Cloning *Snn3*-**B1**

Wheat TILLING resources (Krasileva et al. 2017)

- Cadenza (hexaploid): sequenced 1,200 EMS mutants
- Kronos (tetraploid): sequenced 1,535 EMS mutants





here are currently 59 tracks turned off.

nsembl Plants Triticum aestivum version 102.4 (IWGSC) Chromosome 5B: 7,019,450 - 7,026,800

Variant: Cadenza0604.chr5B.7024123

more about Cadenza0604.chr5B.7024123

Class	SNP
Location	5B:7024123
Alleles	G/A
Ambiguity code	R
Consequence	stop gained
Source	EMS-induced mutation

Zengcui Zhang **Katherine Running**

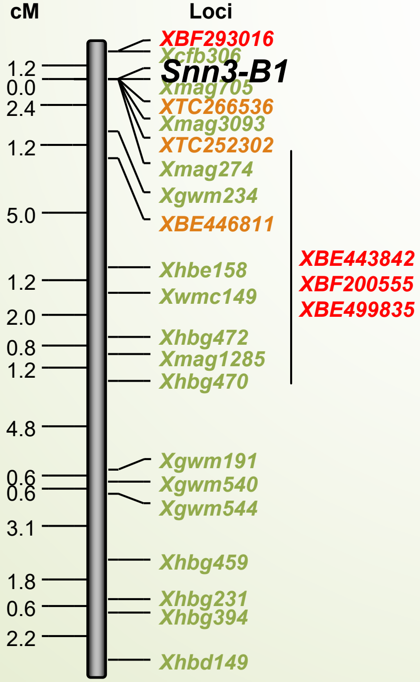
Ordered and tested 13 Cadenza mutants:

- **9 were completely insensitive or segregating for reaction to SnTox3 infiltrations**

Cloning *Snn3-**B1***

- We previously developed 13 SnTox3-insensitive mutants in wheat line Sumai3 (Shi et al. 2015).
- Sequencing of *Snn3-B1* from the mutants indicated that NONE of them had mutations within the *Snn3-B1* coding region.

CS x Sumai3 5B



<u>Allelism test</u>

Sumai3 x BG220 (SnTox3 diff.)

↓

Screened 176 F_2 plants:

174 sensitive : 2 insensitive

↓

Suggesting different genes!

Zengcui Zhang

Katherine Running

What is the SnTox3 sensitivity gene in Sumai3?

- **MutChromSeq** (Sánchez-Martín et al. 2016)
- Jaroslav Doležel Lab

Chromosome sorting (5B) from wt and each mutant

↓

Sequencing and assembly

↓

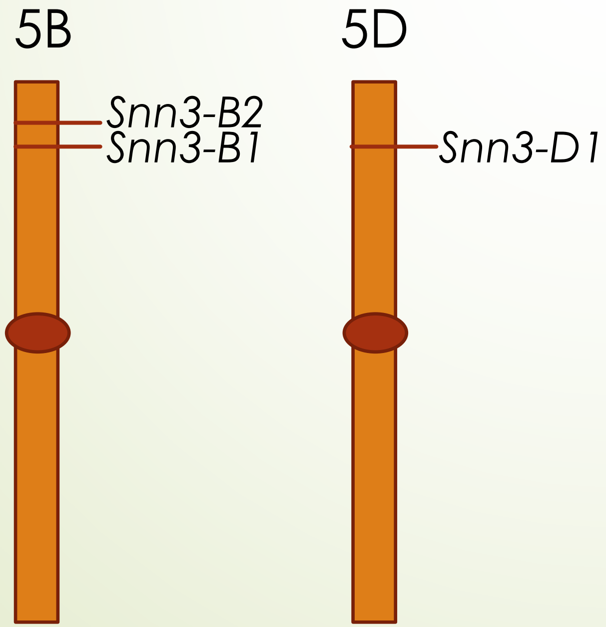
Sequence comparisons to identify SNPs within common scaffolds

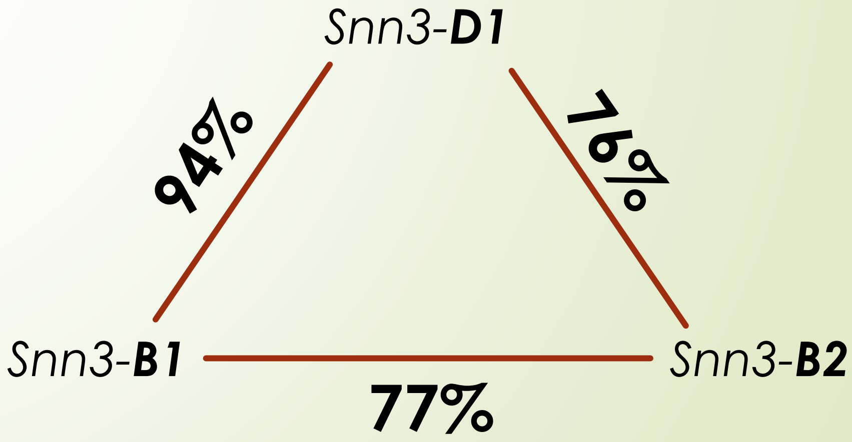
↓

Snn3-B2

Snn3-B2

- PK-MSP
- 77% identity to *Snn3-B1*
- Located about 1 Mb distal to *Snn3-B1*





Zengcui Zhang

Katherine Running

Tsn1 vs *Snn1* vs ***Snn3***

Tsn1 = NLR

- In plant-**biotroph** systems, recognition of effectors by NLRs activates effector-triggered immunity (ETI)
- *P. nodorum* uses ToxA to subvert *Tsn1* to activate **ETI** pathway

