# Novel Approaches in Fighting Sudden Death Syndrome in Soybean

Madan K. Bhattacharyya
Agronomy Department
Iowa State University

# Why to enhance sudden death syndrome (SDS) resistance in soybean?

# Why to enhance sudden death syndrome (SDS) resistance in soybean?

- The estimated soybean yield suppression from SDS in 2010 was 2.1% of total yield, which was valued at $0.82 billion.
- Growing SDS resistant cultivars is the major method of controlling this disease.
- Unfortunately, SDS resistance encoded by a large number QTL; and breeding SDS resistance is challenging.
- We need novel single-genes encoding SDS resistance.

# Two Novel Approaches to Enhance SDS Resistance in Soybean

1) Understand how the pathogen causes SDS and apply the knowledge to create SDS resistant soybean lines.
2) Identify and express plant genes to enhance SDS resistance.

# Pathogen remains in infected roots.

## SDS symptoms – (i) root necrosis and (ii) foliar SDS

Young seedlings


Mature plants

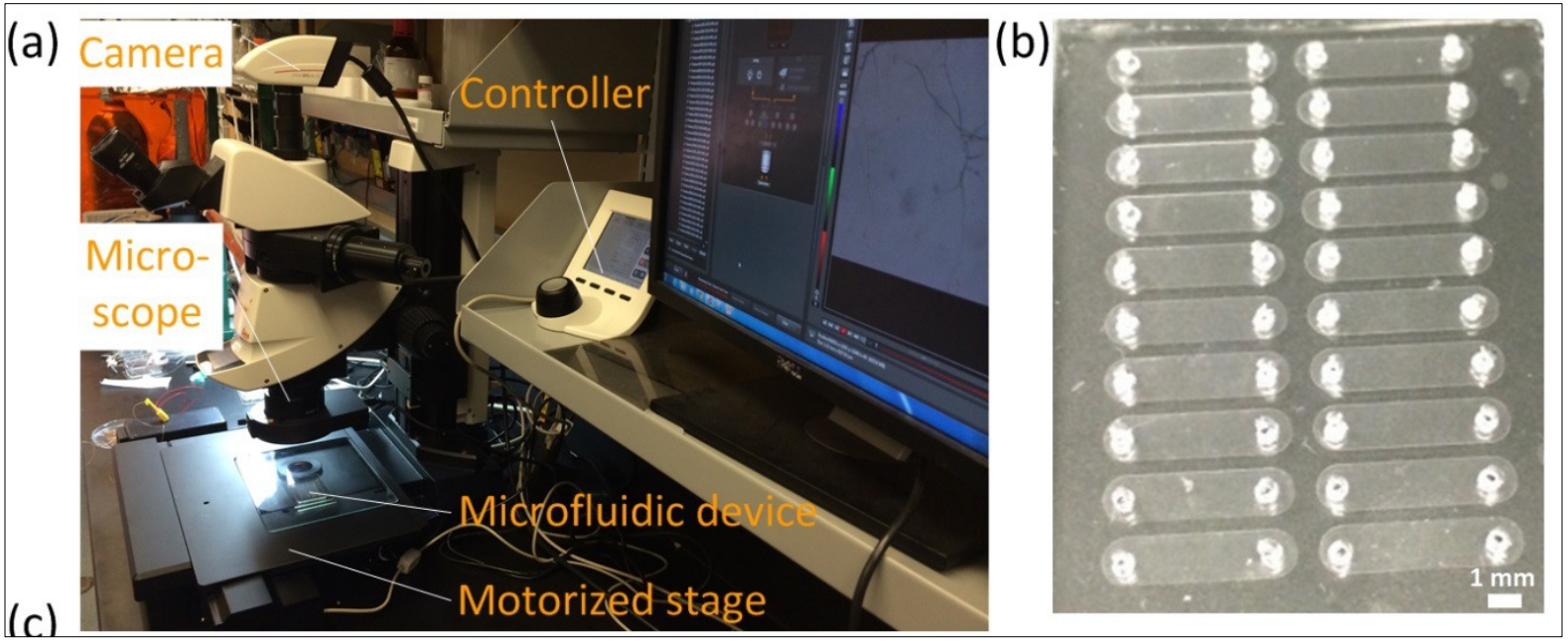
Small chlorotic spots on leaves

Dead interveinal tissue falls off

Leaf drop – followed by flower and pod drop

- In North America, *Fusarium virguliforme* causes SDS.
- In South America, *F. tucumaniae* is the major causal agent of SDS.
- Both are haploid organisms.
- They produce sickle-shaped conidia for spread of the disease.
- They overwinter as chlamydospores.

# Microfluidic Assays

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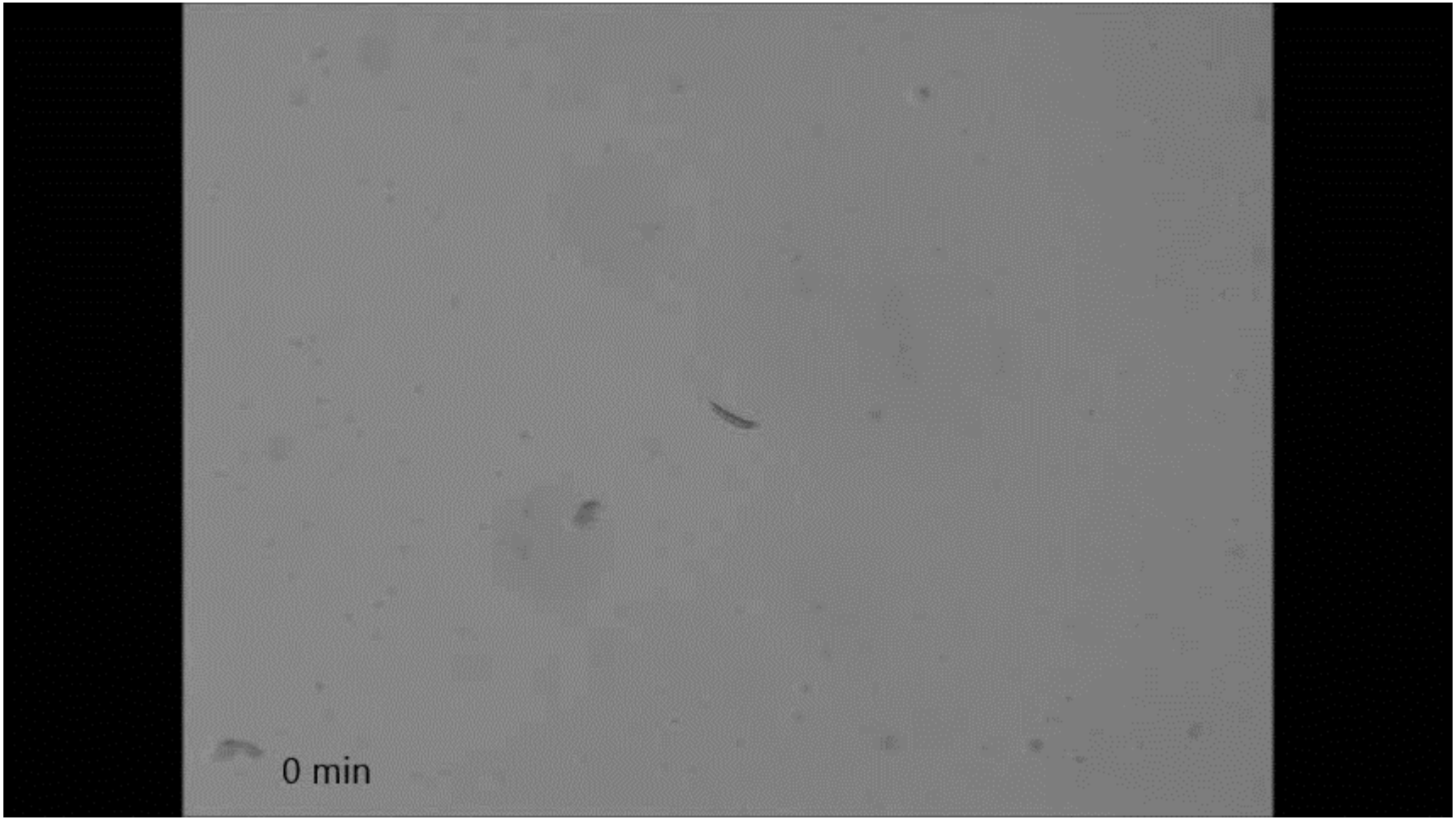
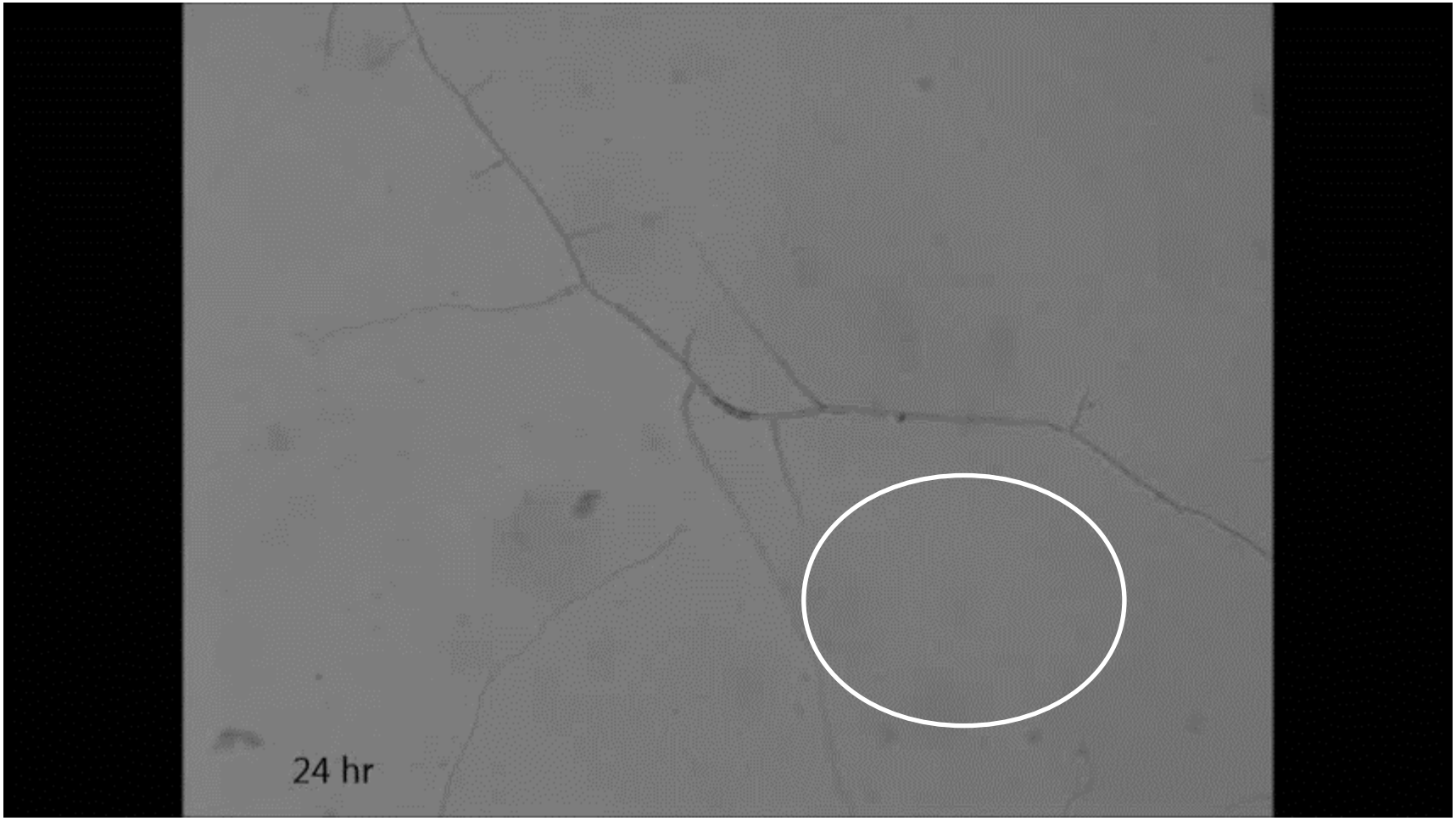
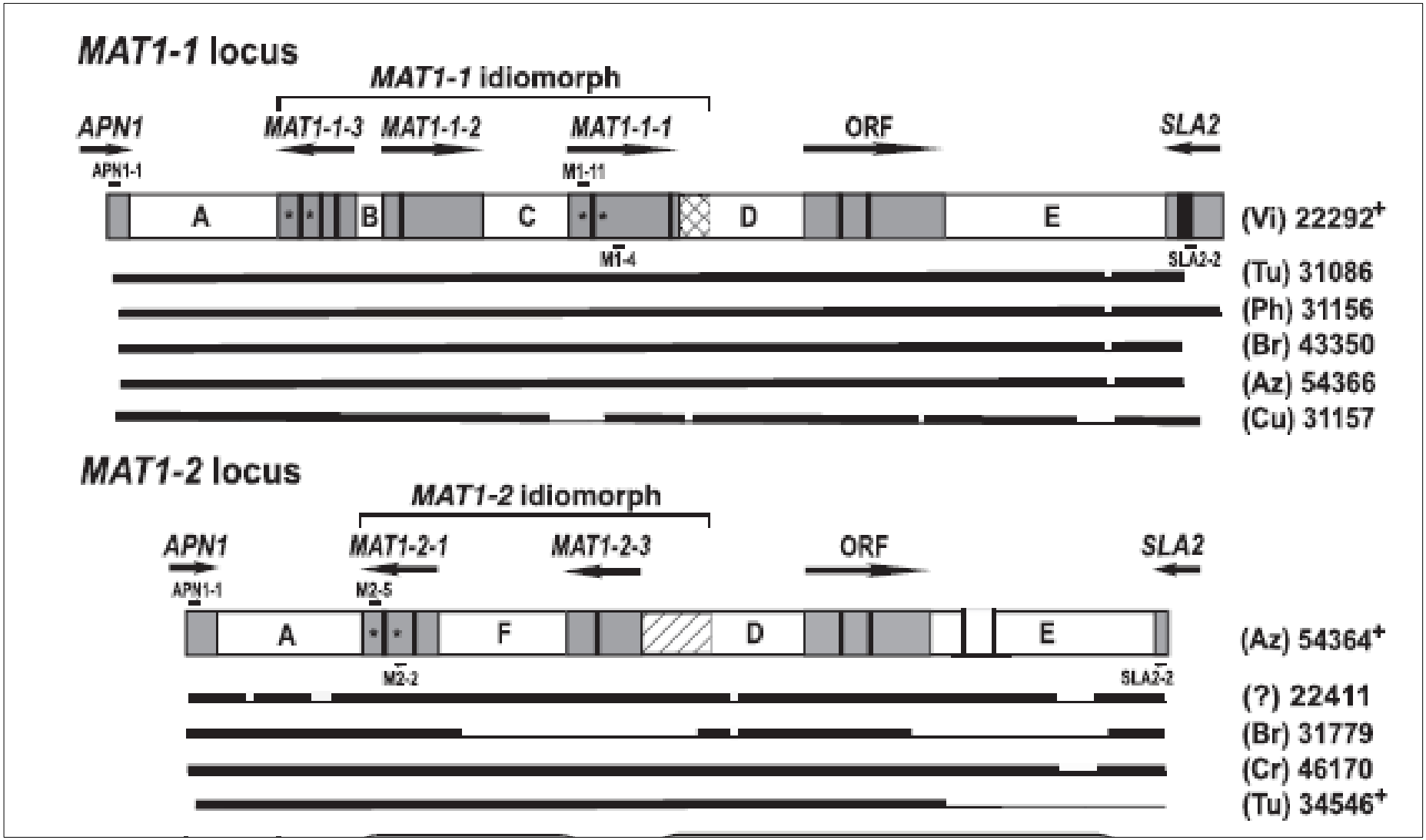
# How quickly conidium germinate?



