

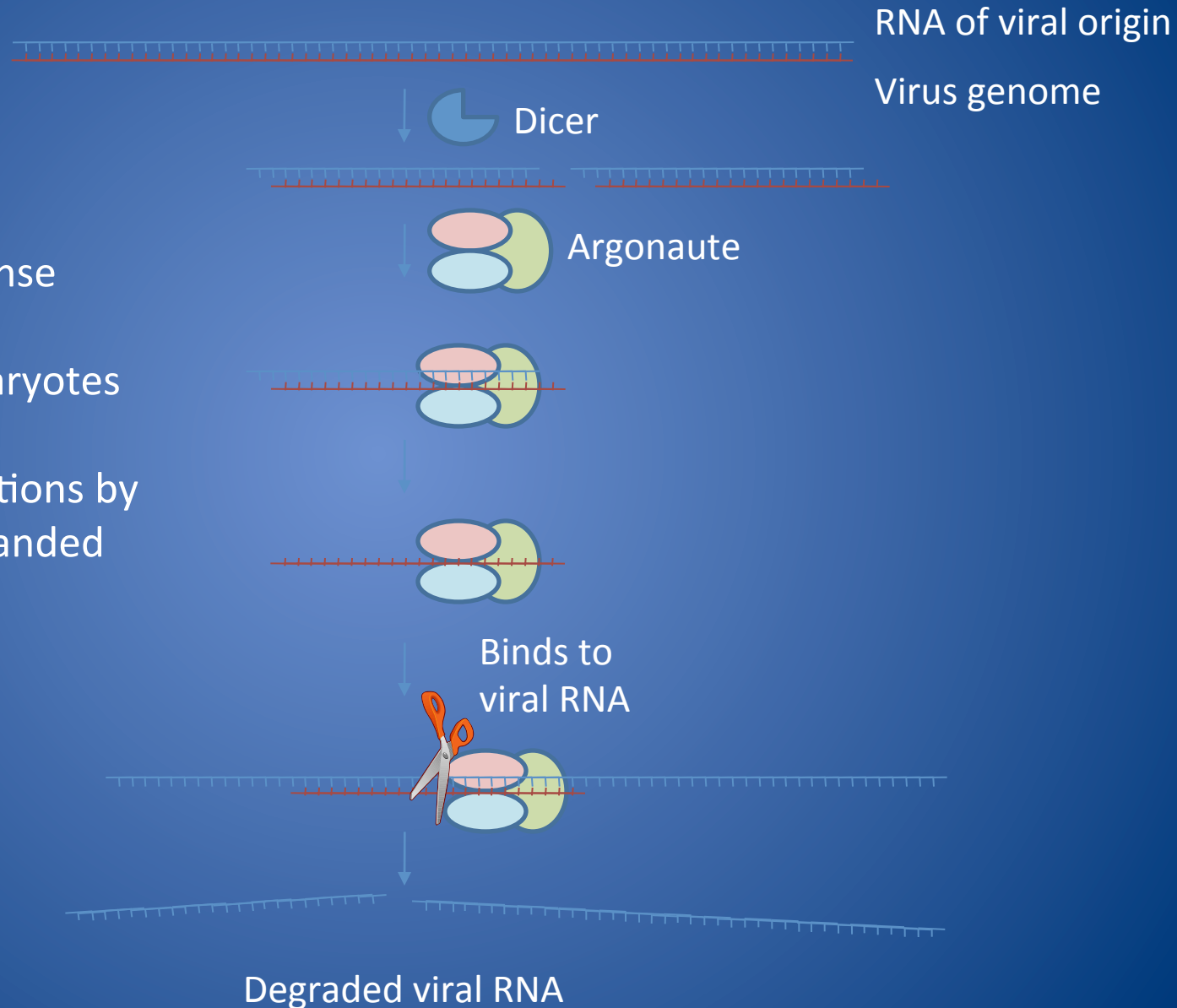
Utilization of VIGS to identify genes involved in resistance to stress in soybeans

Soybean Breeder's Workshop –
February 2013

Outline

- BPMV VIGS system
- Soybean rust defense gene networks
- Soybean mosaic virus defense gene networks
- Other pathosystems
- Conclusions

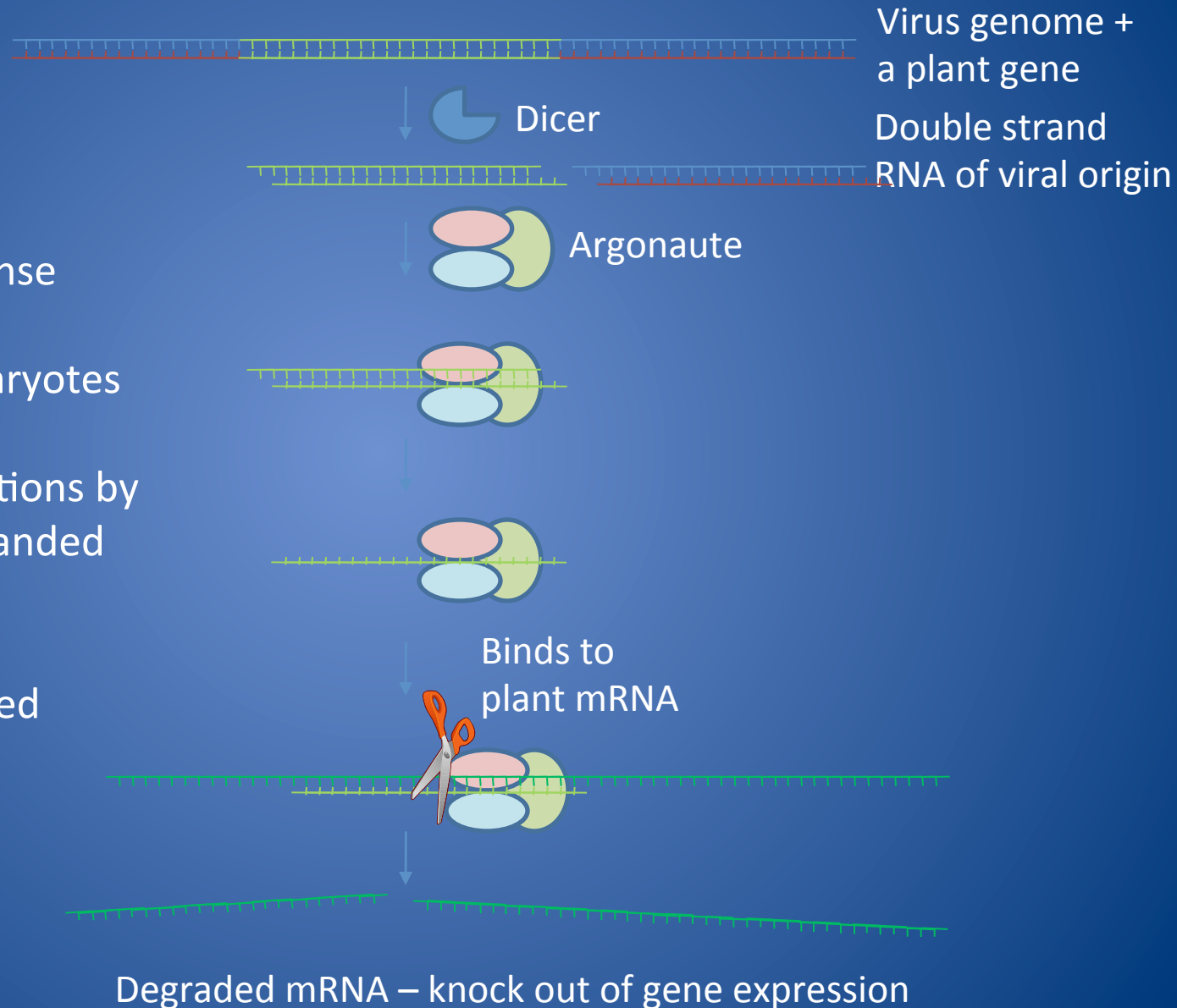
What is VIGS?