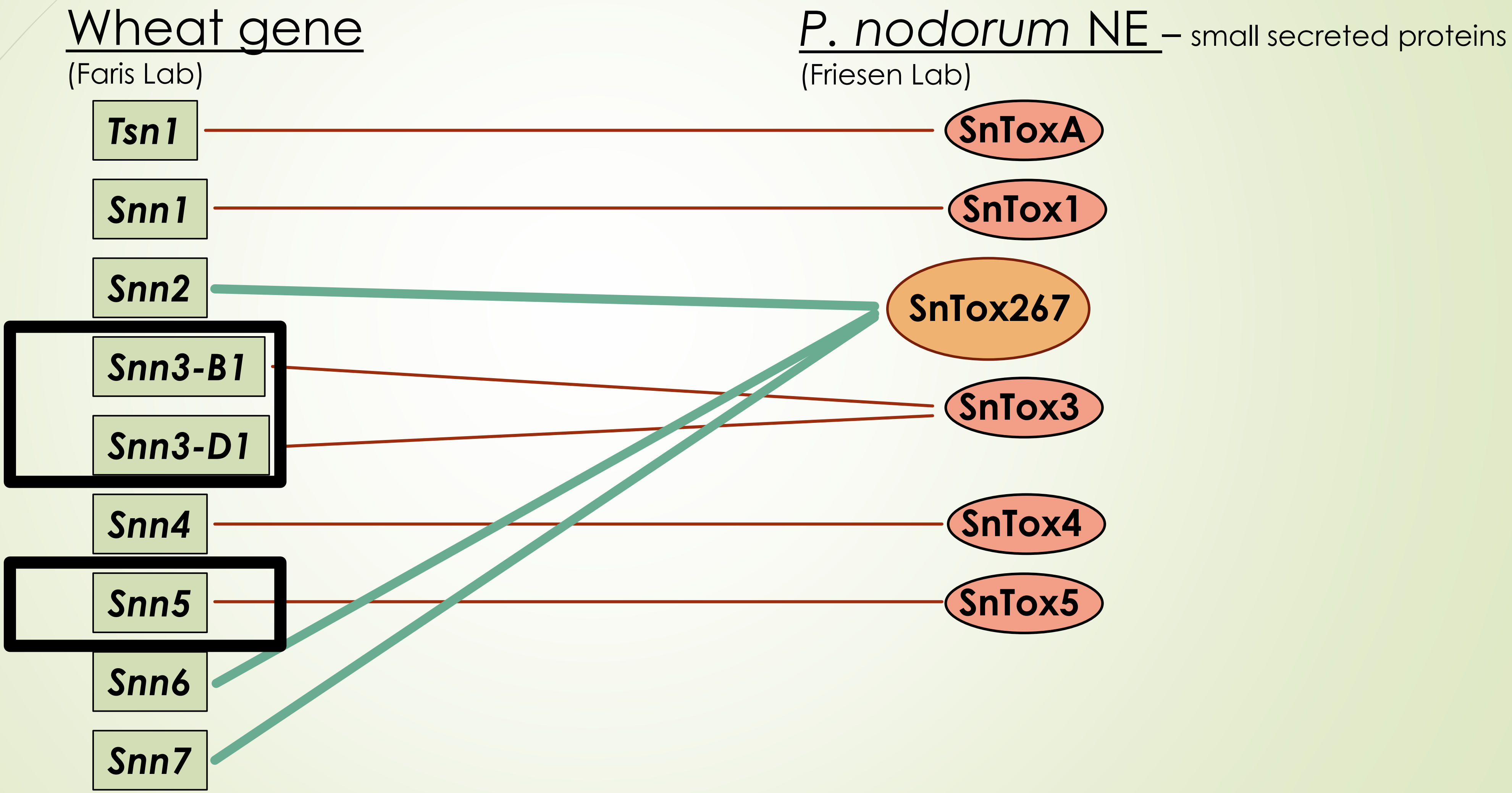
Snn1 = RLK (WAK)

- In plant-**biotroph** systems, recognition of PAMPs by RLKs activates PAMP-triggered immunity (PTI)
- *P. nodorum* uses SnTox1 to subvert *Snn1* to activate **PTI** pathway

Snn3 = PK-MSP

- ???

The known interactions

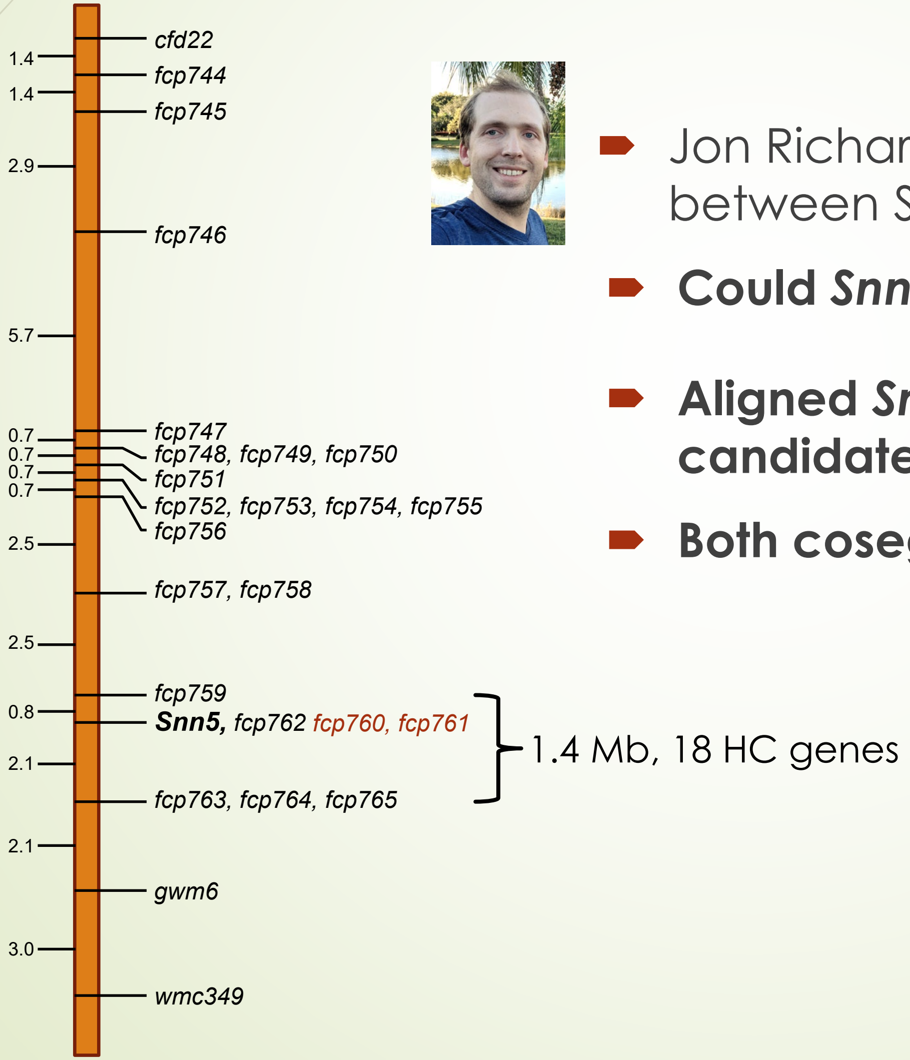


<u>Wheat gene</u>
(Faris Lab)

