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**
  - Biotrophs: feed on living tissue
  - Necrotrophs: feed on dead/dying tissue
- **Nematodes**
- **Viruses**

**Generalists**
- Stem rust
- Powdery mildew
- 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

Flor's Classic Gene-for-Gene Model

Host

RR  rr

+Bvr  -Bvr

Host-NE Gene-for-Gene Model

Host

SS  ss

+NE  -NE

USDA Fargo, ND 1931-1969

Harold H. Flor

Harold H. Flor
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
SnTox3
SnTox267
SnTox4
SnTox5
SnTox6
SnTox7
Genomic locations of SNB susceptibility genes

- Snn1
- Snn2
- Snn3-B1
- Snn3-D1
- Snn4
- Snn5
- Snn6
- Snn7
- Tsn1
Genetic dissection of host gene-NE interactions

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.

Infiltrate purified NE into wheat leaves.

Score mapping population for reaction to the NE.

Place NE sensitivity locus on genetic linkage map.
Cloning the Genes
The known interactions

Wheat gene
(Faris Lab)

\[Tsn1\]

\[Snn1\]

\[Snn2\]

\[Snn3-B1\]

\[Snn3-D1\]

\[Snn4\]

\[Snn5\]

\[Snn6\]

\[Snn7\]

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

\[SnToxA\]

\[SnTox1\]

\[SnTox267\]

\[SnTox3\]

\[SnTox4\]

\[SnTox5\]
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<th>Year</th>
<th>Title</th>
<th>Authors</th>
<th>Journal</th>
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<tr>
<td>1987</td>
<td>Cultivar-Specific Toxicity of Culture Filtrates of <em>Pyrenophora tritici-repentis</em></td>
<td>A. Tomás and W. W. Bockus</td>
<td>Physiology and Biochemistry</td>
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<td>1996</td>
<td>Chromosomal Location of a Gene Conditioning Insensitivity in Wheat to a Necrosis-Inducing Culture Filtrate from <em>Pyrenophora tritici-repentis</em></td>
<td>J. D. Faris, J. A. Anderson, L. J. Francl, and J. G. Jordahl</td>
<td>Genetics</td>
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<td>2006</td>
<td>Emergence of a new disease as a result of interspecific virulence gene transfer</td>
<td>Timothy L. Friesen, Eva H Stuenbrock, Zhaohui Liu, Steven Meinhardt, Hua Ling, Justin D Faris, Jack B Rasmussen, Peter S Solomon, Bruce A McDonald &amp; Richard P Oliver</td>
<td>nature genetics</td>
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<td>2019</td>
<td>Transposon-Mediated Horizontal Transfer of the Host-Specific Virulence Protein ToxA between Three Fungal Wheat Pathogens</td>
<td>Megan C. McDonald, Adam P. Taranto, Erin Hill, Benjamin Schwessinger, Zhaohui Liu, Steven Simpfendorfer, Andrew Milgate, Peter S. Solomon</td>
<td>mBio</td>
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</table>

- **Tsn1-ToxA in wheat-tan spot system**
- **Tsn1-ToxA in wheat-*Septoria nodorum* blotch system**
- **Tsn1-ToxA in wheat-spot blotch system**
Map-based cloning of \textit{Tsn1}

![Diagram of Chromosome 5B with genetic and physical maps, showing markers Xfcg24, Xfcg29, Xfcg25.2, Xfcg30, Xfcg31, Xfcg26, Xfcg33, Xfcg32, Xfcg30, Xfcg22, Xfcg17, and ATG, TAG, Protein kinase domain, NB domain, and LRR domain.]

Huangjun Lu
Zengcui Zhang
A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens

Plant disease susceptibility conferred by a "resistance" gene

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

Tsn1 = PK-NLR

LOV1 = NLR

Pc = NLR
The known interactions

Wheat gene (Faris Lab)

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

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

- SnToxA
- SnTox1
- SnTox267
- SnTox3
- SnTox4
- SnTox5
Map-based cloning of Snn1

Chromosome 1B

Xfcp618

Gongjun Shi

2016: Snn1 = WAK
**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)

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

P. nodorum NE – small secreted proteins
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- 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

**ITMI Snn3-B1 locus Chromosome 5BS**

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**Ae. tauschii Snn3-D1 locus Chromosome 5DS**

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**Zengcui Zhang**

*Two putatively homologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to Stagonospora nodorum*
Map-based cloning of Snn3-D1 in Ae. tauschii

Chromosome 5D

Genetic map
16,918 gametes

161 kb

200 kb insertion

231 kb

362 kb

AL8/78 scaffold (519,647 bp)

