




How does the fungal pathogen,  
*Fusarium virguliforme* cause  
SDS?

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# Current Projects

- Isolation and characterization of a toxin that induces SDS in soybean - **USB**
- Development of a plant antibody against this toxin - **NCSR**
- Identification of FvTox1-interacting soybean protein(s) - **Syngenta**
- Identify pathogenicity genes involved in SDS development - **ISA**





The fungus *Fusarium virguliforme* has never been isolated from the above ground diseased plants.

Toxins have been implicated in foliar SDS development.



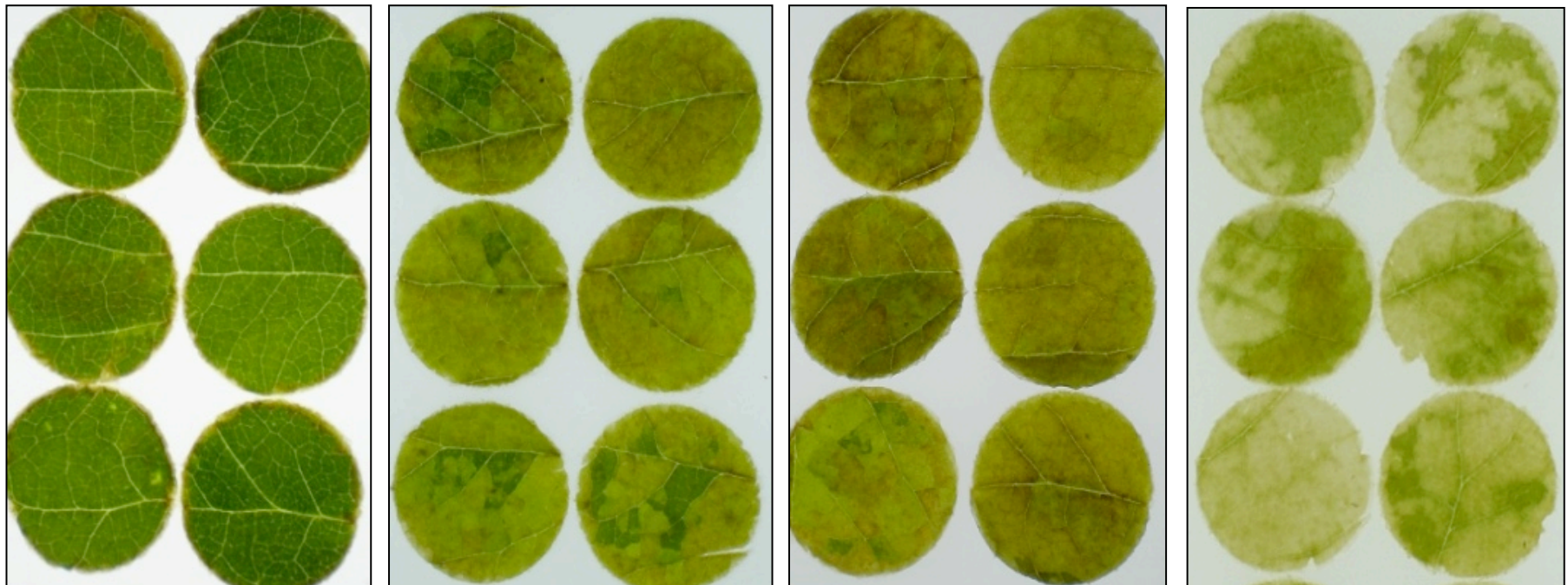
Phytotoxin, FvTox1 causes chlorophyll losses or chlorosis (as in foliar SDS) in leaf discs.