# Conidia development



# Structure of the MAT locus






Hughes et al. 2013: Mycologia 106:686–697.

# Frequency distributions of alleles at the Mat locus among the *Fusarium spp.*

TABLE I. Summary of *MAT* multiplex assay

| *Fusarium* species | Disease[a] | No. of isolates | | | Percentage of isolates | |
|---|---|---|---|---|---|---|
| | | Total | *MAT1-1* | *MAT1-2* | *MAT 1-1* | *MAT1-2* |
| *F. azukicola* | BRR | 8 | 3 | 5 | 38 | 62 |
| *F. brasiliense* | SDS | 12 | 5 | 7 | 42 | 58 |
| *F. crassistipitatum* | SDS | 13 | 0 | 13 | 0 | 100 |
| *F. cuneirostrum* | BRR | 6 | 6 | 0 | 100 | 0 |
| *F. phaseoli* | BRR | 2 | 1 | 1 | 50 | 50 |
| *Fusarium* sp.[b] | BRR | 1 | 0 | 1 | 0 | 100 |
| *F. tucumaniae* | SDS | 268 | 92 | 176 | 34 | 66 |
| *F. virguliforme* | SDS | 129 | 129 | 0 | 100 | 0 |
| Total | | 439 | 236 | 203 | — | — |

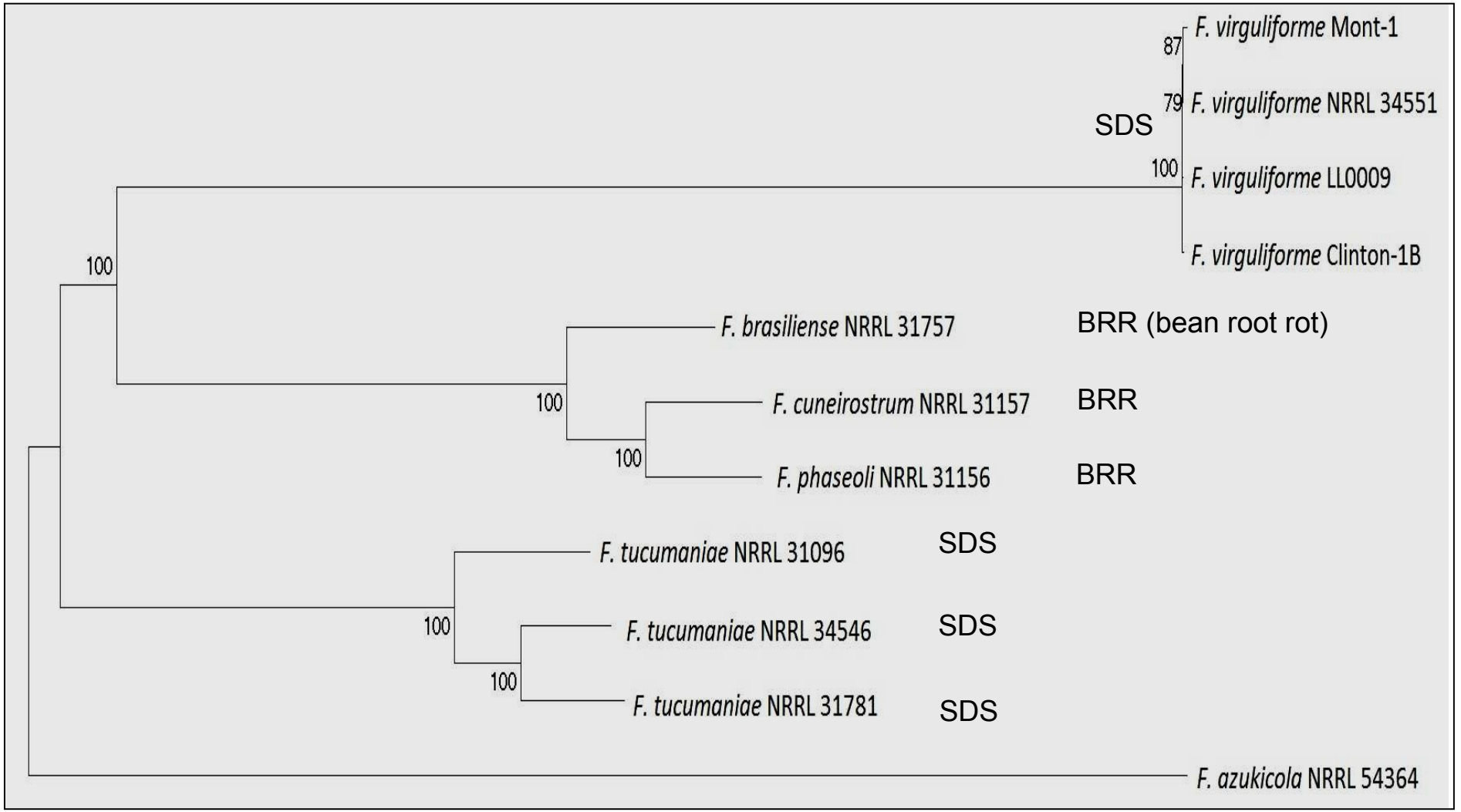
[a] BRR = bean root rot; SDS = sudden death syndrome.

[b] Isolate identified previously as *F. phaseoli* using a multilocus genotype assay (O'Donnell et al. 2010). Current study sugge isolate NRRL 22411 may be a hybrid with *F. phaseoli* and *F. brasiliense*-like parents.