VIGS is based on a natural antiviral defense mechanism that is intrinsic to most eukaryotes

This mechanism functions by degrading double stranded RNA molecules

What is VIGS?

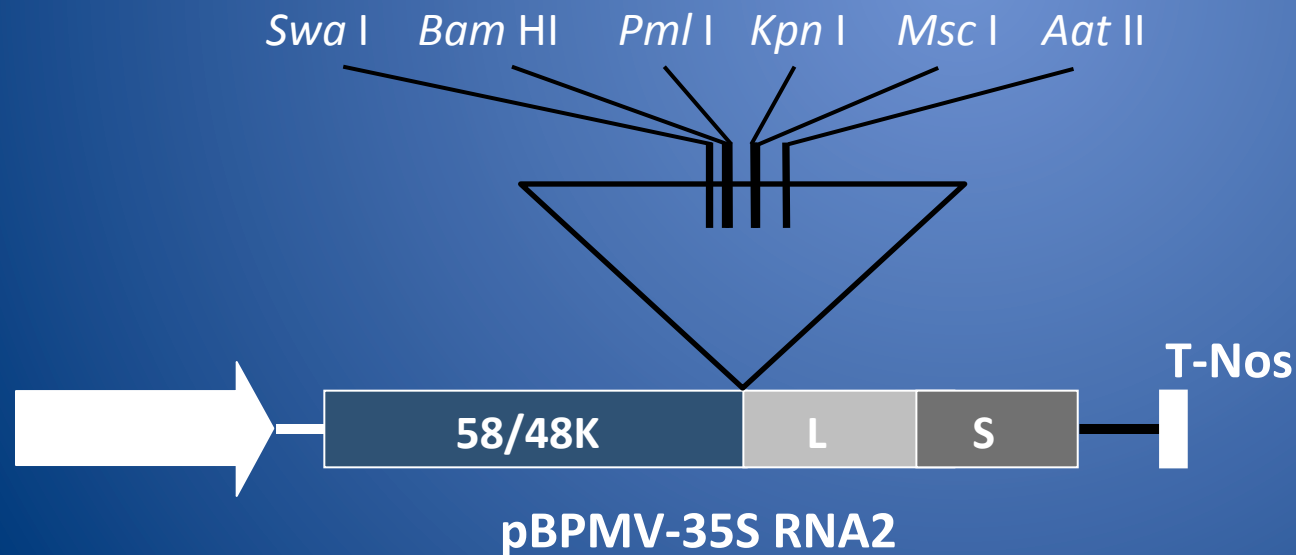
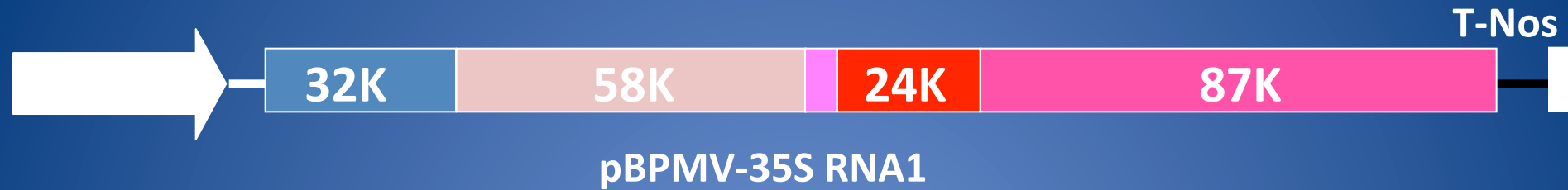


VIGS is based on a natural antiviral defense mechanism that is intrinsic to most eukaryotes

This mechanism functions by degrading double stranded RNA molecules

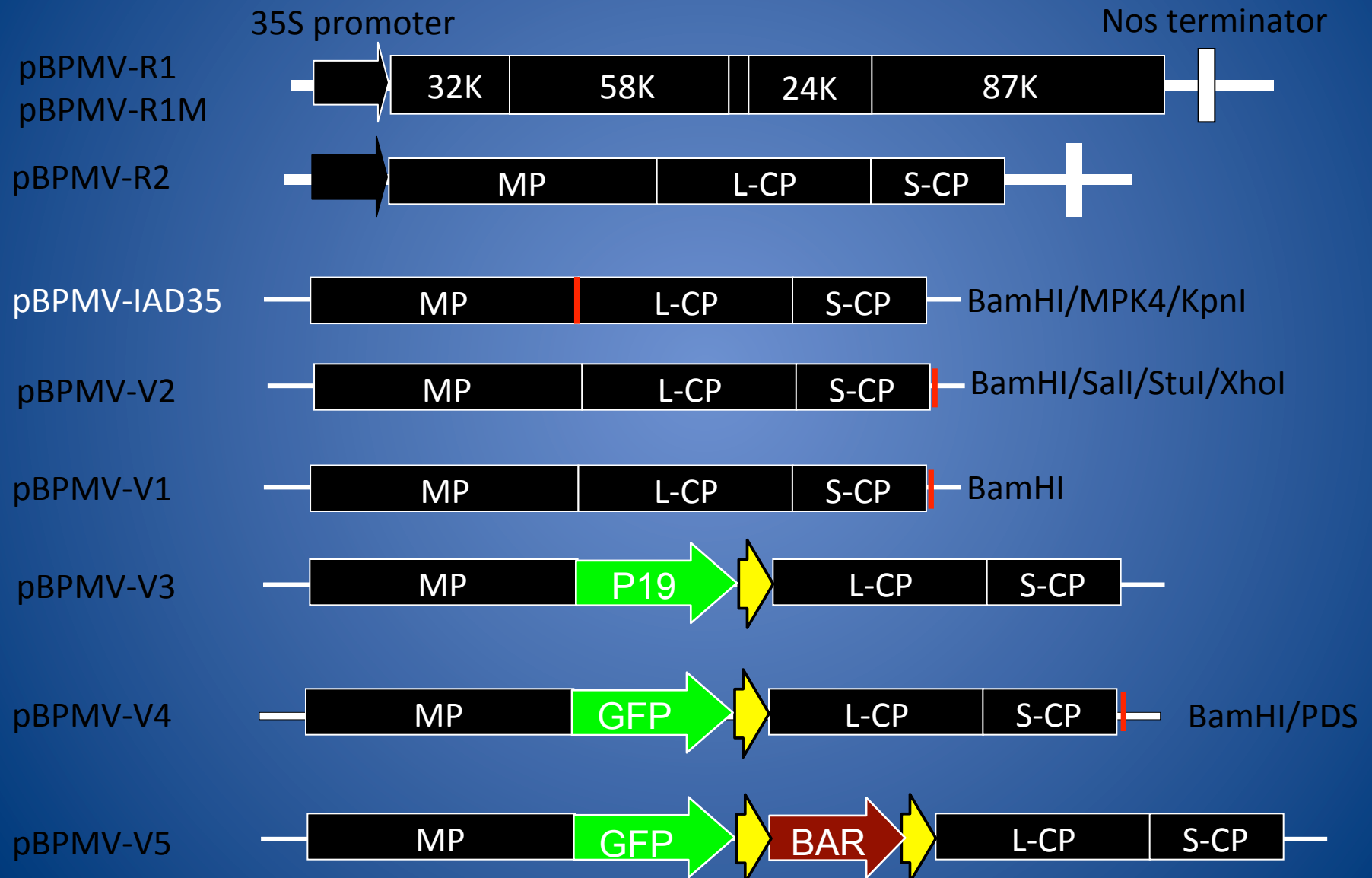
System can be targeted to degrade any RNA including host mRNA