Empty vector control

Pro-FvTox1

Mature FvTox1

Culture filtrate

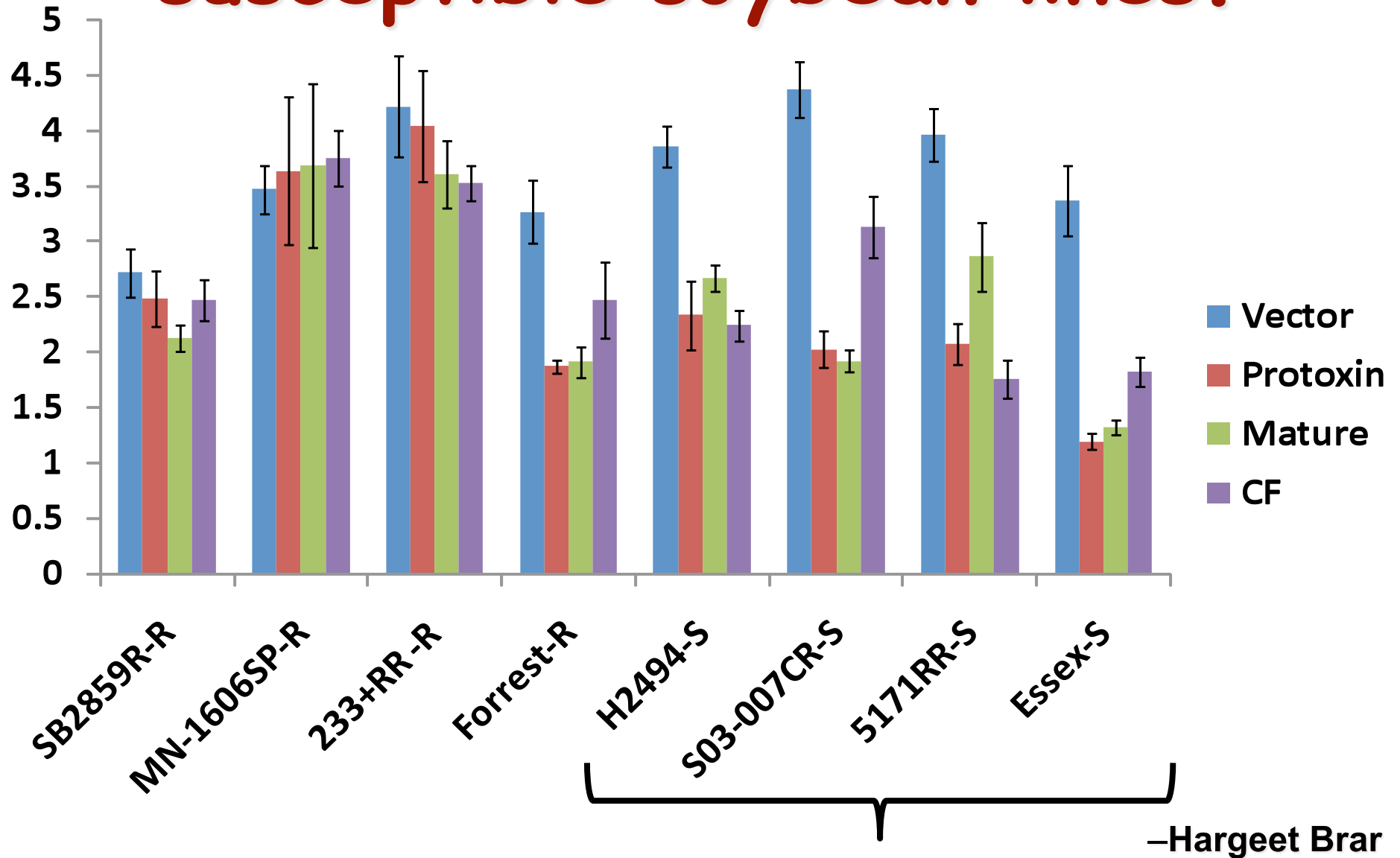


SDS-susceptible cultivar, Essex

–Hargeet Brar and Siva Swaminathan

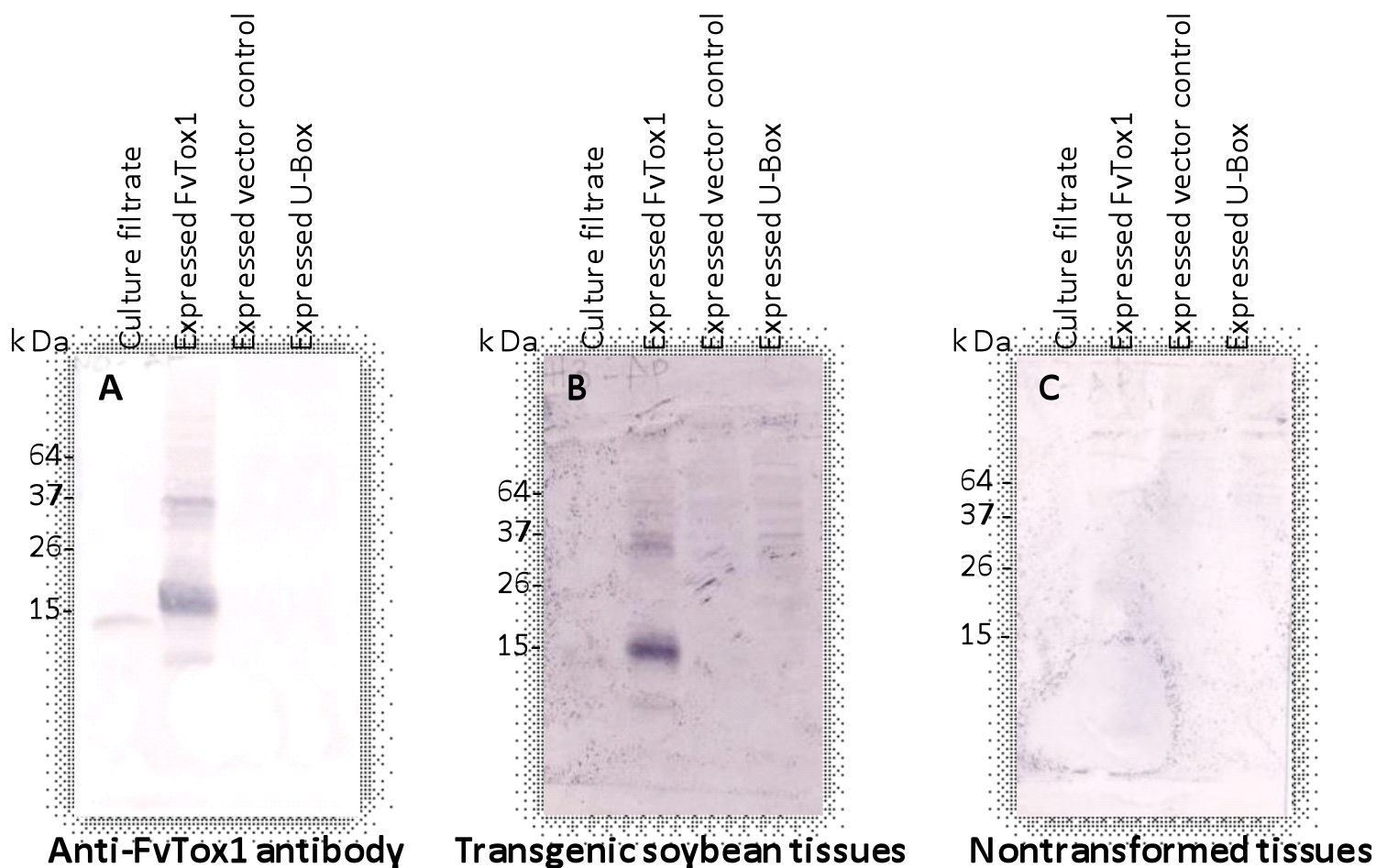


# Losses of chlorophyll in SDS-susceptible soybean lines.





# Created anti-FvTox1 plant antibody gene and expressed in transformed roots.



–Hargeet Brar



## Genomics approach for identification of additional pathogenecity factors

- Genome of the pathogen is being sequenced.
- Candidate pathogenicity or virulence genes are being identified.
- Candidate genes will be investigated for their possible roles in SDS development.



# Genome sequence of the SDS pathogen, *Fusarium virguliforme*

## Collaborators:

### *Iowa State University*

- Madan K. Bhattacharyya
- Xiaoqiu Huang

### *Southern Illinois University*

- Ahmad Fakhoury

### *University of Arkansas*

- Burton Bluhm

# Genome sequence of the SDS pathogen, *Fusarium virguliforme*

The project is funded jointly by:

- Illinois Soybean Association
- Iowa Soybean Association
- North Central Soybean Research Program
- Soybean Research Development Council



## Objectives of the sequencing project are:

1. Generate high quality *Fusarium virguliformae* genome sequence.
2. Sequence transcripts of the pathogen.
3. Annotate (name) the genes.
4. Construct a bacterial artificial chromosome library.
5. Sequence additional strains of the pathogen.