P. nodorum NE – small secreted proteins
(Friesen Lab)

Molecular cloning of *Snn5*

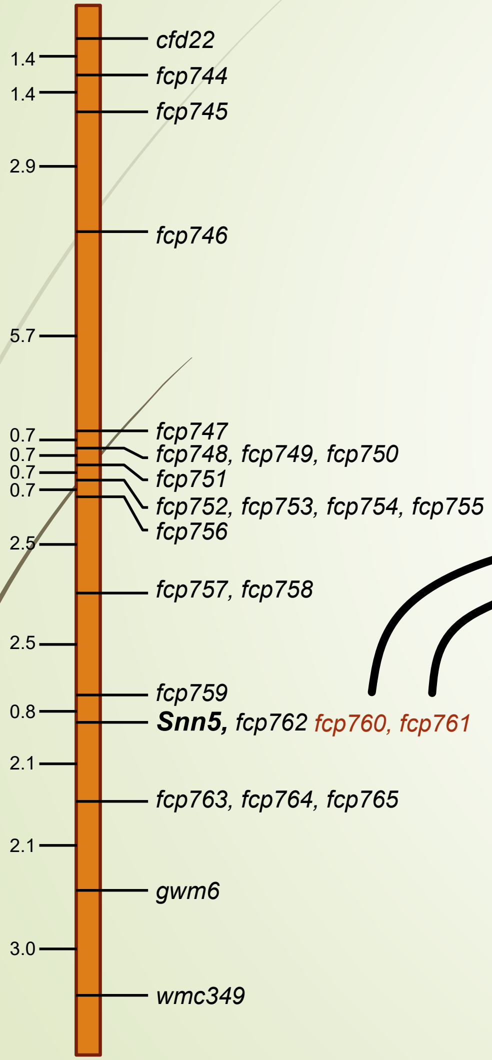
Chromosome 4B

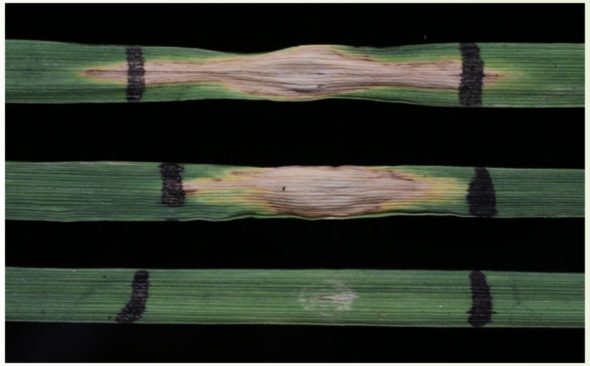


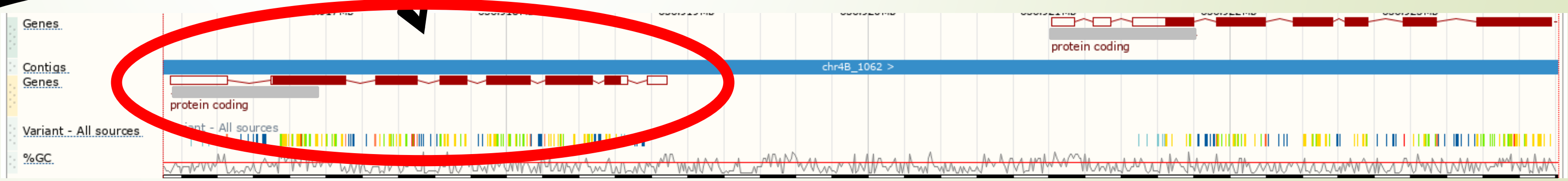
- Jon Richards: found structural similarities between SnTox3 and SnTox5
- **Could *Snn5* be a homolog of *Snn3*?**
- **Aligned *Snn3* sequence to *Snn5* candidate region, found two candidates**
- **Both cosegregated with *Snn5***

Sapna Sharma

Molecular cloning of *Snn5*







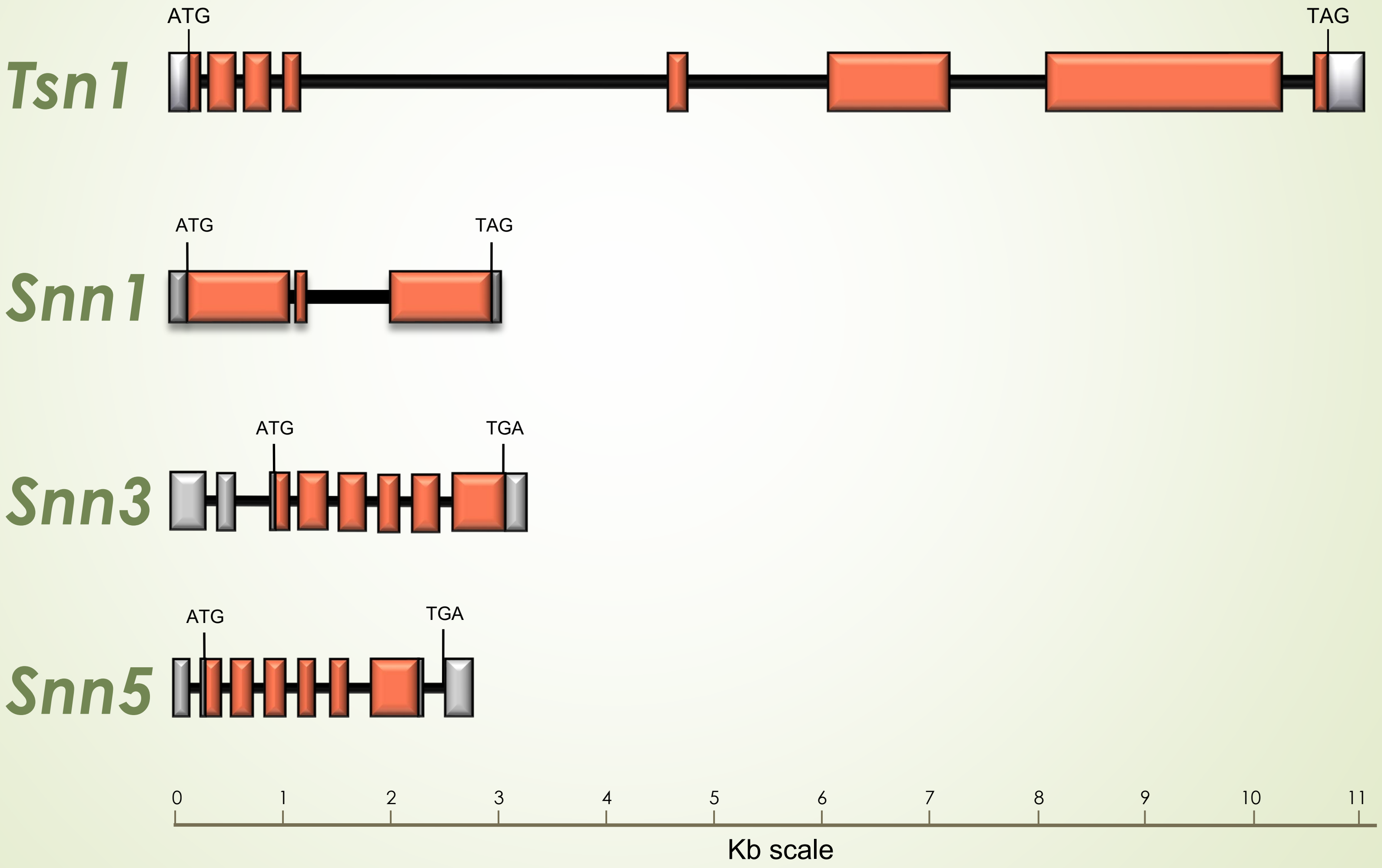
Katherine Running

<u>TaPKMSP-1</u>: 7 of 15 mutant families were completely insensitive to SnTox5 or segregated for sensitivity