Virus induced gene silencing – *Bean pod mottle virus*



Chris Zhang

BPMV vectors



VIGS procedure

Insert fragment of target gene into
BPMV VIGS vector



Infect unifoliolate leaves of soybean
plants with BPMV-VIGS



3 weeks later initiate pathogen assays
on 3rd or 4th trifoliolate leaves



PDS silencing in Williams 82 leaves

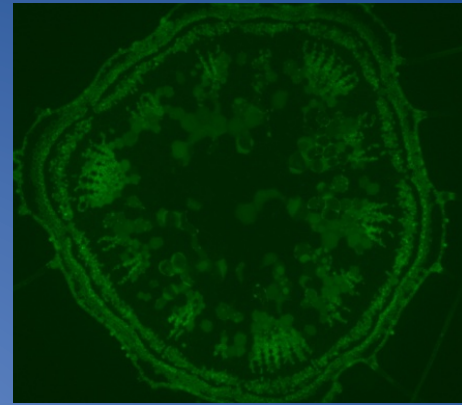
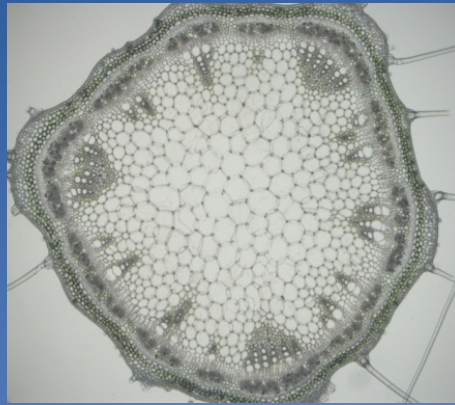


Stem

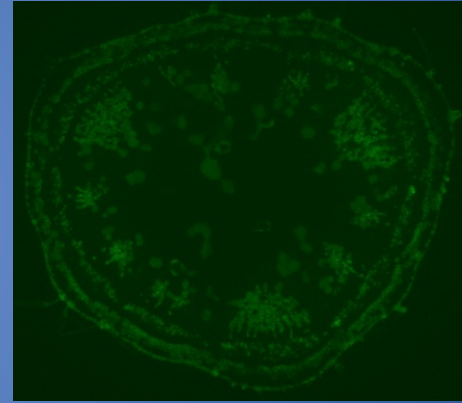
Bright field

GFP

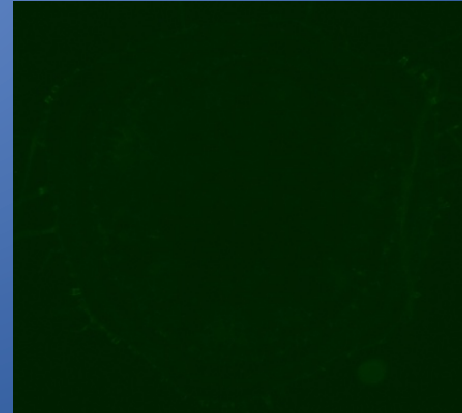
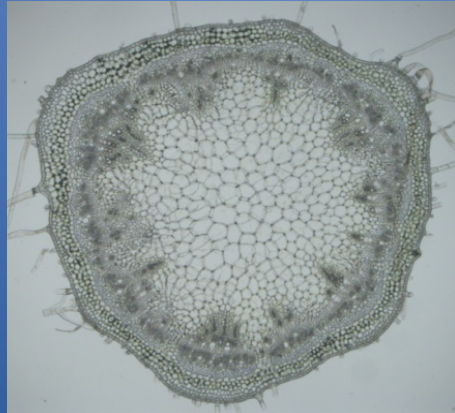
Mock



Empty
vector



Silencing
vector



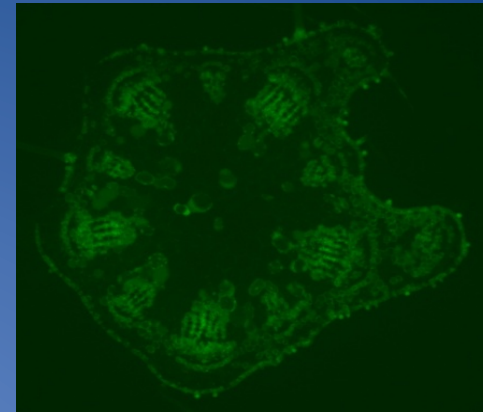
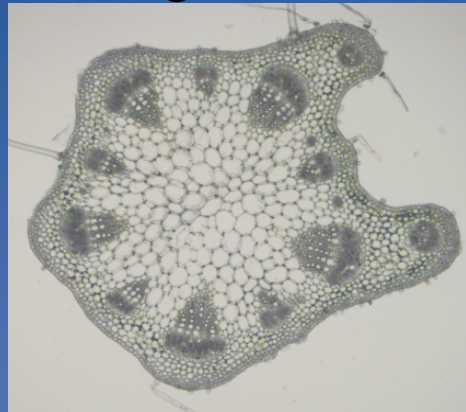
Section taken between 2nd and 3rd trifoliolate
on 21st day post inoculation.
80 micrometer thick sections.
Uniform exposure (500 ms)
Uniform magnification