# *Fusarium virguliforme* Mont 1 is being sequenced.

- Mont 1 isolates were generated from single conidia and virulence of the selected isolates were tested.
- One of the single conidia-derived isolates was selected for sequencing.

Hargeet Brar

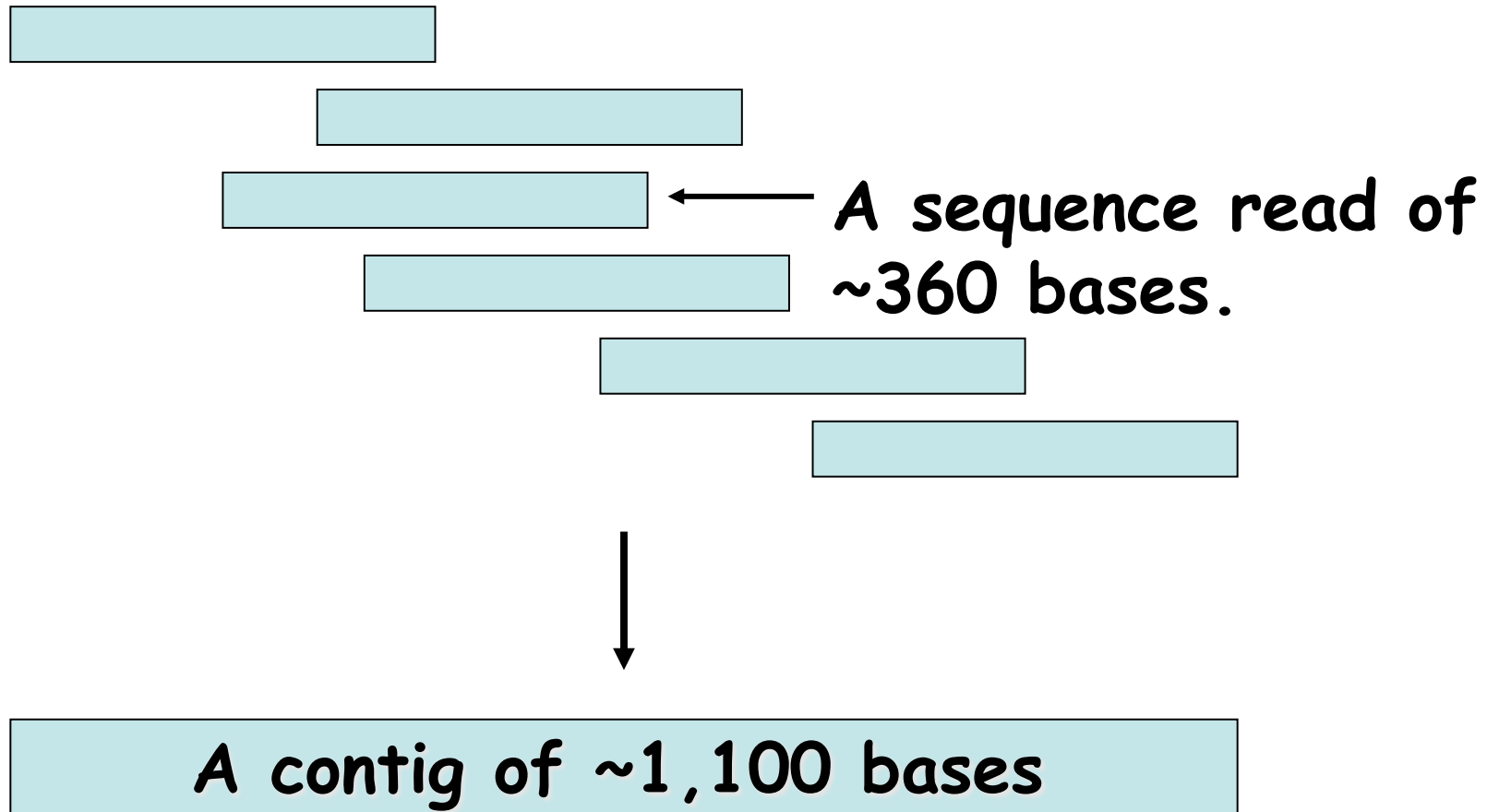


# Sequencing the *Fusarium virguliforme* Mont-1 genome

- Shotgun sequence of sheared DNA.
- Paired-end sequence of ~ 3 kb DNA fragments.
- ~1 giga bases sequence (20X genome equivalents) assembled into contiguous (contig) sequences.