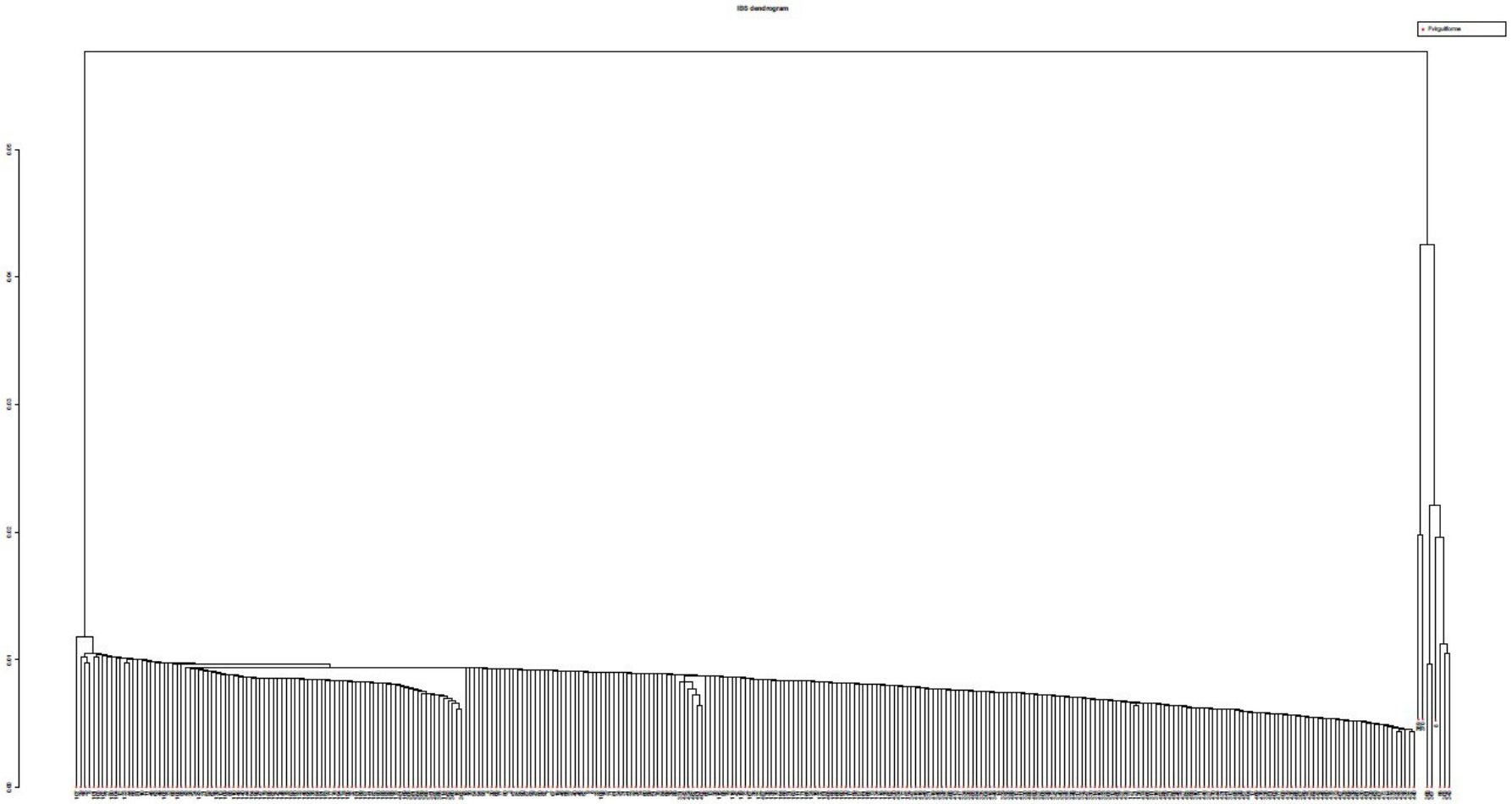
Hughes et al. 2013: Mycologia 106:686–697.

# A maximum-likelihood tree of *Fusarium species* (based on genome-wide SNP data)

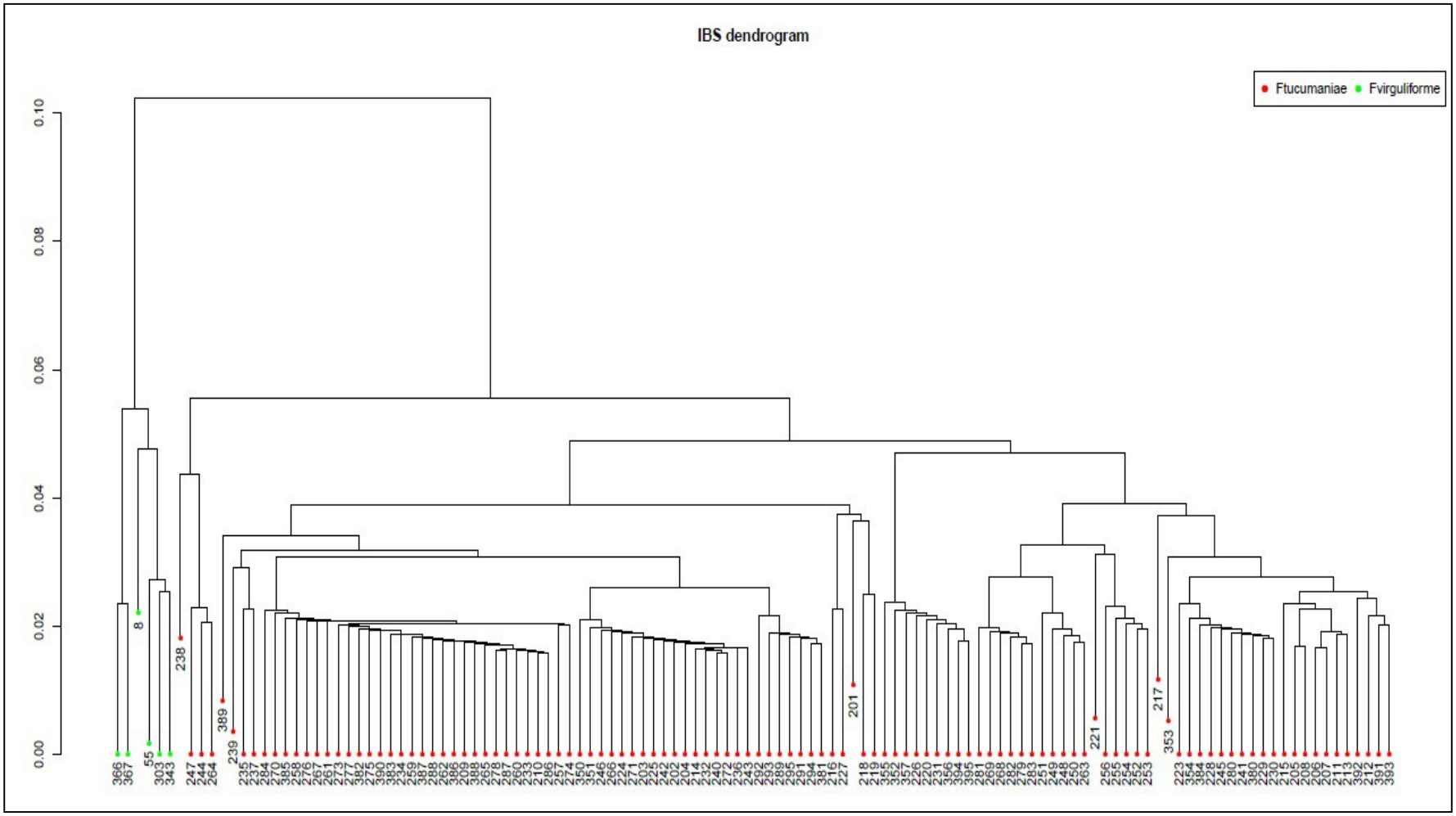



Huang et al. 2016: PloS One, Under Revision

# Phylogenetic tree of *F. virguliforme* isolates.



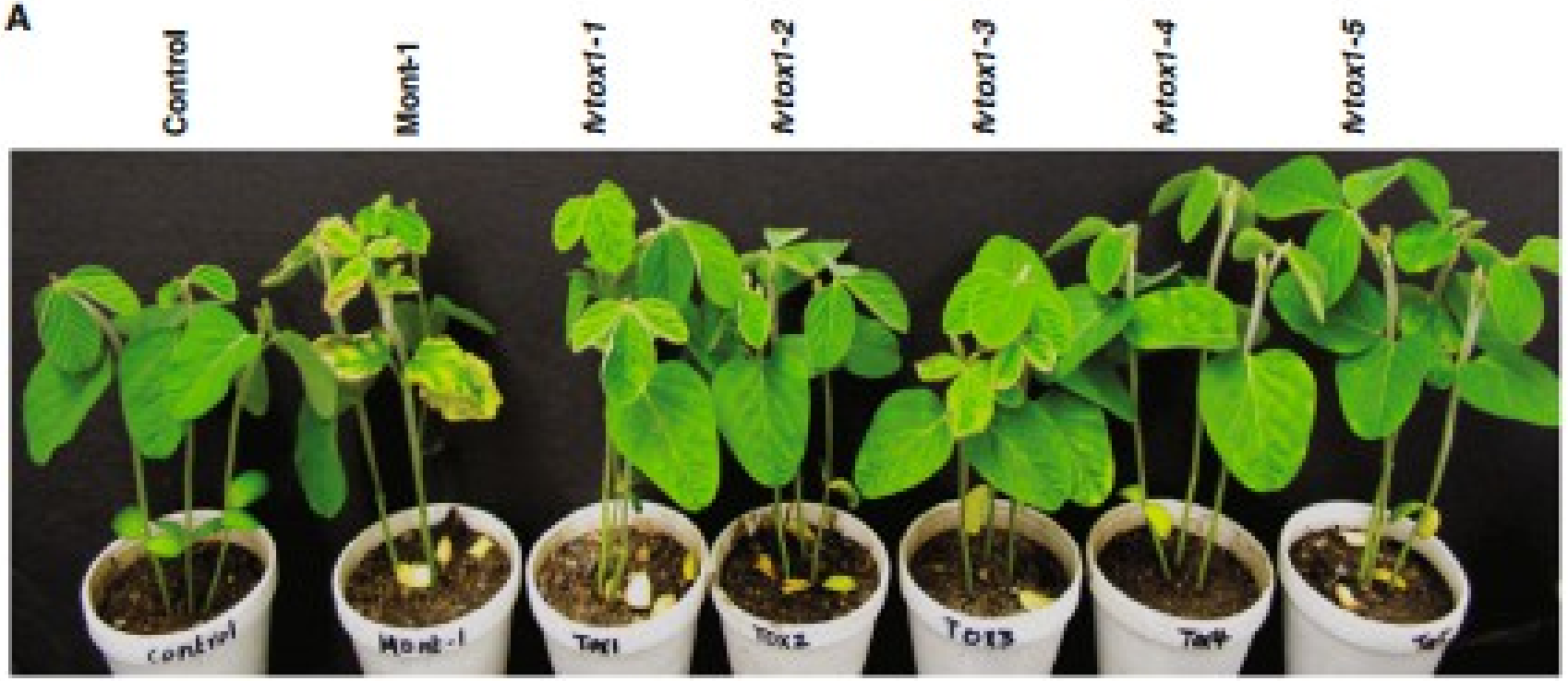
# Phylogenetic tree of *F. tucumaniae* isolates.