Leaf petiole

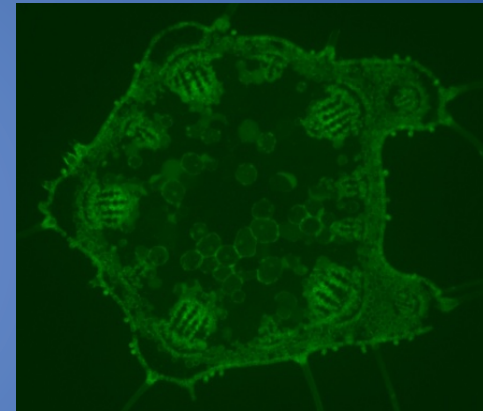
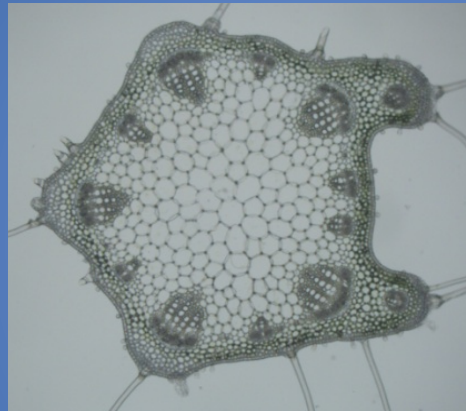
Bright field

GFP

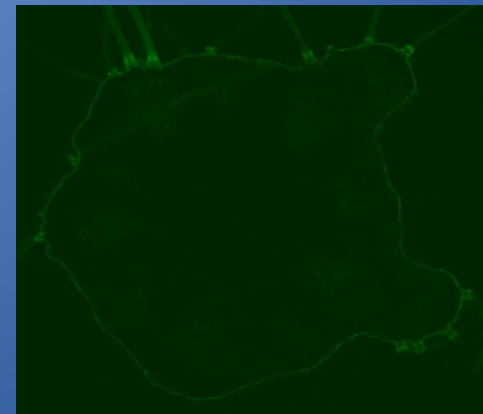
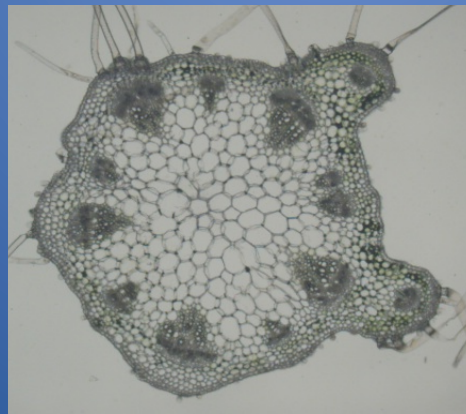
Mock