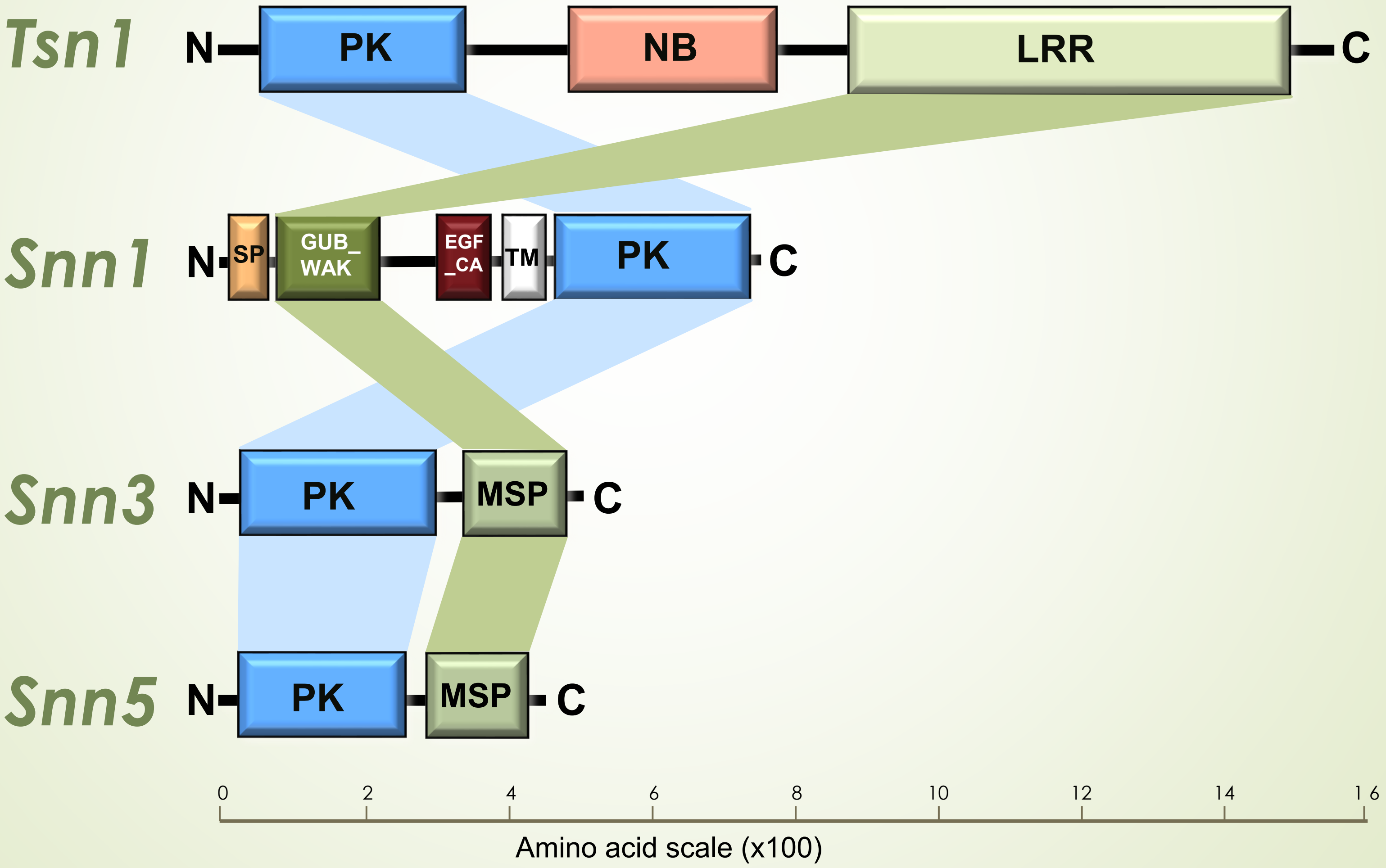
<u>TaPKMSP-2</u>: All of 17 mutant families were completely sensitive