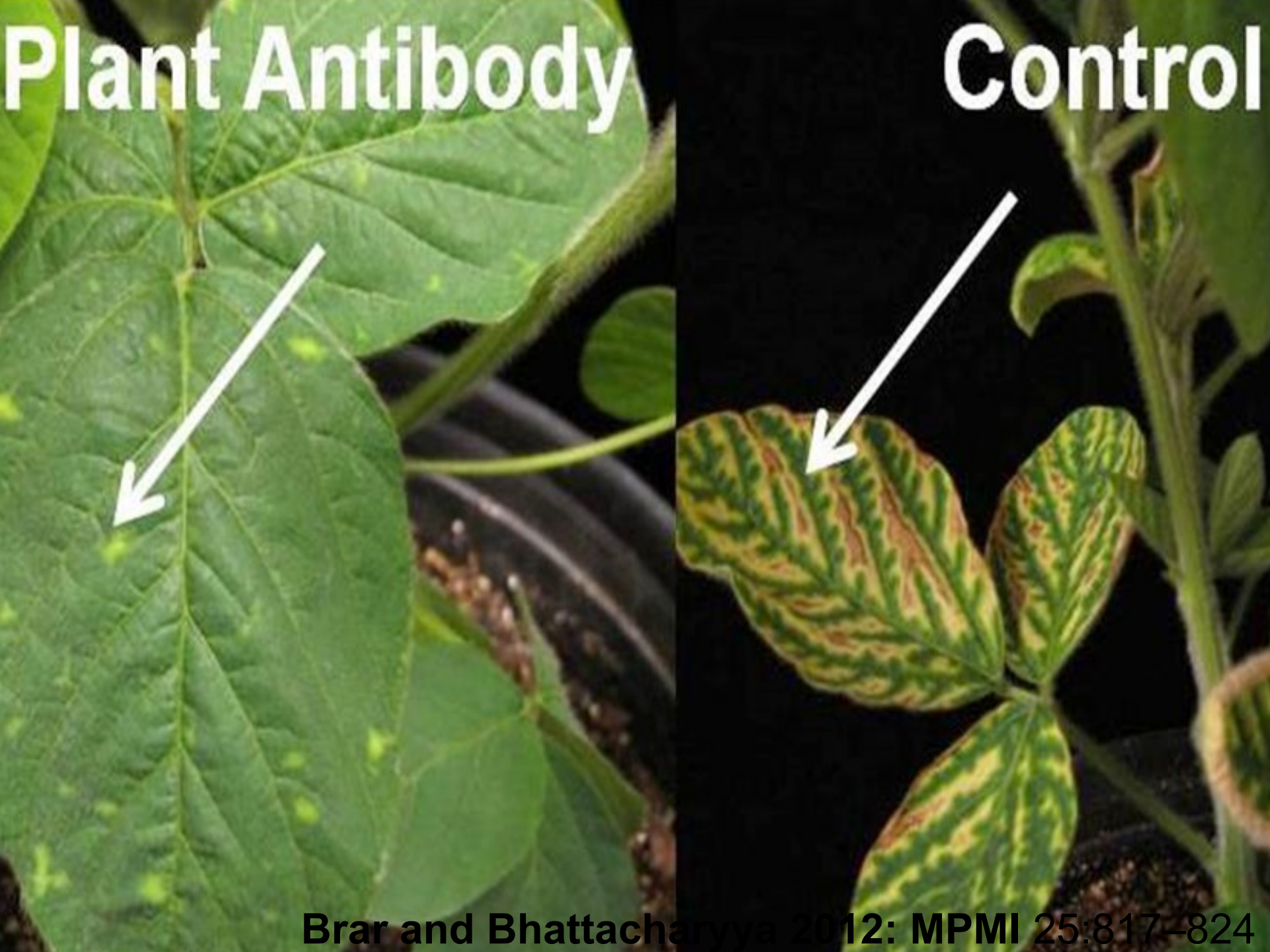
# Two Approaches to Enhance SDS Resistance in Soybean

1) Understand how the pathogen causes SDS and apply the knowledge to create SDS resistance.
2) Express plant genes to enhance SDS resistance.

# FvTox1 – acidic protein causes foliar SDS

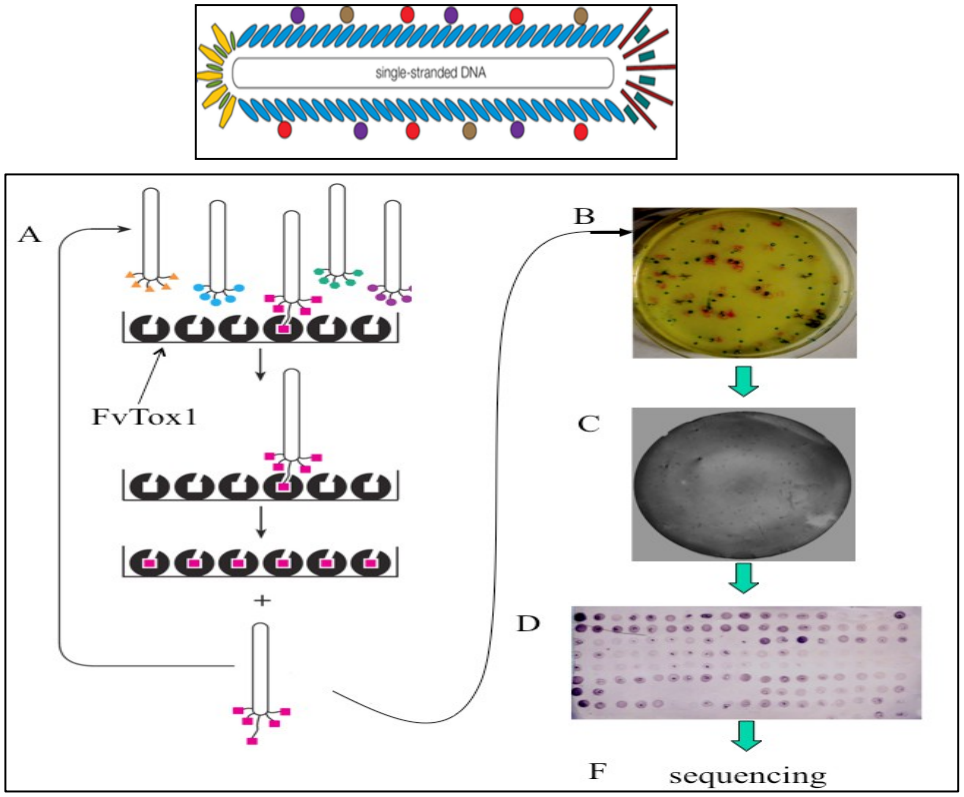



Brar et al. (2011) MPMI 24: 1179-1188
Pudake et al. (2013) Current Genetics, 59:107-117