Empty vector

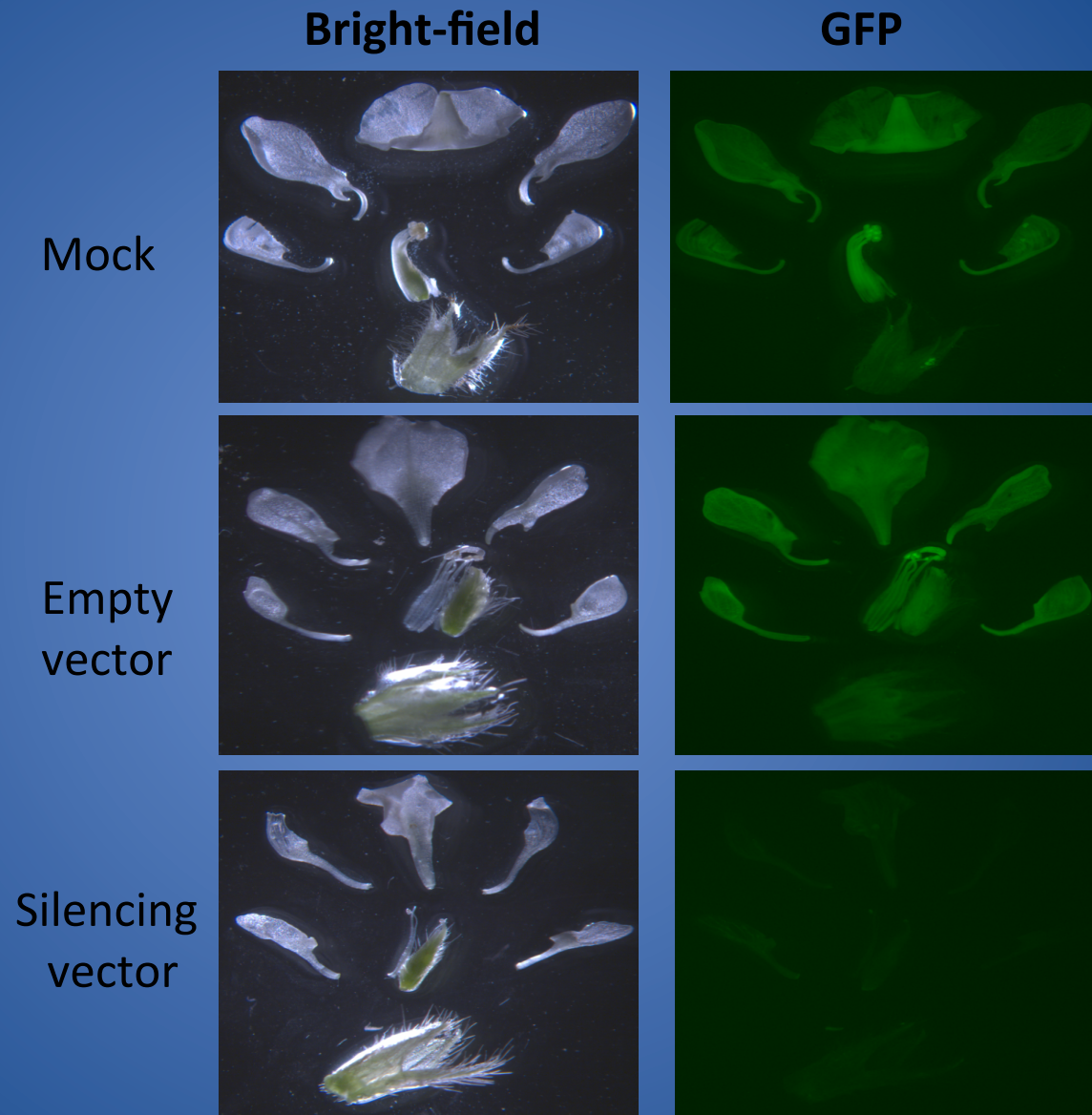


Silencing vector



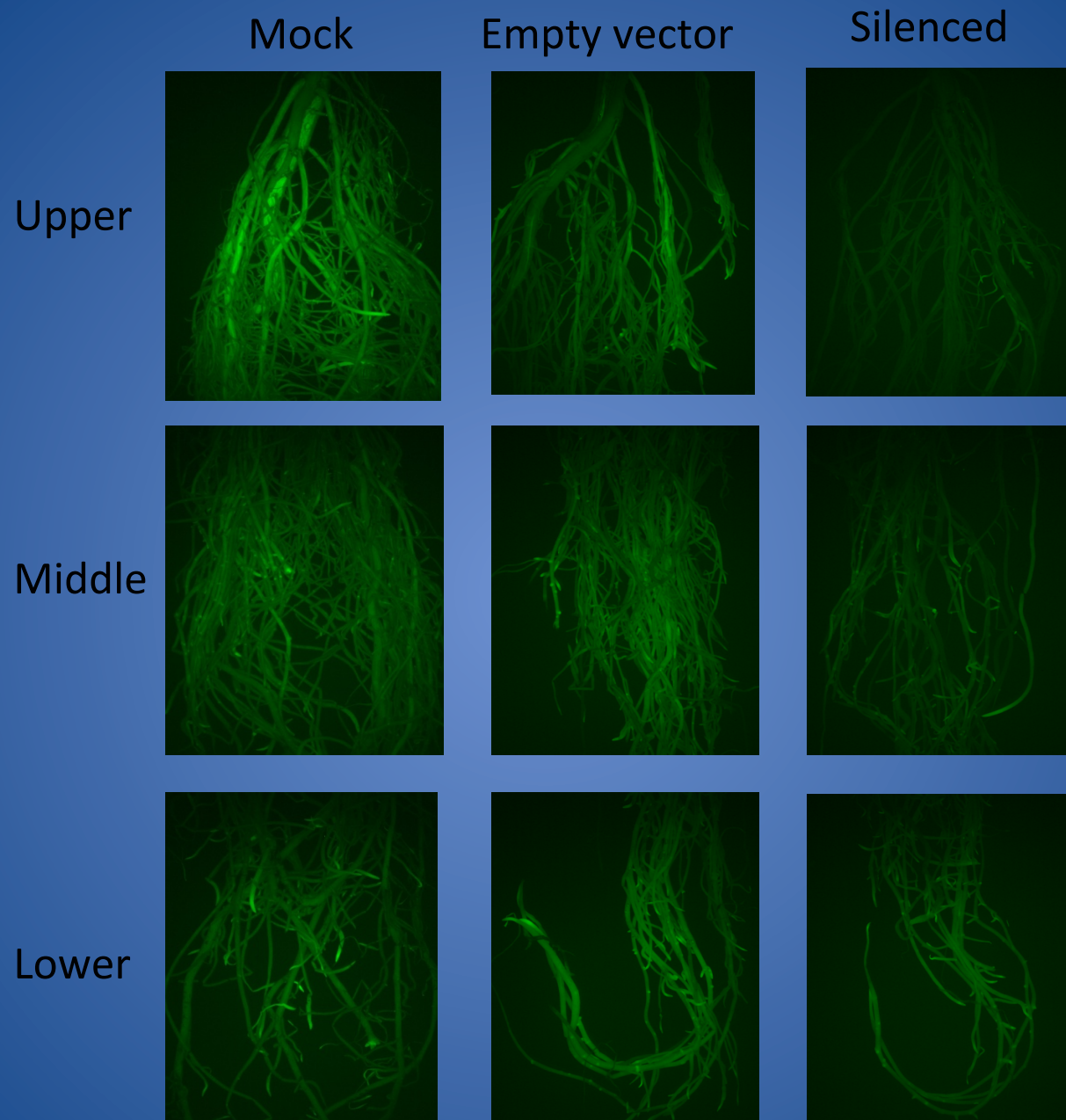
Petiole from second trifoliolate was sectioned on 21st day post inoculation.
80 micrometer thick sections
Uniform exposure (500 ms)
Uniform magnification

Flowers



Equal exposure (.5 sec)
Equal magnification

Roots



Exposure for GFP: 5 sec

Juvale et al. (2012) *Mol Plant Path.* 13:1140-1148

Discovery of genes involved in soybean defense

**Candidate
defense
genes**

Microarray profiling
susceptible and resistant
interactions

Known Arabidopsis
defense genes

Disease resistance traits
(genetic map)

Reverse genetics with
BPMV VIGS system

[VIGS library](#)

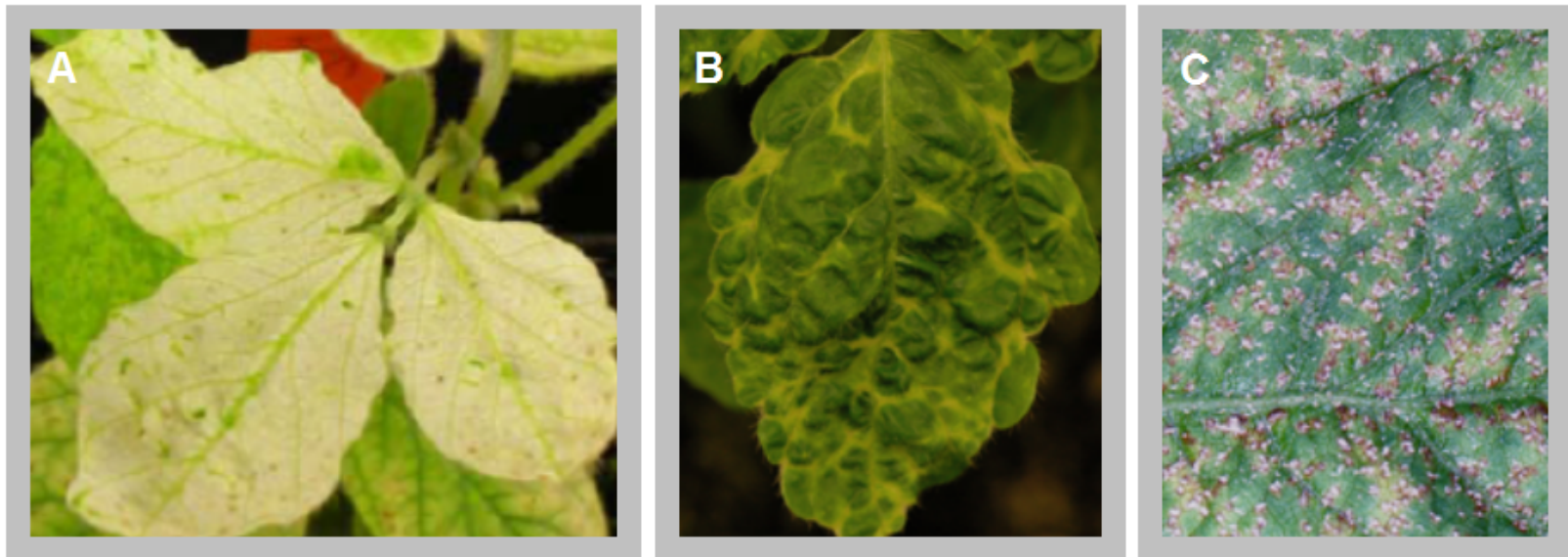
Loss of function phenotypes
(molecular & macroscopic)

**Gene
function
analysis**

**Soybean
defense
networks**

Regulatory modules

SoyVIGS: Virus Induced Gene Silencing in Soybean



Examples of VIGS in Soybean

- A. BPMV VIGS of phytoene desaturase (*Pds*) results in a photobleaching phenotype.
- B. BPMV VIGS of a target gene causes a distorted leaf phenotype
- C. BPMV VIGS of candidate *Rpp4* resistance genes causes lost of resistance to *Phakopsora pachyrhizi* (Asian Soybean Rust).