SeqWright

**454-sequence reads were ~360 bases.**





# *Fusarium virguliforme* genome is composed of ~47 Mb DNA.

>400 kb	1
>300 kb	3
>200 kb	7
>100 kb	92

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102 contigs = 15,395,638 bases

>50-99.99 kb	197
>10-49.99 kb	650
>5-9.99 kb	360

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1,207 contigs = 31,478,950 bases

**Total = 46,874,588 bases (~47 Mb)**

Subodh Srivastava

# Current Activities

To accomplish genome assembly

- Paired-end sequencing of 8 kb DNA fragments (SeqWright)
- Bacterial artificial chromosome-end sequencing (Lucigen)

To facilitate annotation

- Sequencing of the pathogen transcripts (ISU)



*F. virguliforme* contains 47 Mb  
DNA and 15,244 genes

*Fusarium graminearum*

35.3 Mb, 14,034 genes

*Fusarium verticillioides*

40.5 Mb, 14,195 genes

*Fusarium oxysporum* f. sp. *lycopersici*

59.5 Mb, 17,608 genes

# Identification of Candidate Pathogenecity Genes

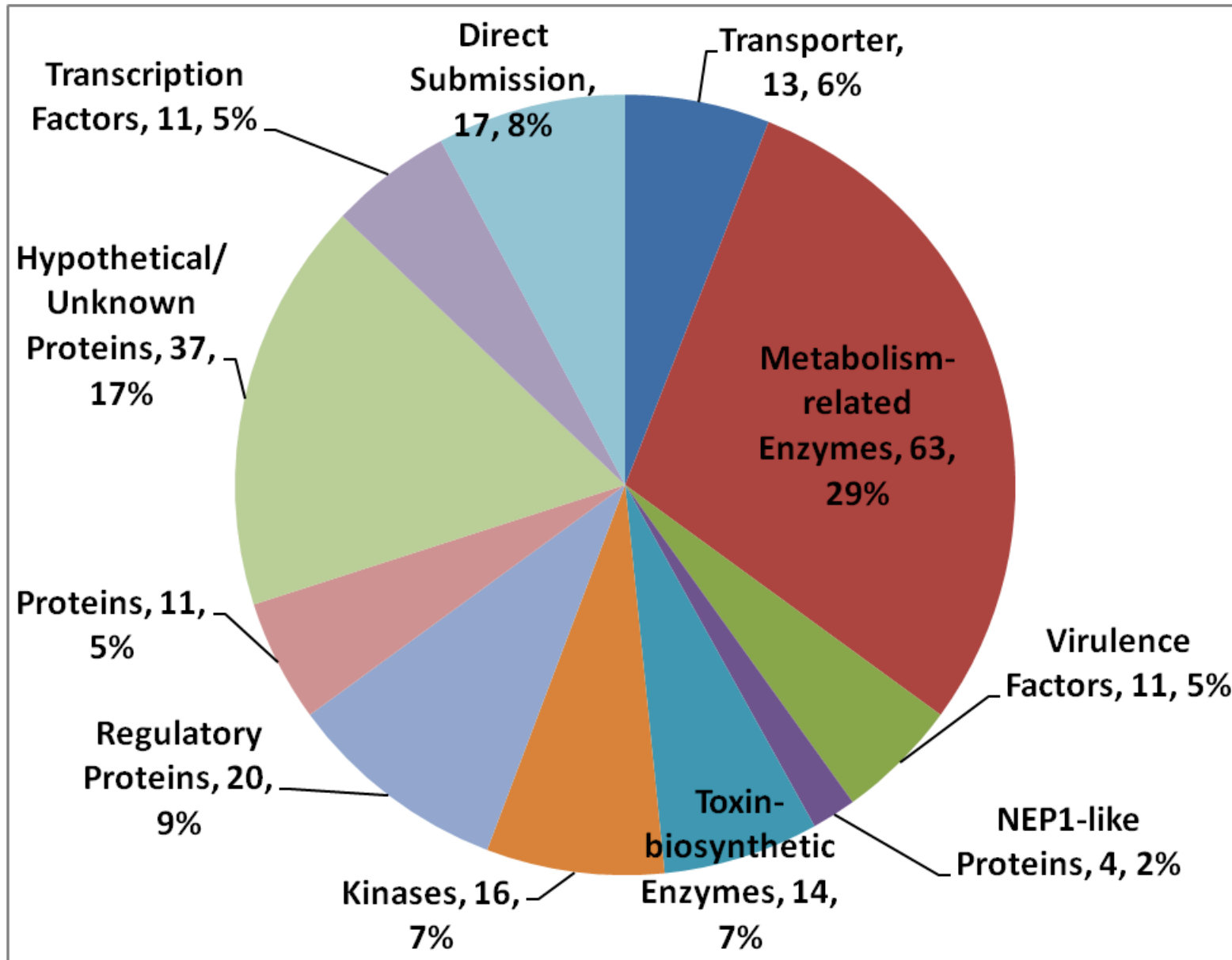
- We investigated *F. virguliforme* 15,244 genes for genes that show high similarities to known pathogenecity genes.
- We used the pathogenecity proteins included in pathogen-host interaction database (PHI database) for this study.