Brar and Bhattacharyya 2012: MPMI 25:817–824

# Work flow for identifying peptides from phage display peptides libraries



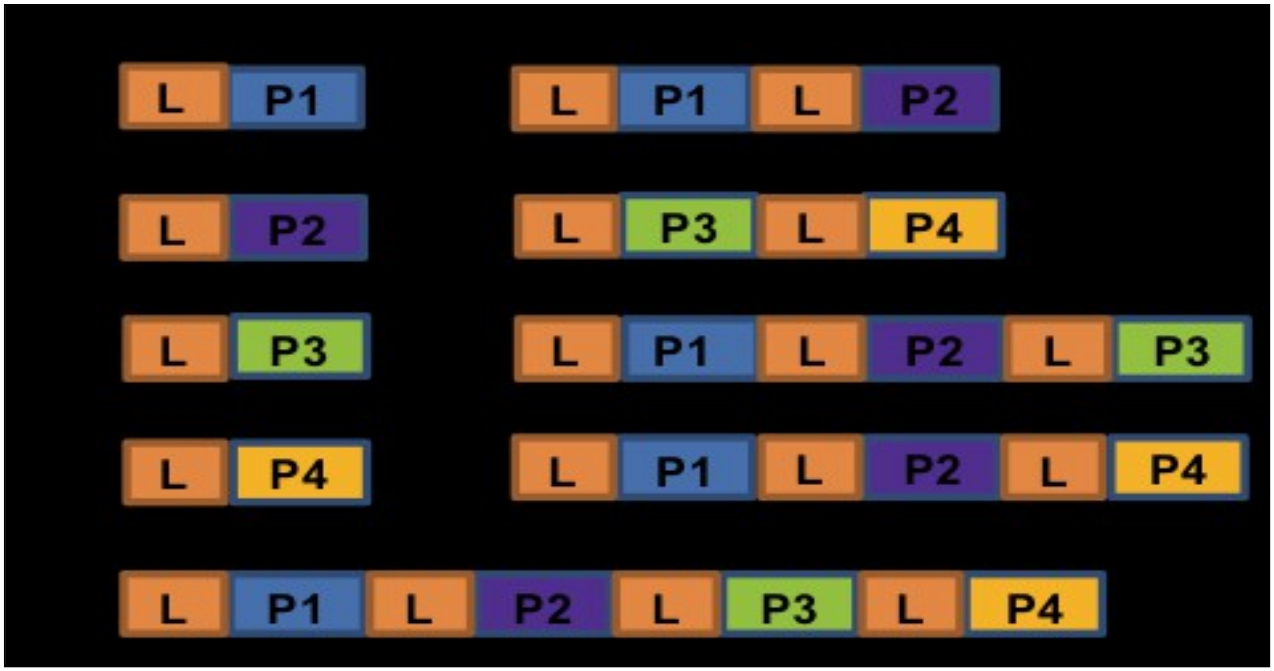



Wang et al. (2015) PLoS OneDOI:10.1371/journal.pone.0145156

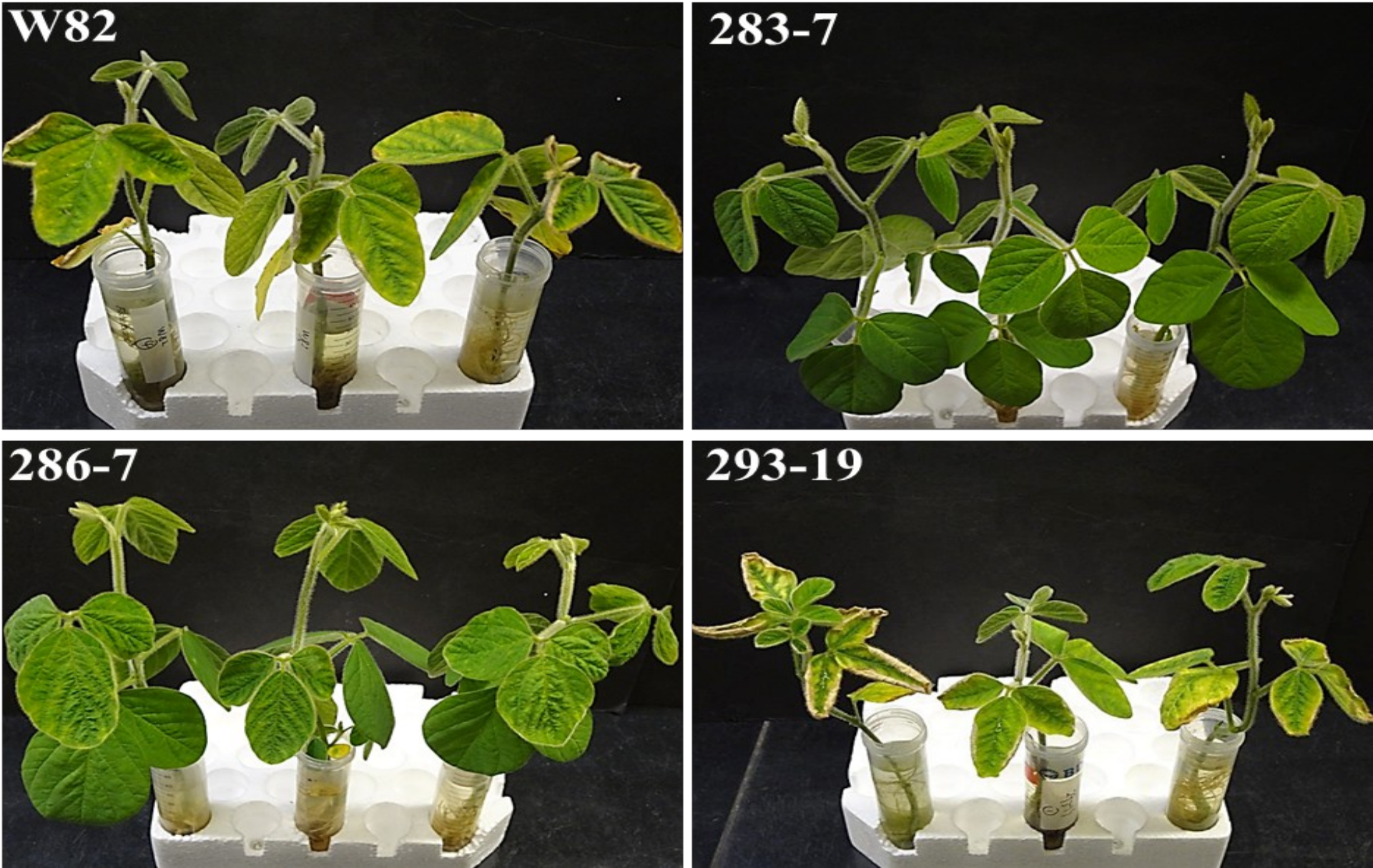
# Four classes of M13 phage clones displayed FvTox1-interacting peptides.

| Class (sequence) | Clone Number | Peptide Library |
|---|---|---|
| Class I (SYLPETIYEYRL) | 1,2,4,5,6,7,8,10,11,12,13,14,15,16,17,18,19,20,23,28,29,30,36,38,39 | Ph.D.-12 |
| Class II (VENKTRYHDREV) | 3,9,22,25,27,37 | Ph.D.-12 |
| Class III (HEGAWHNYARSV) | 24 | Ph.D.-12 |
| Class IV (SNGRVAD) | 31 | Ph.D.-7 |

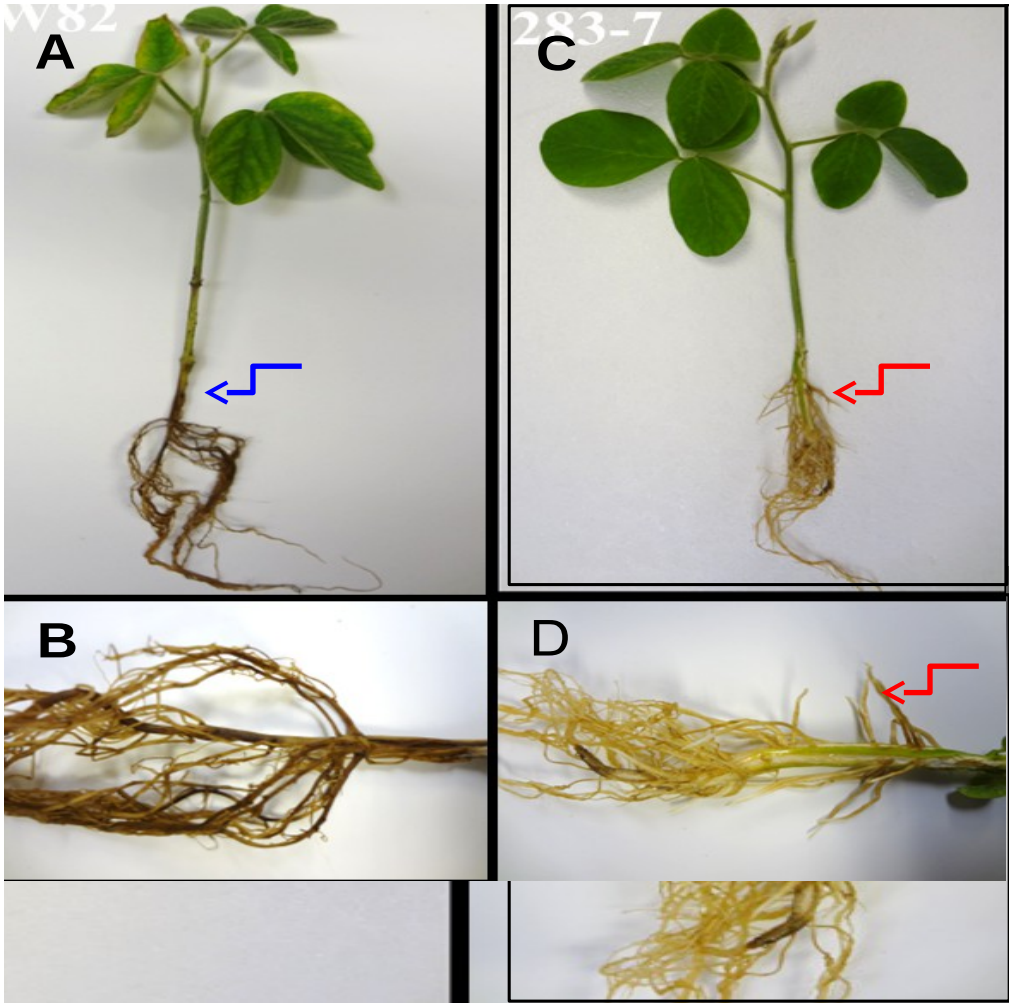
# Nine synthetic genes encoding FvTox1-interacting peptides