Virus Induced Gene Silencing for Soybean

SoyVIGS Database

In the spaces below you will be able to browse or search the SoyVIGs database for entries based on their Glyma (e.g. Glyma06g05260) or laboratory (e.g. 21H11) identifiers.

Browse the SoyVIGS database:

The button below will allow you to retrieve a HTML representation of the complete database.

Search the SoyVIGS database:

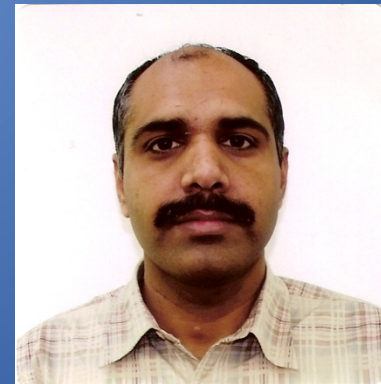
To search for one or more constructs based on various fields choose a field from the list below:

Cut-and-paste a list of identifiers in the input field below:

Asian soybean rust



- Rpp1* – Immune reaction
- Rpp1b* – Red-brown reaction
- Rpp2* – Red-brown reaction
- Rpp3* – Red-brown reaction
- Rpp4* – Red-brown reaction
- Rpp5* – Immune & Red-brown reaction



Ajay Pandey

Expected phenotypes

Incompatible interaction

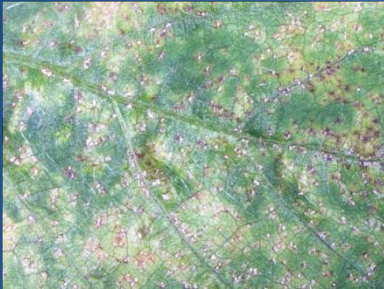


Compatible interaction



Loss-of-resistance phenotypes of 11 candidate genes in *Rpp2* plants (140 screened)