Gene Comparisons

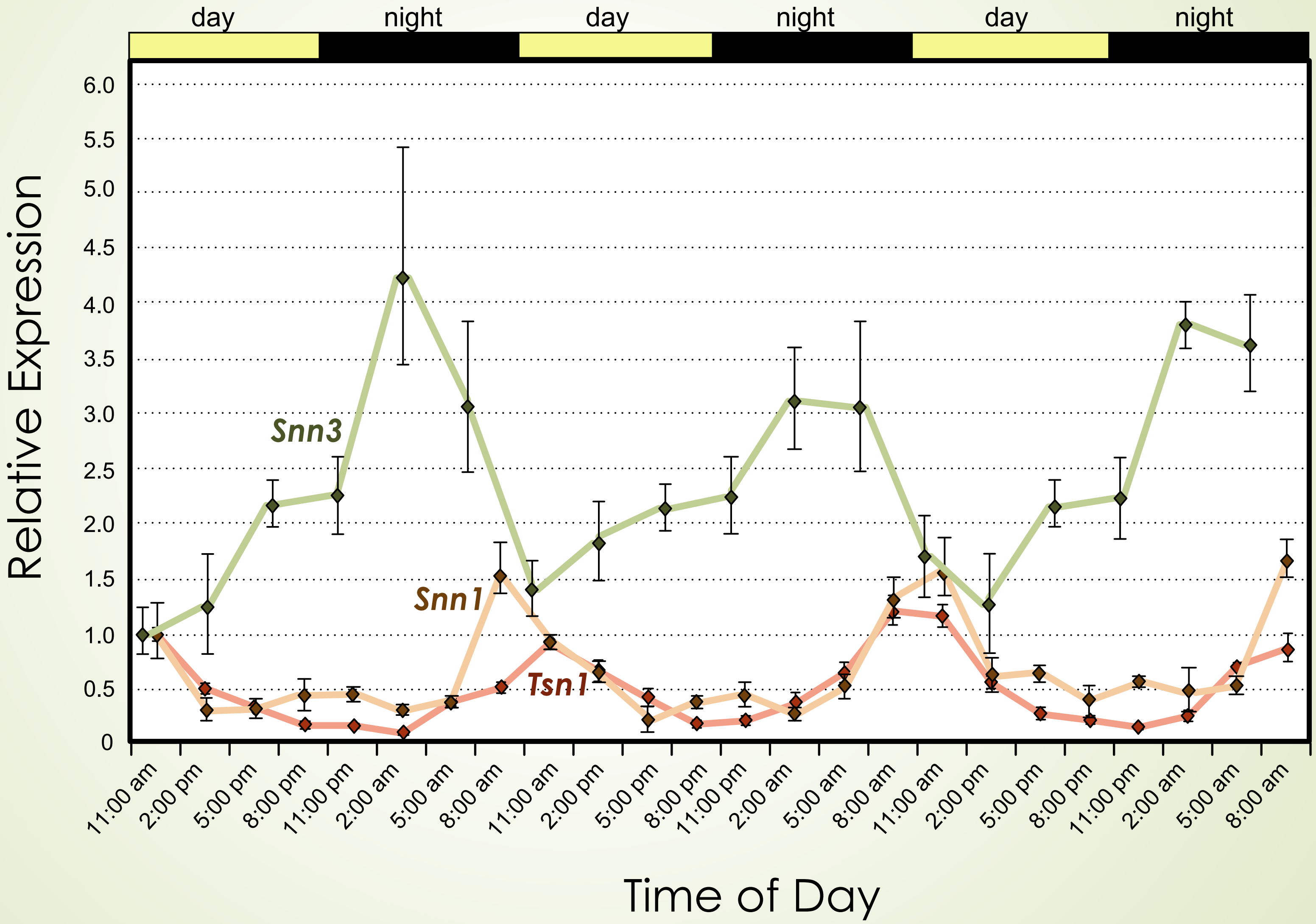
Four-gene comparison: Structure



Four-gene comparison: Functional domains

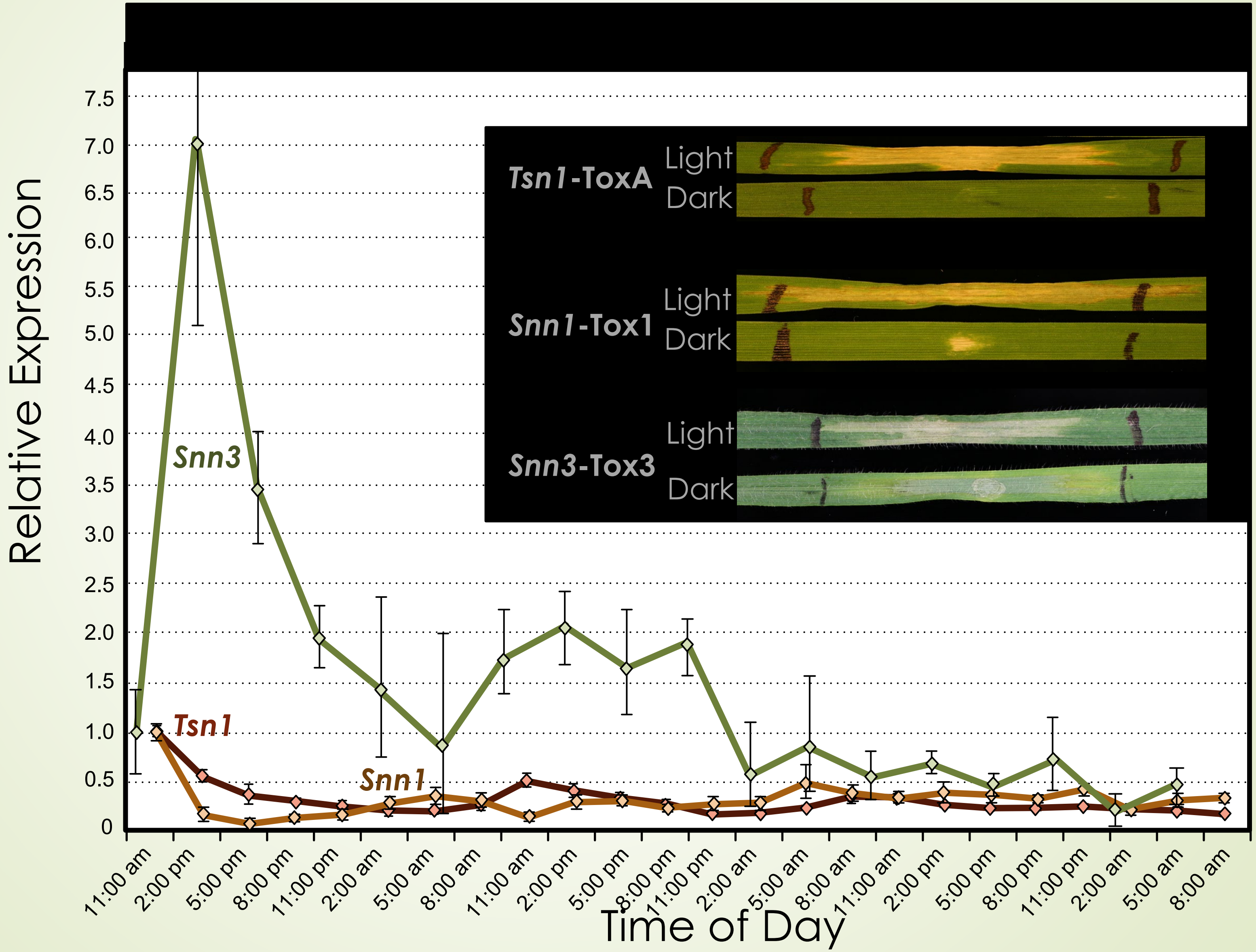


Three-gene comparison: Expression patterns



Zengcui Zhang

Three-gene comparison: Expression patterns



Function of the interactions in disease expression

- Evaluated expression of disease conferred by compatible gene-for-gene interactions
  - Mapping population segregated for four NE sensitivity genes (*Tsn1, Snn1, Snn3, Snn5*)
  - Inoculated population with pathogen isolates that produced various combinations of NEs (ToxA, Tox1, Tox3, Tox5)
  - Used regression to determine the role of individual interactions in disease development
  - RNAseq and RT-qPCR to look at expression of NEs

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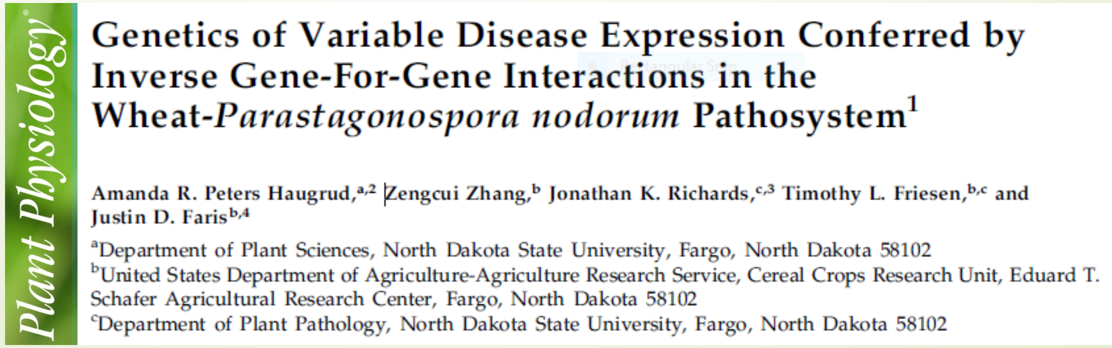

Plant Physiology

Genetics of Variable Disease Expression Conferred by Inverse Gene-For-Gene Interactions in the Wheat-*Parastagonospora nodorum* Pathosystem[1]