# Transgenic soybean lines carrying FvTox1-interacting Pep -1 showed enhanced foliar SDS resistance.

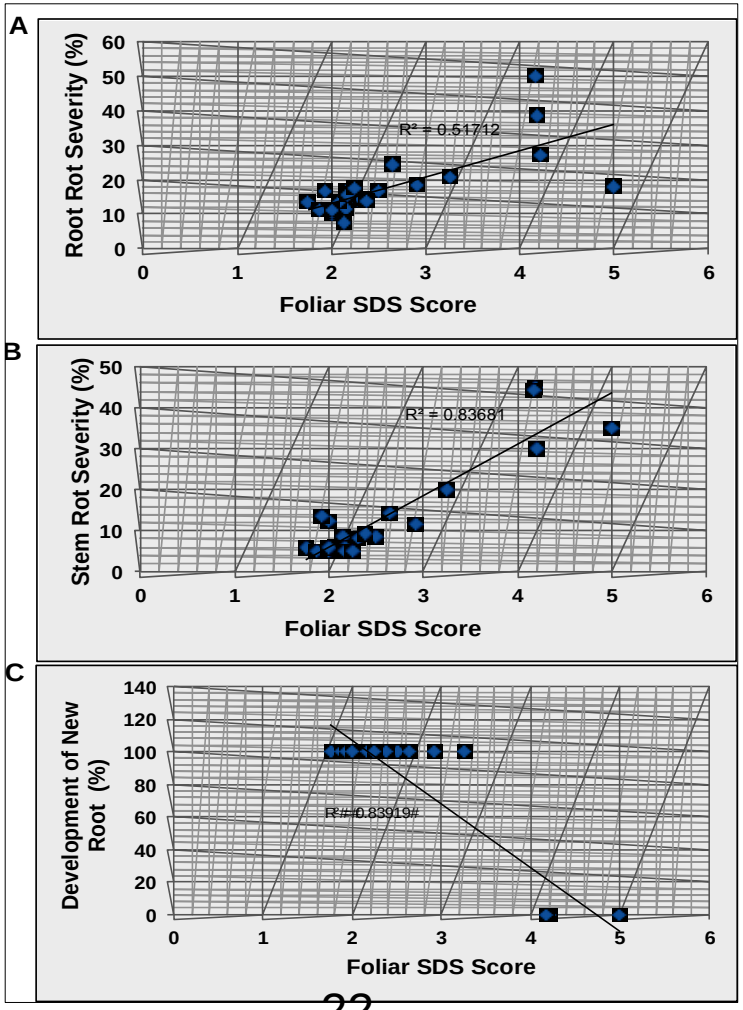


# FvTox1-interacting peptides confer enhanced tolerance of roots to FvTox1.

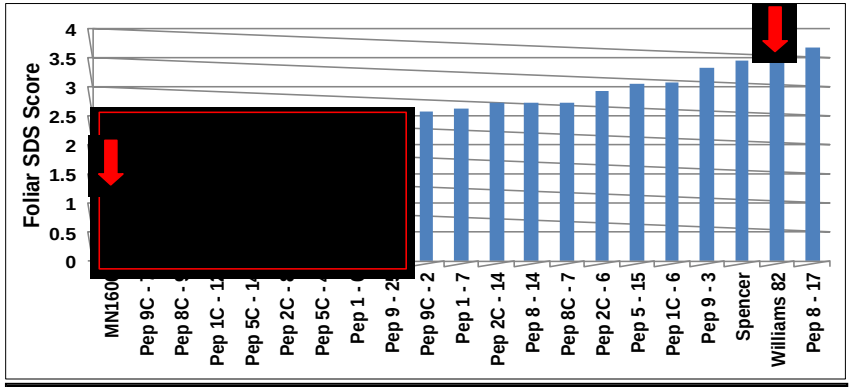


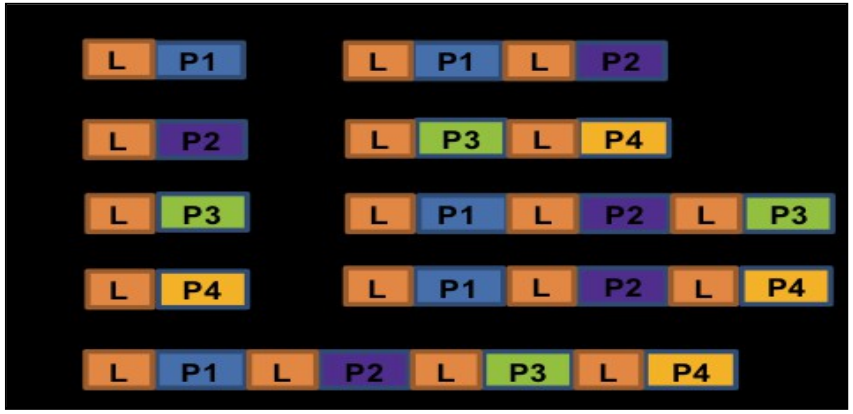

Williams 82

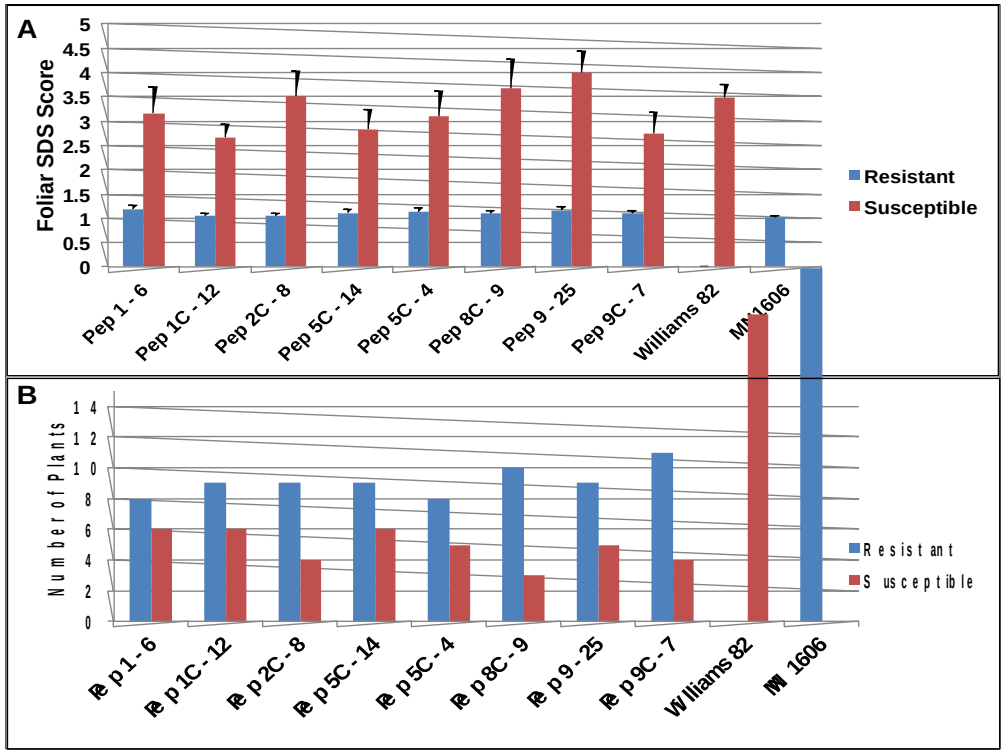
Williams 82 + Pep 1



# Transgenic soybean lines showed enhanced resistance to *F. virguliforme* infection.






B. Wang and M.K. Bhattacharyya, unpublished

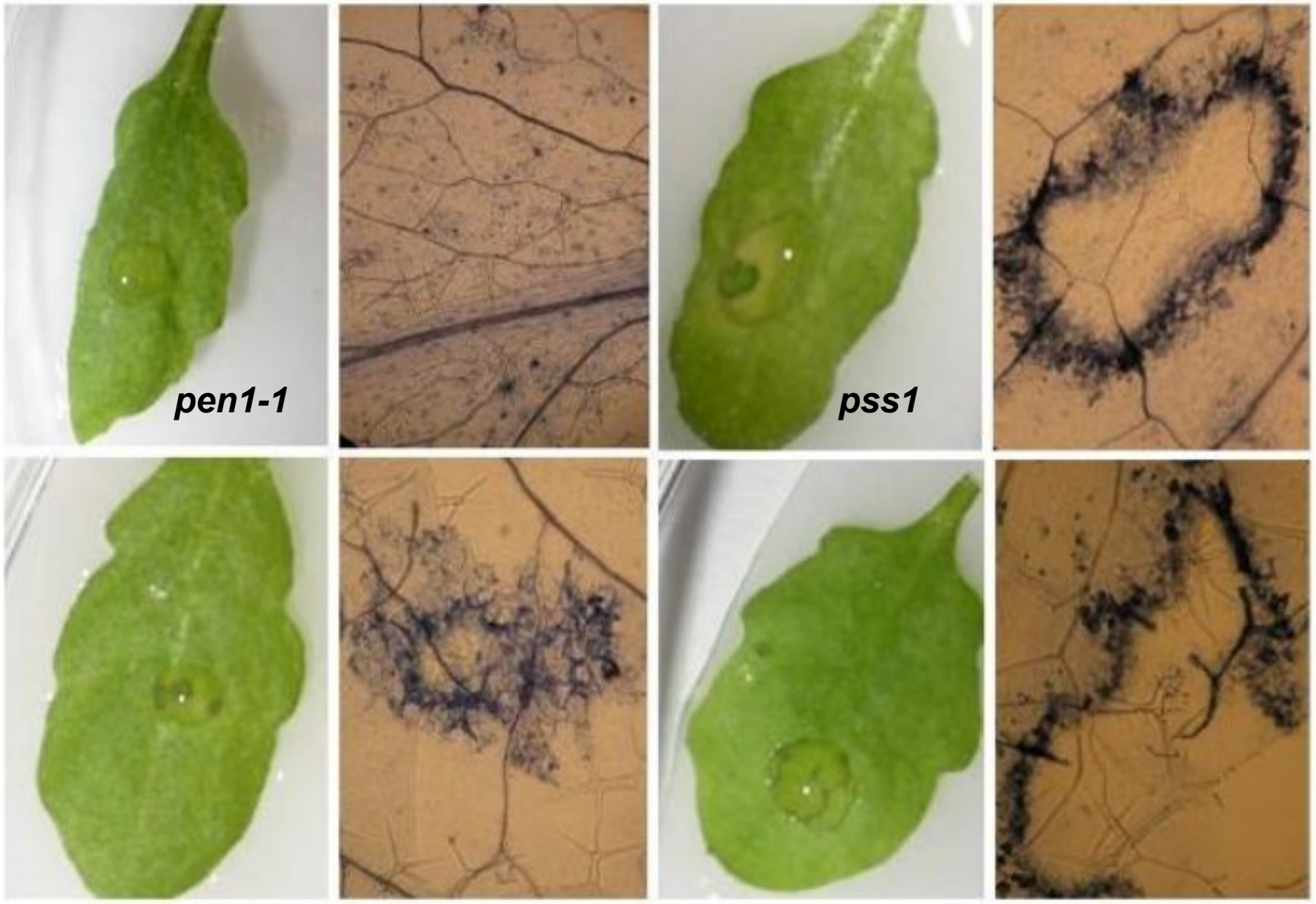
# Summary

1. Demonstrated that FvTox1-interacting Pep-1 enhances foliar SDS resistance.
2. FvTox1-interacting peptides conferring enhanced foliar SDS resistance also showed improved tolerance of roots to FvTox1.
3. FvTox1 most likely causes suppression of root growth and root necrosis in addition to foliar SDS.

# Two Approaches to Enhance SDS Resistance in Soybean

1) Understand how the pathogen causes SDS and apply the knowledge to create SDS resistance.
2) Identify and express plant genes to enhance SDS resistance.
    1. Arabidopsis nonhost resistance genes
    2. Altered expression of soybean genes

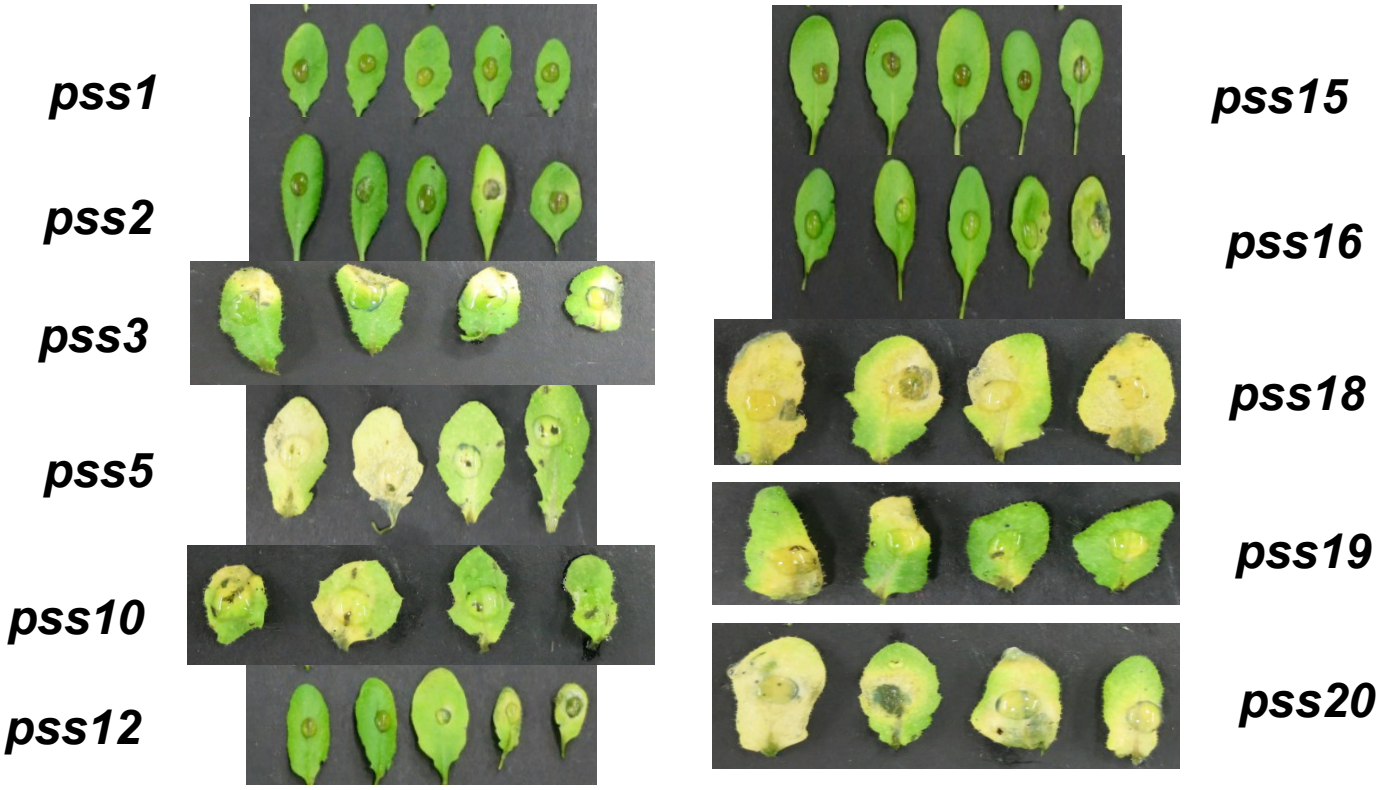
# *Phytophthora sojae* susceptible (*pss*) mutants