GmEDS1



GmPAD4



GmNPR1



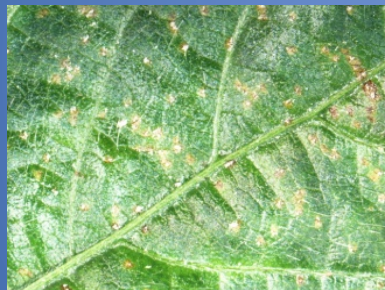
GmWRKY36



GmWRKY40



GmWRKY45



GmDBTF



GmMYB84



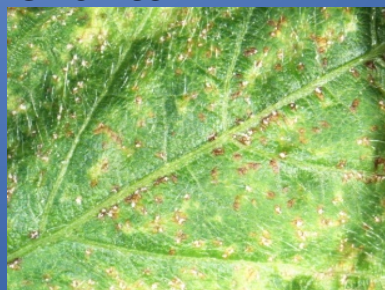
GmO-MT



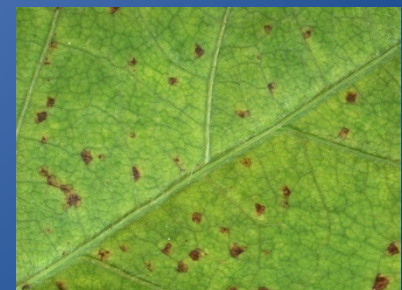
GmPAL1



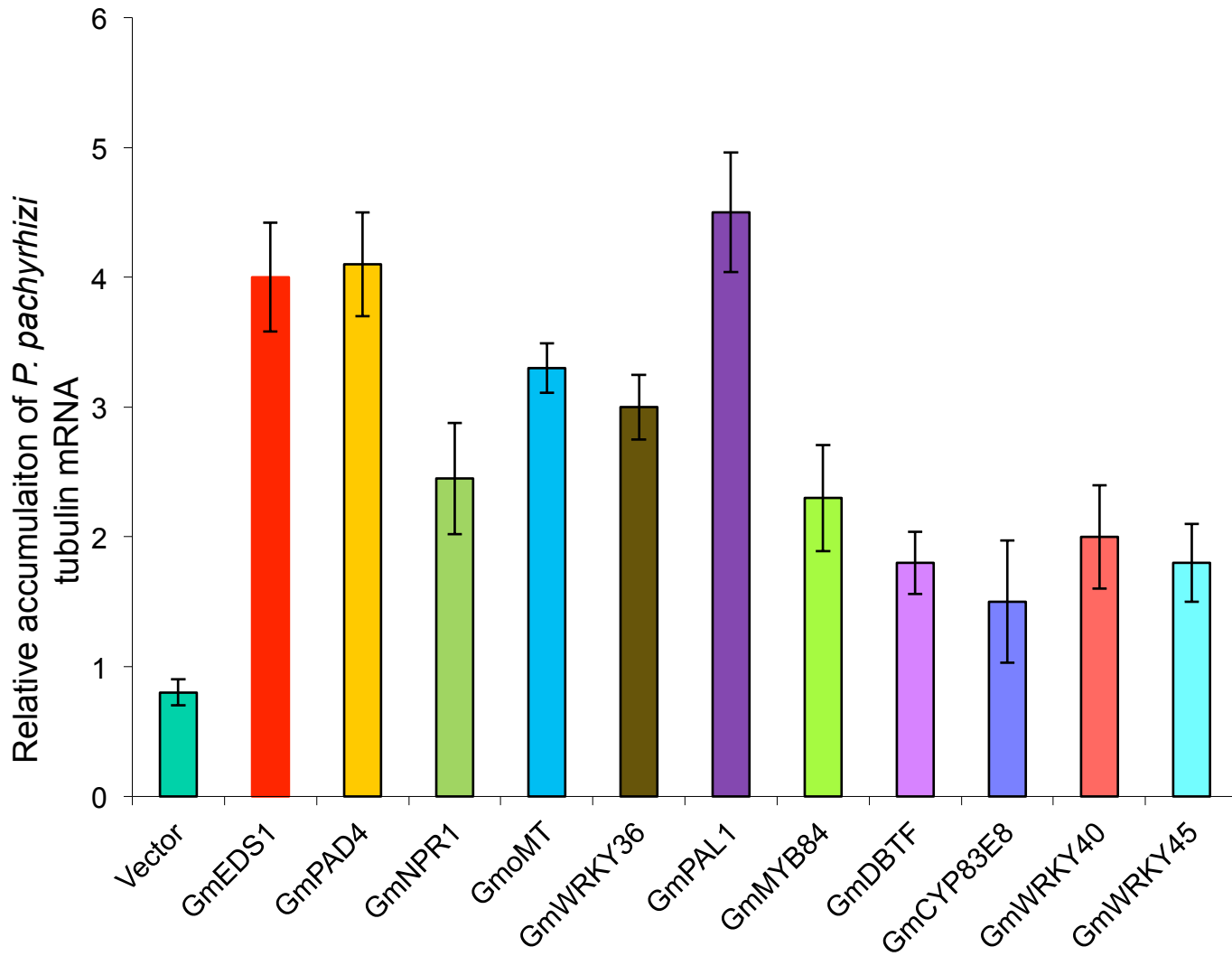
GmCYP83E12



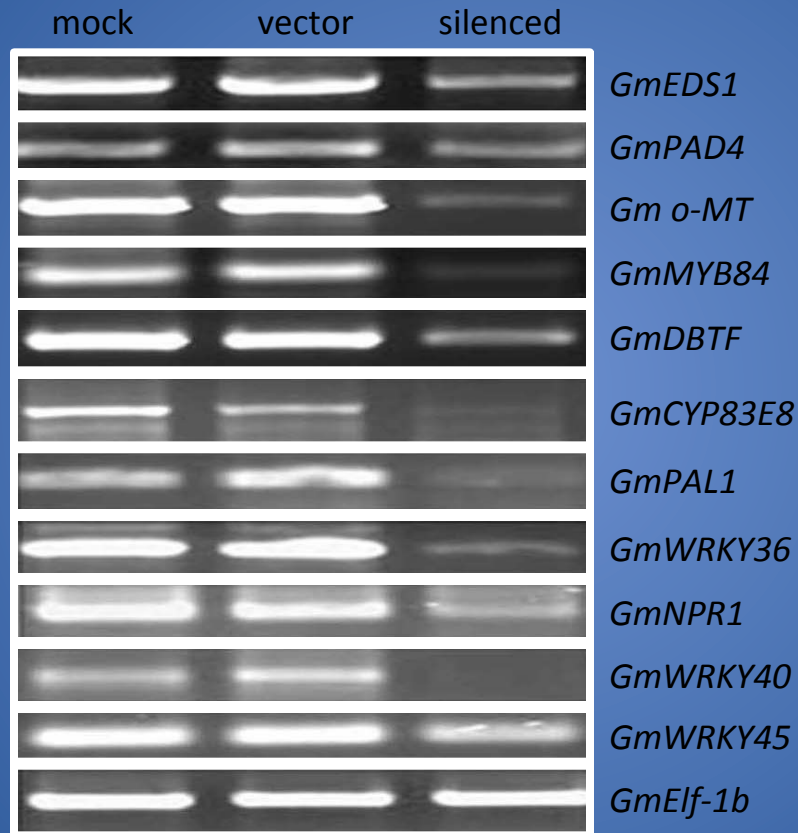
Vector



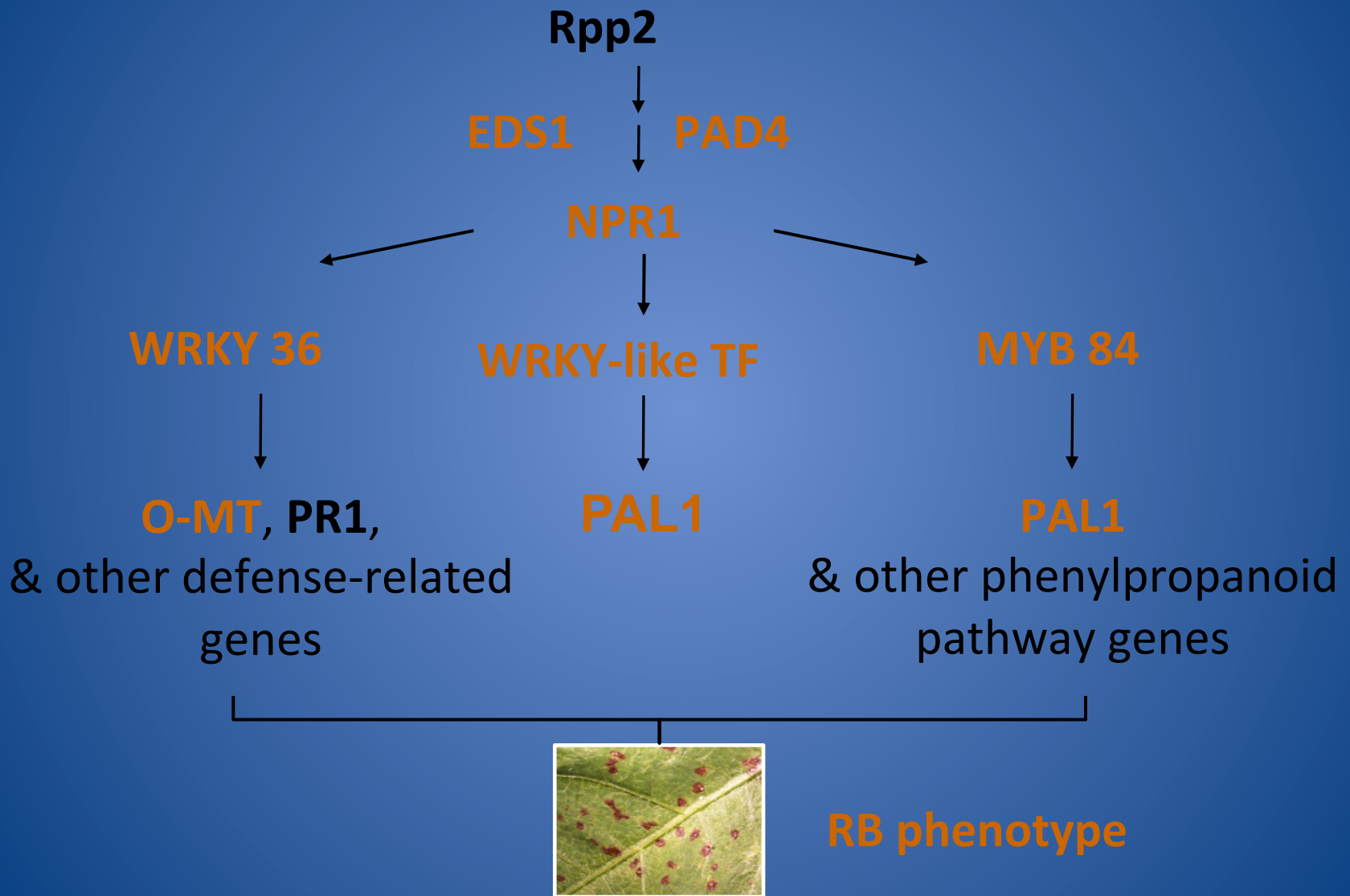
Relative fungal growth in silenced plants



Verification of gene silencing



Rpp2 resistance network



Soybean mosaic virus



Rsv1 – Extreme resistance

Rsv3 – Restrict long-distance movement

Rsv4 – Delayed susceptibility



Chris Zhang

SMV – soybean interaction

- Rsv1*
- Confers extreme resistance to SMV-N
 - No detectable virus replication or movement
 - PI 96983, L78-379 (*Rsv1*), Williams (*rsv1*)