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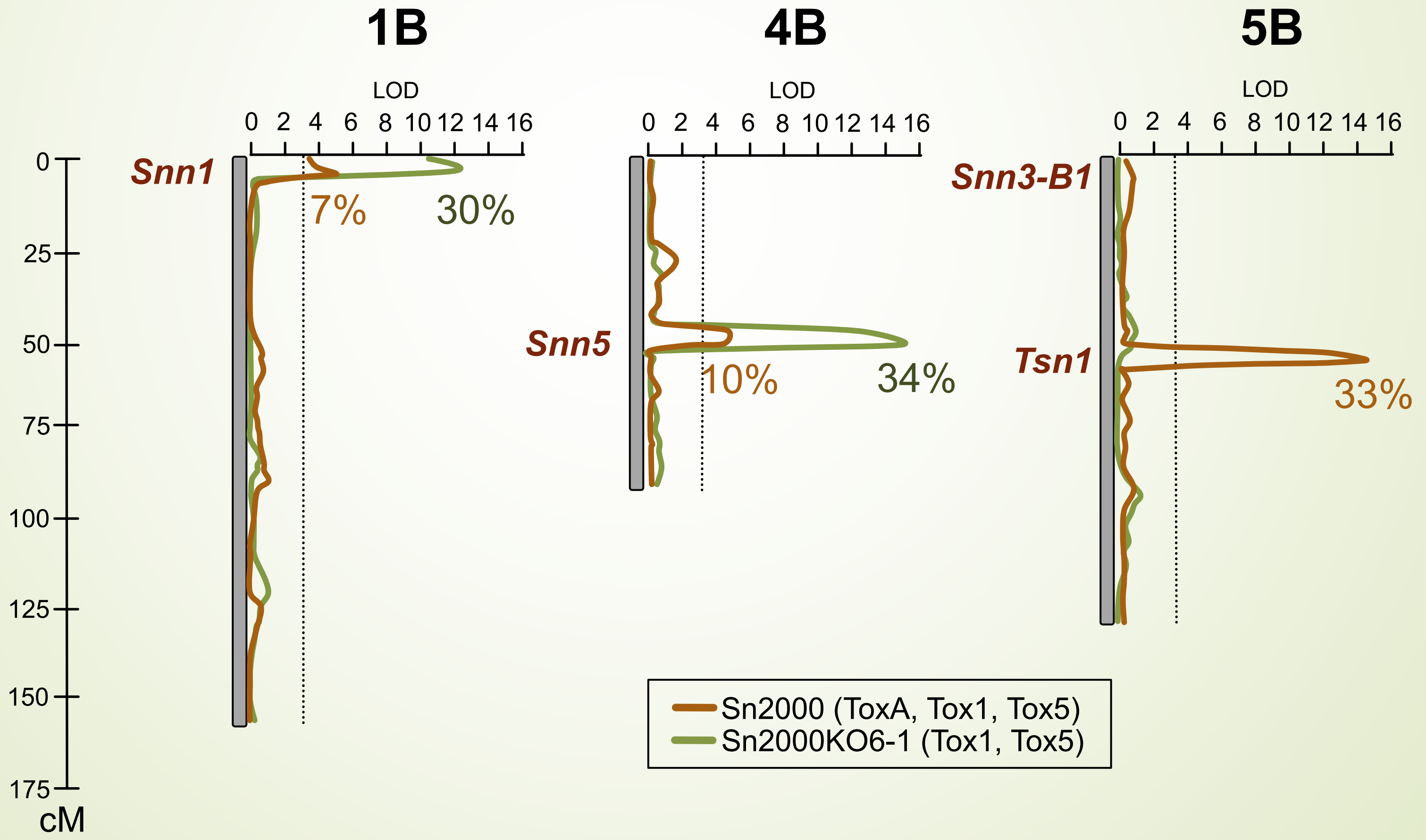
Variable expression of *Snn1*-Tox1 and *Snn5*-Tox5 in the presence/absence of *Tsn1*-ToxA



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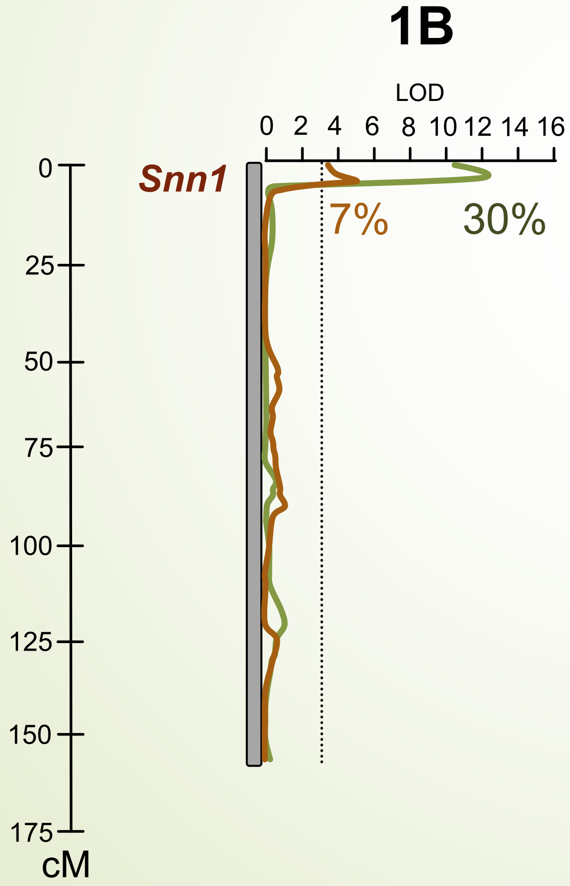
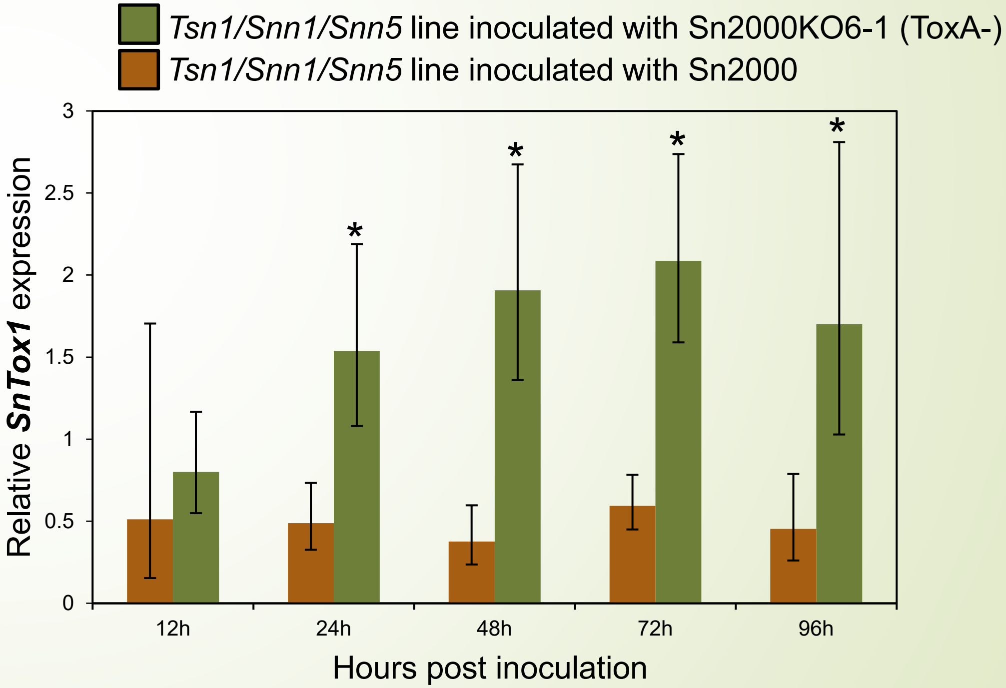
Variable expression of *Snn1*-Tox1 and *Snn5*-Tox5 in the presence/absence of *Tsn1*-ToxA