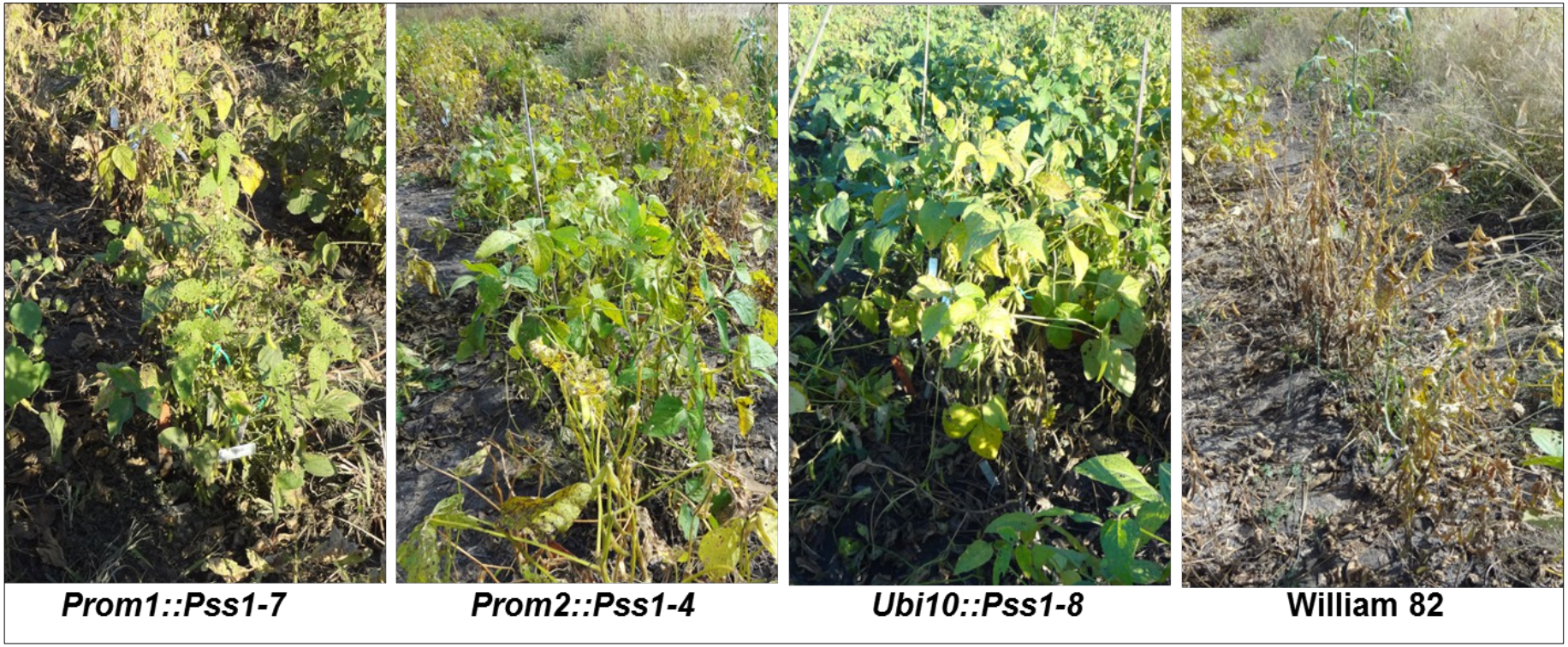
# Identified 30 *pss* mutants.

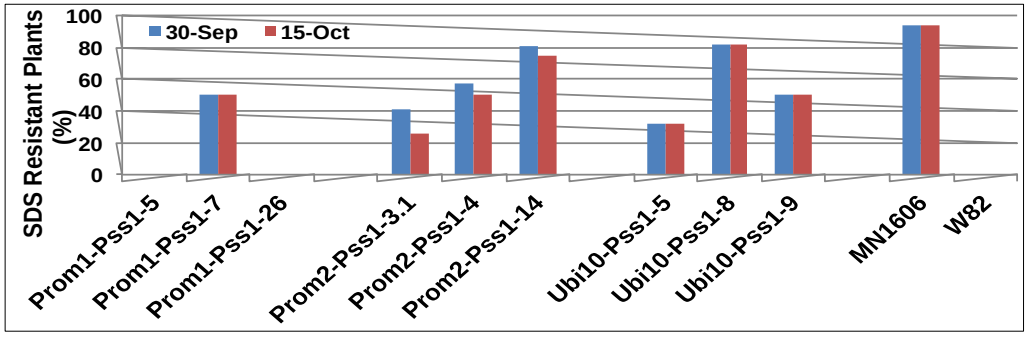
| *Mutant* | Family | *Mutant* | Family | *Mutant* | Family |
|---|---|---|---|---|---|
| *pss1* | 0.2B17I9 | *pss11* | 0.25B8C1 | *pss21* | 0.25B9D7 |
| *pss2* | 0.2B3D2 | *pss12* | 0.25 B9A1 | *pss22* | 0.25B9F1 |
| *pss3* | 0.3B6A7 | *pss13* | 0.25B8H5 | *pss23* | 0.2B20I2 |
| *pss4* | 0.2B21F1 | *pss14* | 0.3B6E8 | *pss24* | 0.2B9G4 |
| *pss5* | 0.2B15I6 | *pss15* | 0.3B6H8 | *pss25* | 0.2B8B7 |
| *pss6* | 0.2B4G5 | *pss16* | 0.25B7F8 | *pss26* | 0.3B1C7 |
| *pss7* | 0.2B21B9 | *pss17* | 0.25B7G4 | *pss27* | 0.3B8B8 |
| *pss8* | 0.2B4A5 | *pss18* | 0.25B9C3 | *pss28* | 0.25B9E3 |
| *pss9* | 0.2B21D6 | *pss19* | 0.3B6E4 | *pss29* | 0.2B1A9 |
| *pss10* | 0.25B7I5 | *pss20* | 0.3B6C8 | *pss30* | 0.25B7D2 |

# Fourteen *pss* mutants are also susceptible to *F. virguliforme.*



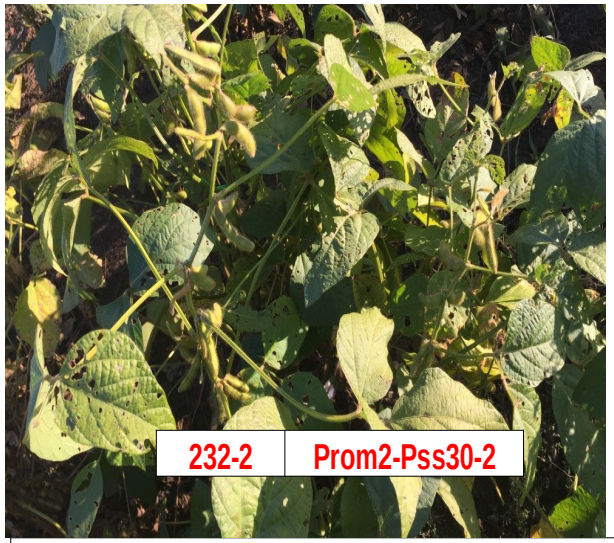
# Field evaluation of transgenic soybean plants carrying Arabidopsis nonhost resistance *Pss1* gene