# **Pathogen-host interaction database (PHI database)**

- **There are 1,100 proteins in the PHI database.**
- **They are experimentally verified for pathogenicity, virulence and effector proteins**
- **They are from bacteria, fungi and *Oomycete* pathogens,**
- **that Infect plants, human, animals, insects, fishes and fungi.**

**<http://www.phi-base.org/>**

**A total of 217 candidate pathogenicity genes were detected.**





## **Fv proteins with similarities to enzymes involved in toxin biosynthesis**

<b>Fv Protein</b>	<b>Protein ID</b>	<b>Enzyme</b>	<b>E Value</b>	<b>Organism</b>
g13093	ABB90284	FAD/FMN-containing dehydrogenases	0	<i>Fusarium graminearum</i>
g11245	AAD43562	Fum1p	0	<i>Gibberella monilif...</i>
g6149	AAX09988	nonribosomal peptide synthetases	0	<i>Cochliobolus heterostrophus</i>
g10139	ABK64180	O-methyltransferase	2.00E-042	<i>Cercospora nicotianae</i>
g1999	ABK64184	oxidoreductase	2.00E-053	<i>Cercospora nicotianae</i>
g8583	ABK64182	oxidoreductase	2.00E-036	<i>Cercospora nicotianae</i>
g10141	AAS57292	polyketide synthase	0	<i>Fusarium graminearum</i>
g13393	ABB90282	polyketide synthase	0	<i>Fusarium graminea...</i>
g13398	ABB90283	polyketide synthase	0	<i>Fusarium graminear...</i>
g13712	AAB08104	polyketide synthase	0	<i>Cochliobolus heterostrophus</i>
g3411	AAC39471	polyketide synthase	0	<i>Aspergillus fumigatus</i>
g8421	ABB90283	polyketide synthase	0	<i>Fusarium graminear...</i>
g5613	AAO68047	putative thiosulfate sulfurtransferase	6.00E-031	<i>Salmonella enterica</i>
g6775	ABK64183	reductase	5.00E-044	<i>Cercospora nicotianae</i>

# Pathogenecity in *F. virguliforme*

Regulated by toxins (metabolites and peptides) synthesized by enzymes as well as necrosis and ethylene inducing peptide-1 (NEP1)-like proteins.

1. Nonribosomal peptides,
2. Non-proteinaceous, secondary metabolites such as polyketides.
3. NEP1-like proteins.

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## Collaborators

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Dr. Siva Swaminathan

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Dr. Gladys Mbofung, ISU

Dr. Leonor Leandro, ISU

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*Thank you!*