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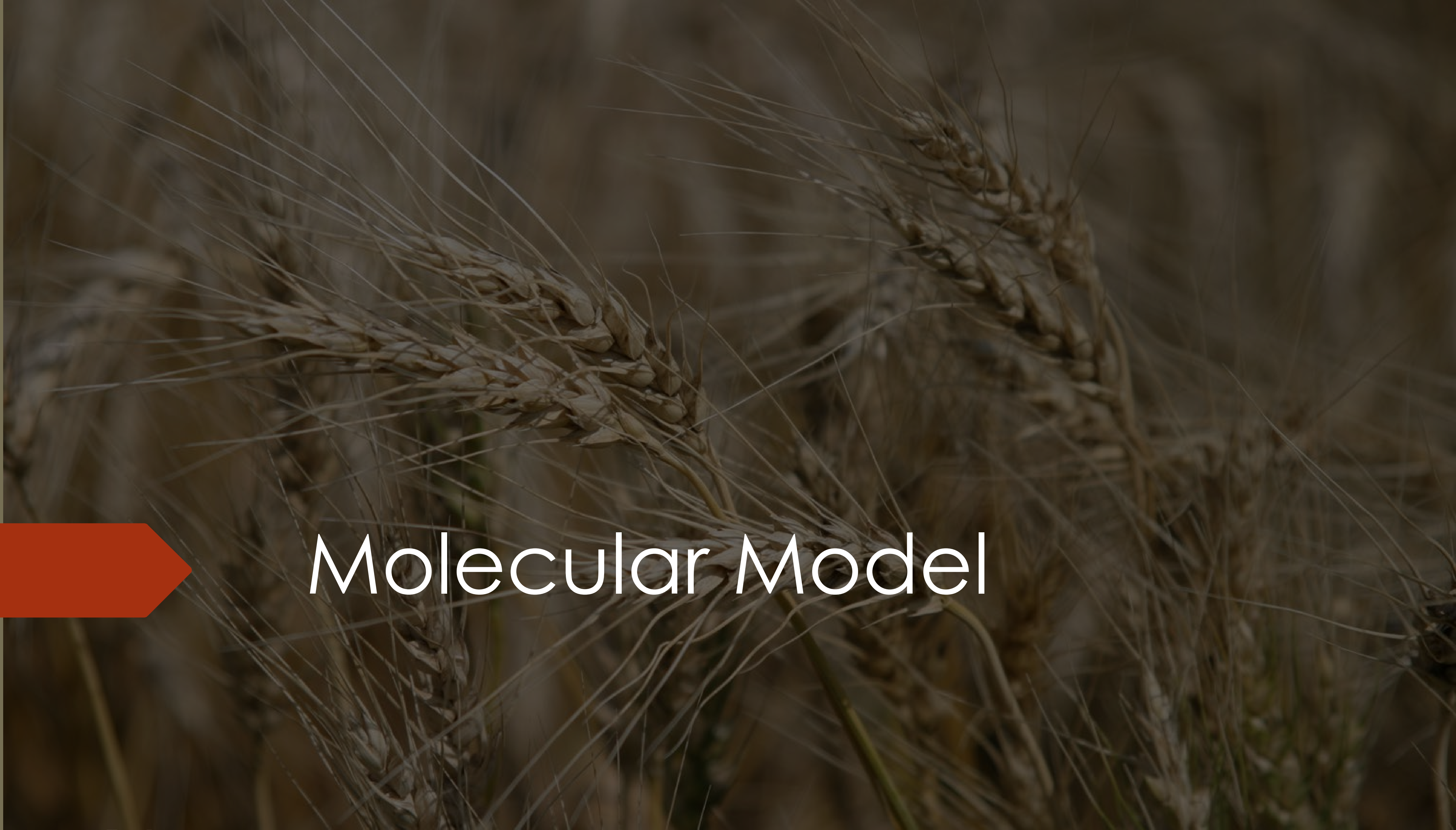
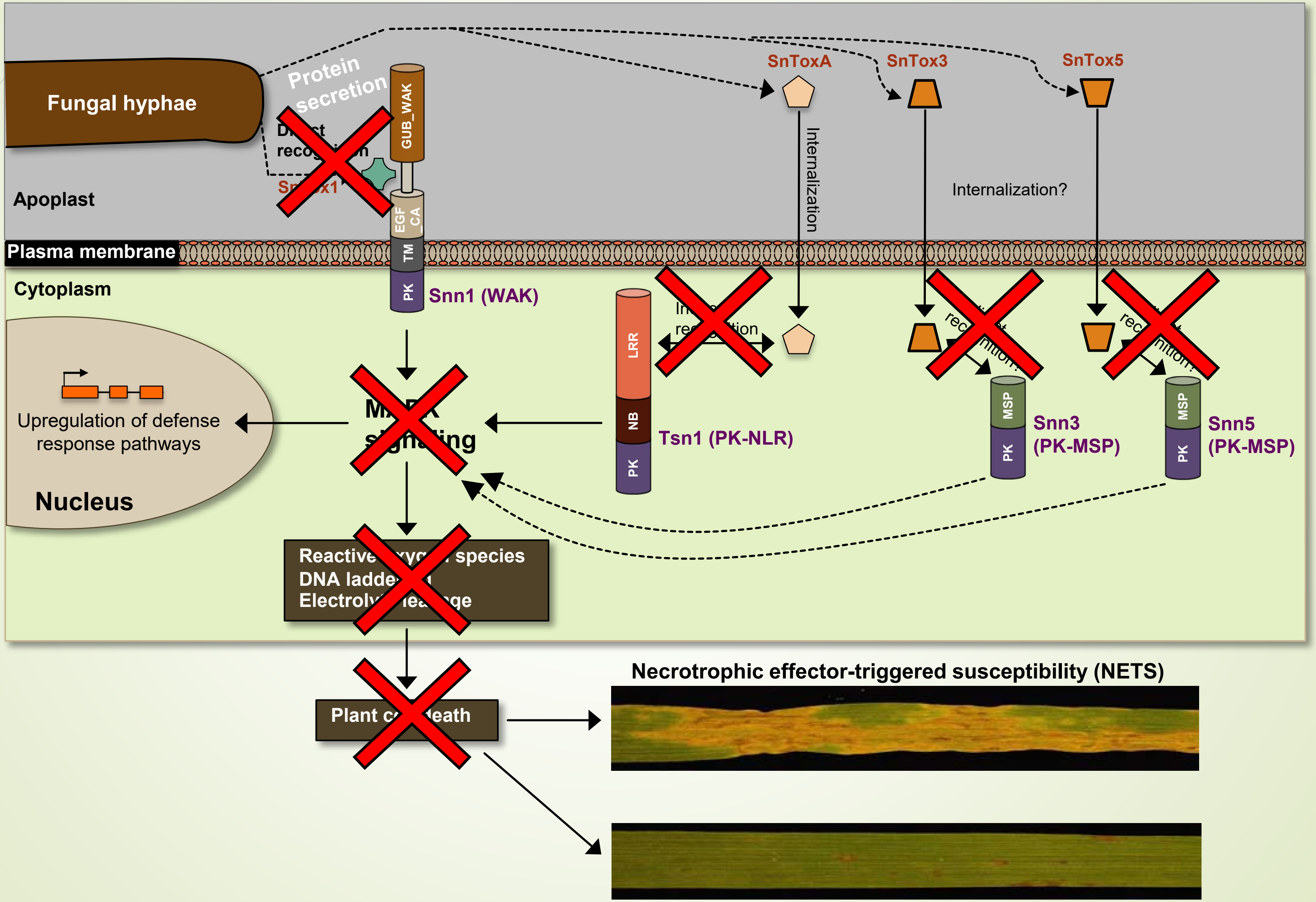
Takeaways regarding the function of the interactions in disease expression