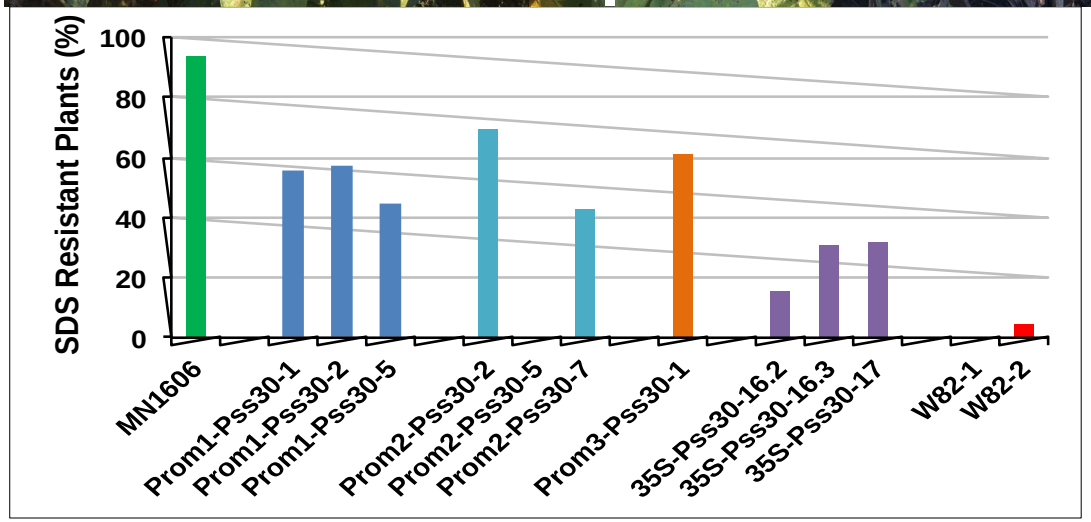




B. Wang et al. unpublished

# Field evaluation of transgenic soybean plants carrying Arabidopsis nonhost resistance *Pss30* gene






S. Kambakam et al., unpublished

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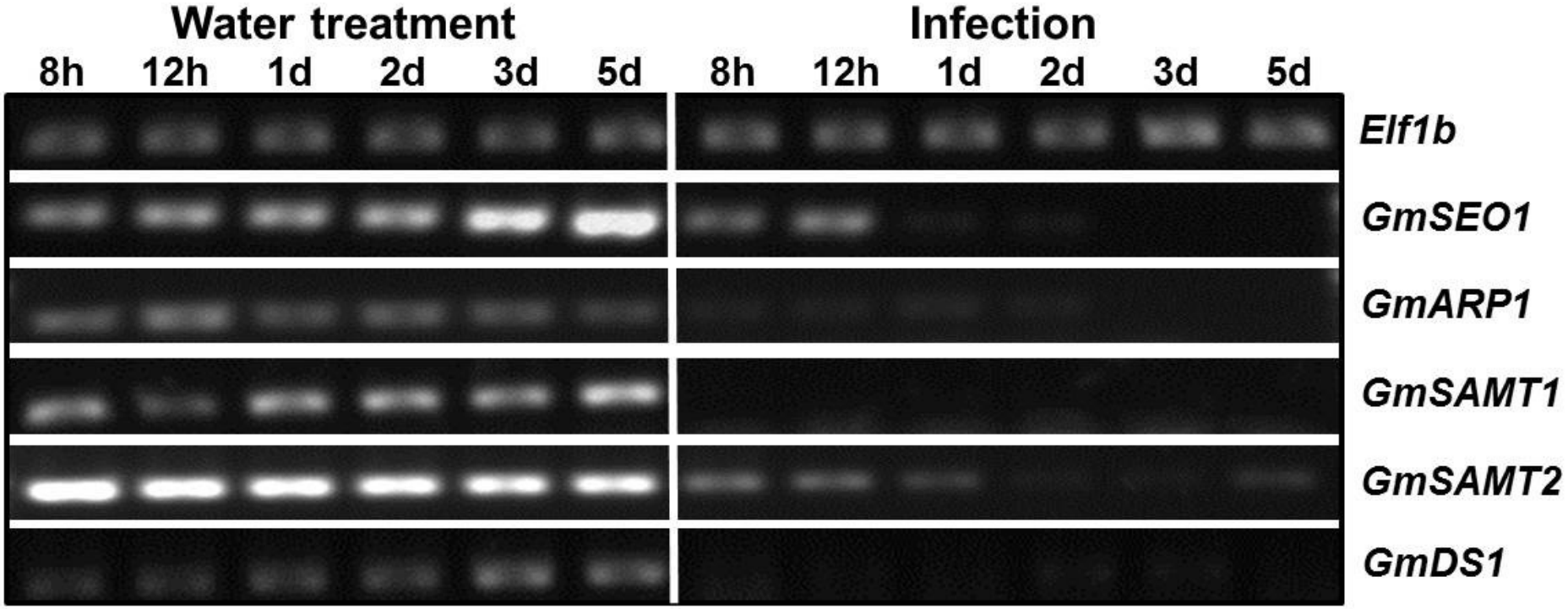
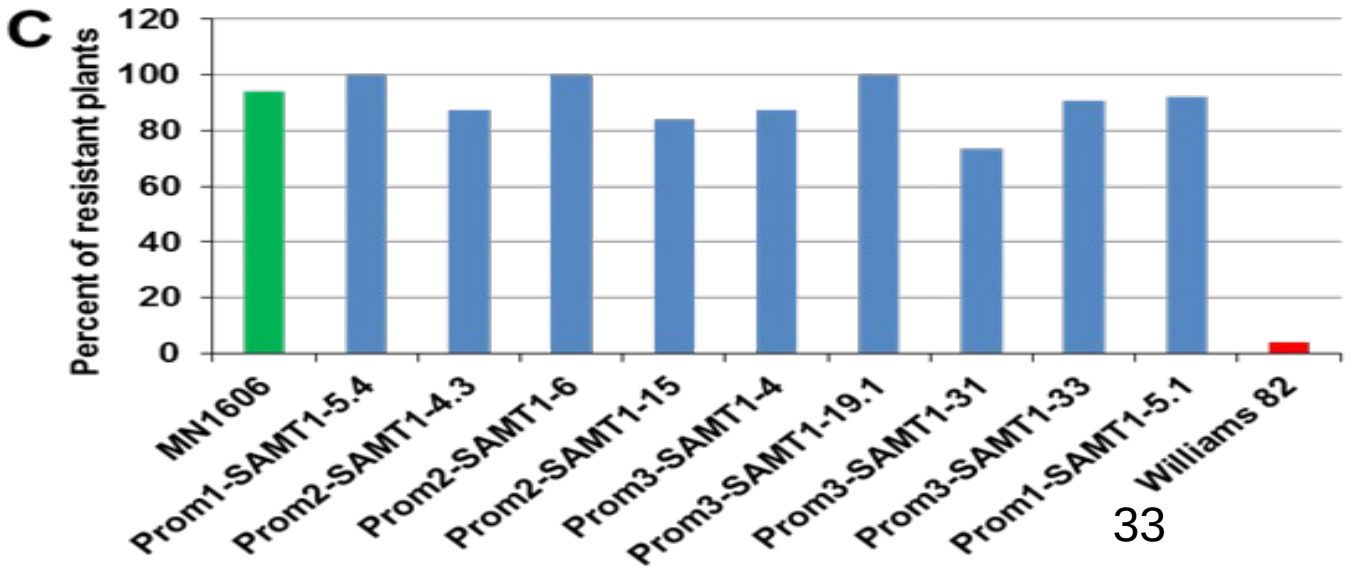
1) Understand how the pathogen causes SDS and apply the knowledge to create SDS resistance.
2) Identify and express plant genes to enhance SDS resistance.
    1. Arabidopsis nonhost resistance genes
    2. Altered expression of soybean genes

# Soybean genes suppressed during *F. virguliforme* infection.

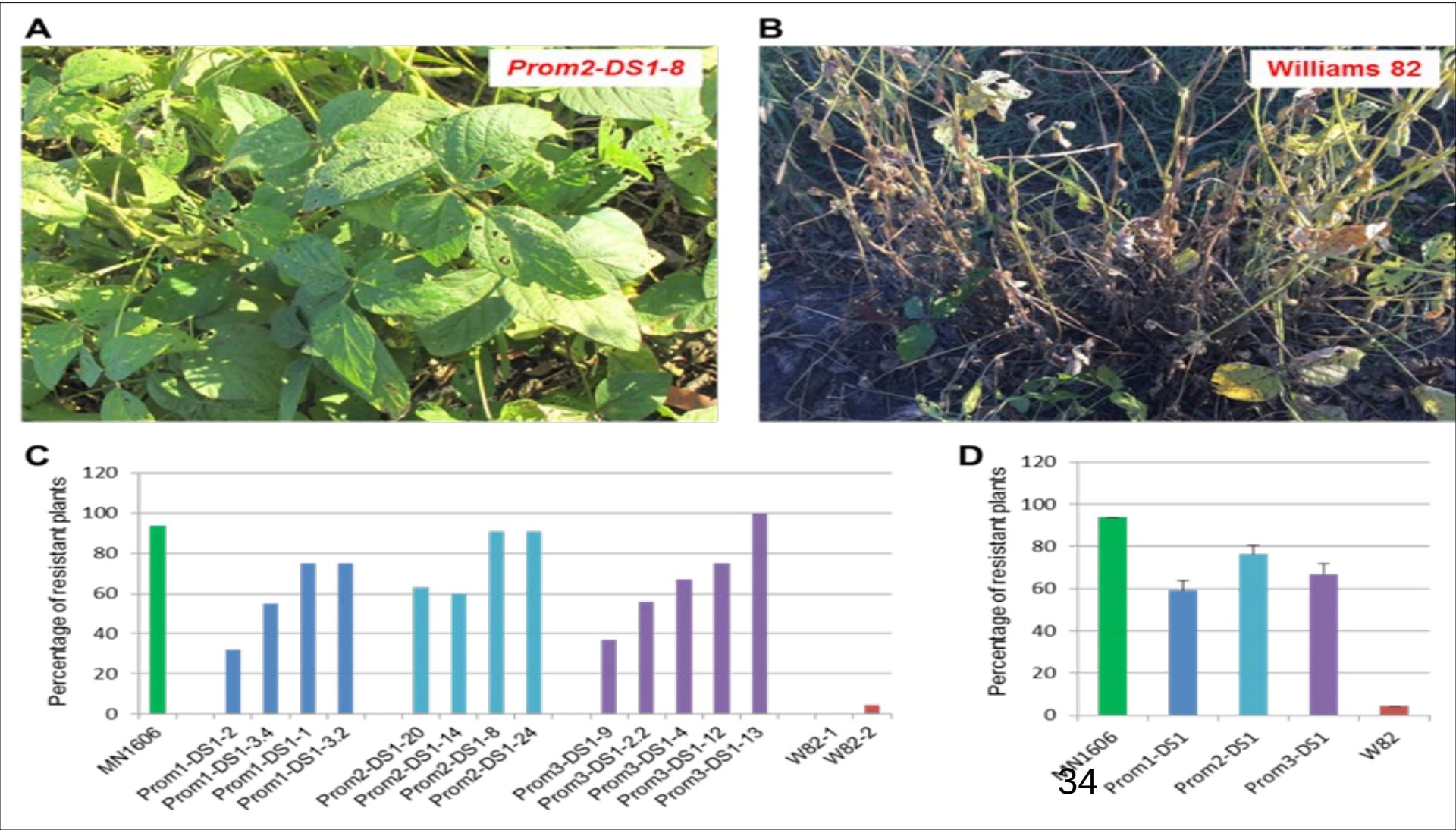


# Expression of *GmSAMT1* enhances SDS resistance in transgenic soybean plants under field conditions





# Expression of *GmDS1* enhances SDS resistance in transgenic soybean plants under field conditions



# Current Status

| Gene Name | Growth Chamber | Field Evaluation |
|---|---|---|
| *Pss1* | Resistant | Resistant |
| *Pss30* | Resistant | Resistant |
| *Pss6* | Resistant | Not Yet |
| *Pss21* | Resistant | Not Yet |
| *Pss25* | Partially Resistant | Not Yet |
| *GmSAMT1* | Resistant | Resistant |
| *GmDS1* | Resistant | Resistant |
| *GmARP1* | Resistant | Resistant |
| *GmSEQ1* | Resistant | Not Yet |

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Thank You!