TA2377 BAC contig

Zengcui Zhang
Snn3-D1 structure and validation

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

Zengcui Zhang

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

Zengcui Zhang, Katherine L.D. Running, Sudesh 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
Cloning Snn3-B1

**Snn3-D1 alignment to best hit on chromosome 5B**

Snn3-D1
- ATGCA GTACCTGTCAGATAAAATGTCCTCGTCGATCCACAAACTATCAAATTATGTTTG
- ATGCA GTACTTGTCAGATAAAATGTCCTCGTCGATCCACAAACTATCAAATTATGTTTG

Snn3-B1
- TTAGA AGCAATCAGAGGAGTTTCAGAGAGATGAAAATTGACGCTGGCTATGGA
- TTAGAGA CAATCAGAGGAGTTTCAGAGAGATGAAAATTGACGCTGGCTATGGA

---

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

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.

Allelism test
Sumai3 x BG220 (SnTox3 diff.)

Screened 176 F2 plants:
174 sensitive : 2 insensitive

Suggesting different genes!
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
**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

Wheat gene
(Faris Lab)

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

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

- SnToxA
- SnTox1
- SnTox267
- SnTox3
- SnTox4
- SnTox5
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

1.4 Mb, 18 HC genes
Molecular cloning of Snn5

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

TaPKMSP-2: All of 17 mutant families were completely sensitive
Gene Comparisons
Four-gene comparison: Structure

Tsn1

Snn1

Snn3

Snn5

ATG

TAG

ATG

TGA

ATG

TAG

0 1 2 3 4 5 6 7 8 9 10 11

Kb scale
Four-gene comparison: Functional domains

Tsn1

Snn1

Snn3

Snn5

PK NB LRR

GUB WAK EGF CA

MSP

C

C

C

C

Amino acid scale (x100)
Three-gene comparison: Expression patterns

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<thead>
<tr>
<th>Gene</th>
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<th>Night</th>
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<tr>
<td>Snn1</td>
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<td>Snn3</td>
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Three-gene comparison: Expression patterns

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<td>2:00 pm</td>
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<tr>
<td>8:00 am</td>
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</table>

- **Tsn1**
  - Light: Increase
  - Dark: Decrease

- **Snn1**
  - Light: Decrease
  - Dark: Increase

- **Snn3**
  - Light: Decrease
  - Dark: Increase

- **Tsn1-ToxA**
  - Light: Increase
  - Dark: Decrease

- **Snn1-Tox1**
  - Light: Decrease
  - Dark: Increase

- **Snn3-Tox3**
  - Light: Decrease
  - Dark: Increase
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

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

Amanda R. Peters Haugrud, a,b Pengxi Zhang, a Jonathan K. Richards, a,b Timothy L. Friesen, b and Justin D. Faris a,c

a, b, c Department of Plant Sciences, North Dakota State University, Fargo, North Dakota 58102
b United States Department of Agriculture-Agriculture Research Service, Cereal Crops Research Unit, Eduard T. Schaler Agricultural Research Center, Fargo, North Dakota 58102
c Department of Plant Pathology, North Dakota State University, Fargo, North Dakota 58102
Variable expression of *Snn1*-Tox1 and *Snn5*-Tox5 in the presence/absence of *Tsn1*-ToxA
Variable expression of *Snn1*-Tox1 and *Snn5*-Tox5 in the presence/absence of *Tsn1*-ToxA

Amanda Peters Haugrud
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**
- **Apoplast**
- **Protein secretion**
- **GUS/WAK**
- **Tsn1 (PK-NLR)**
- **LRRNBPK**
- **SnToxA**
- **Internalization?**
- **SnTox3**
- **SnTox5**
- **Plasma membrane**
- **Cytoplasm**
- **Snn1 (WAK)**
- **Snn3 (PK-MSP)**
- **Snn5 (PK-MSP)**
- **Nucleus**
- **Upregulation of defense response pathways**
- **MAPK signaling**
- **Upregulation of defense response pathways**
- **Tsn1 (PK-NLR)**
- **Reactive oxygen species**
- **DNA laddering**
- **Electrolyte leakage**
- **Necrotrophic effector-triggered susceptibility (NETS)**
- **Plant cell death**
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.

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<th>Gene</th>
<th>Disease score</th>
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<td>Fielder</td>
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<td>BG261</td>
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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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  - Faris Lab
    - Zengcui Zhang
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    - Cayley Steen
    - Gurminder Singh
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- University of California-Berkeley
  - Brian Staskawicz Lab

- University of Saskatchewan
  - Curtis Pozniak

- Institute of Experimental Botany
  - Jaroslav Dolezel Lab
Email: justin.faris@usda.gov
Twitter: @FarisGenLab