- The effects of compatible interactions on disease vary depending on the isolate and host genotype
- Effects range from additive to epistatic
  - The effects of some interactions can be masked or inhibited by others
- **Regulation of interaction expression occurs at the level of NE gene transcription**
- **The pathogen may harbor a repertoire of NE genes but express mainly those that have the corresponding host sensitivity gene present**
  - i.e. the pathogen probably does not waste energy expressing NE genes that will not lead to host cell death



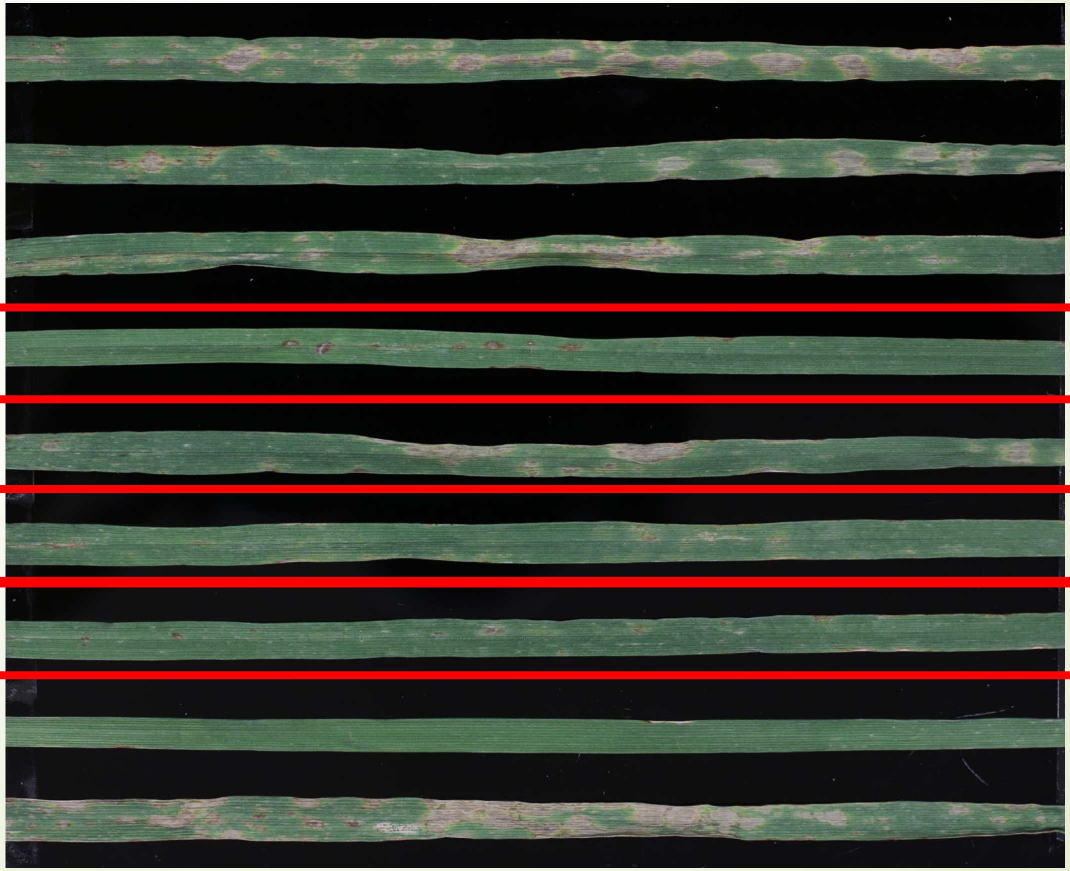
Molecular Model

Model


Fungal hyphae
Protein secretion
Direct recognition
SnTox1
GUB_WAK
EGF_CA
TM
PK
Apoplast
Plasma membrane
Cytoplasm
Snn1 (WAK)
SnToxA
SnTox3
SnTox5
Internalization
Internalization?
Indirect recognition
LRR
NB
PK
Tsn1 (PK-NLR)
recognition?
MSP
PK
Snn3 (PK-MSP)
MSP
PK
Snn5 (PK-MSP)
Upregulation of defense response pathways
Nucleus
MAPK signaling
Reactive oxygen species
DNA laddering
Electrolyte leakage
Plant cell death
Necrotrophic effector-triggered susceptibility (NETS)

CRISPR/Cas9 disruption of susceptibility genes: *Tsn1*

- Single base pair insertion at the CRISPR cut site targeting the NBS domain, creating a frame-shift mutation and a premature stop codon



	Disease score
Fielder	3.5
C11A-2	2.5
C11A-4	3.0
C11A-5	1.5
C11A-8	3.0
C11A-11	1.0
C11A-12	1.5
BR34 : resistant control	0
BG261: SnToxA differential	3.5

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UNIVERSITY OF CALIFORNIA

Caveats for Breeding

- **NE sensitivity genes might also act as 'R' genes for resistance to biotrophic pathogens or insect pests**
  - Example: The oat victorin sensitivity gene (NLR) (Lorang et al. 2007) confers susceptibility to Victoria blight and resistance to crown rust

Breeding for resistance to one disease could result in susceptibility to another

Caveats for Breeding

- **When breeding and introgressing material from uncharacterized sources…**

May result in the incorporation of susceptibility genes inadvertently

Summary

- *P. nodorum* tricks its host (wheat) into inducing cell death through recognition of NEs by sensitivity genes
- The cloning of four sensitivity genes reveals three classes – thus the pathogen can target diverse host targets
- Host gene composition, light-regulated expression, epistasis and additive effects among interactions, and NE gene regulation all contribute to disease expression
- Resistance can be obtained through marker-assisted elimination (MAE) of sensitivity genes or gene editing

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