No GUS staining in *Rsv1* plants

Expected loss-of-resistance phenotype



Rsv1 genotype
Resistant phenotype

VIGS
→

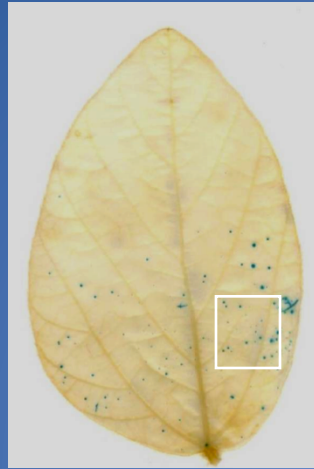


Rsv1 genotype
Susceptible phenotype

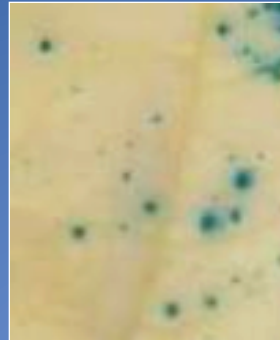
Identification *Rsv1* resistance gene candidates



Mock



rsv1



Rsv1
Vector only



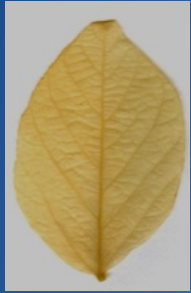
Rsv1
VIGS of *Rsv1*



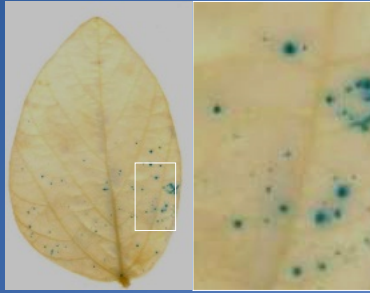
SMV GUS infection foci on
Rsv1 genetic background

Eight candidate genes required for *Rsv1* function (82 BPMV constructs screened)

Rsv1



rsv1



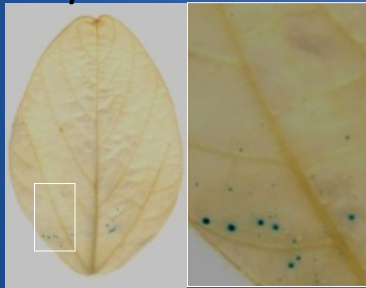
Eds1



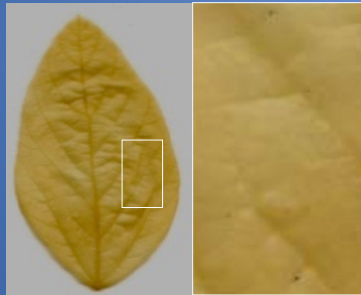
Pad4



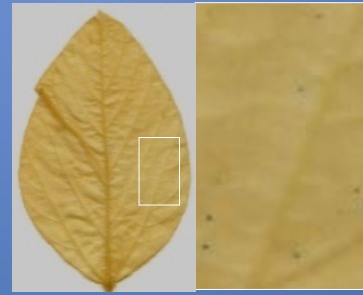
Hsp90



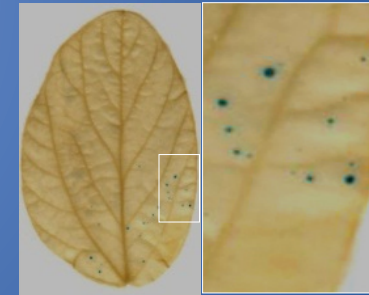
Edr1



Jar1



Edr1/Jar1



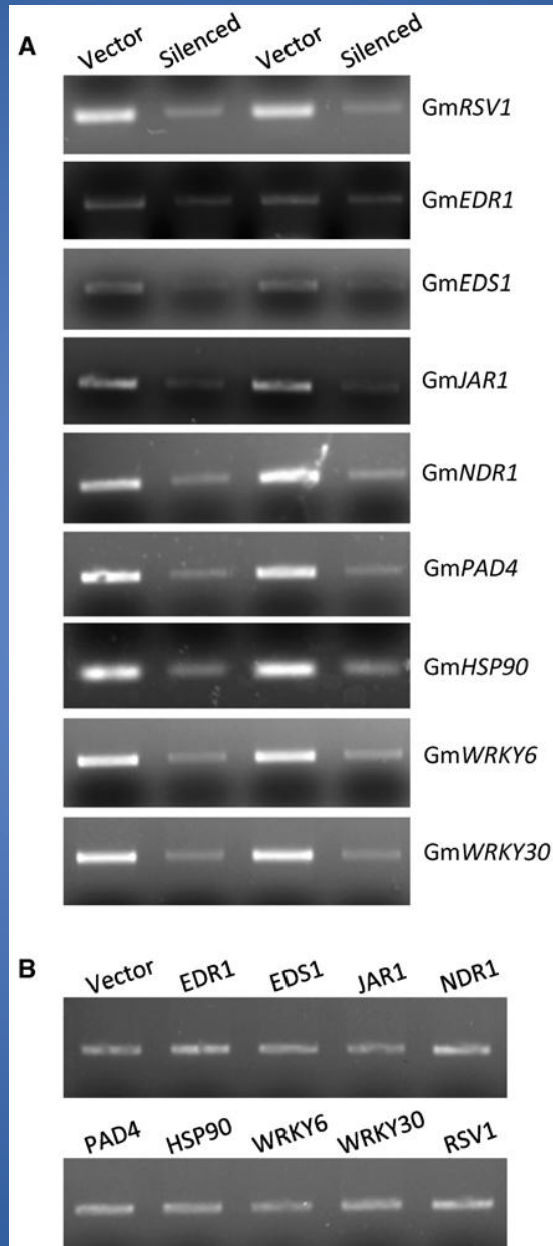
WRKY6



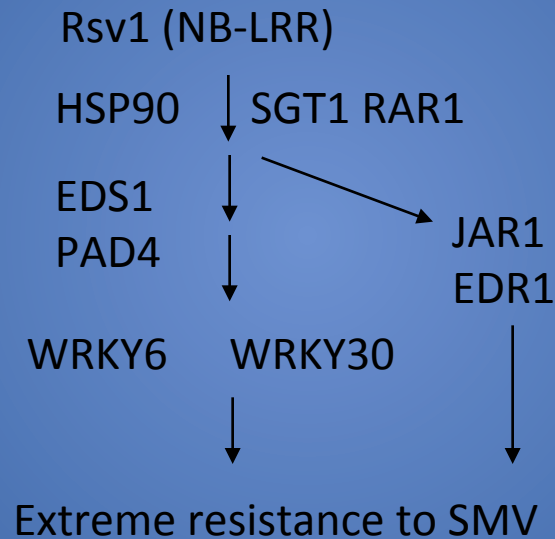
WRKY30



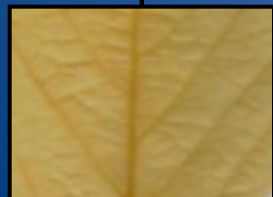
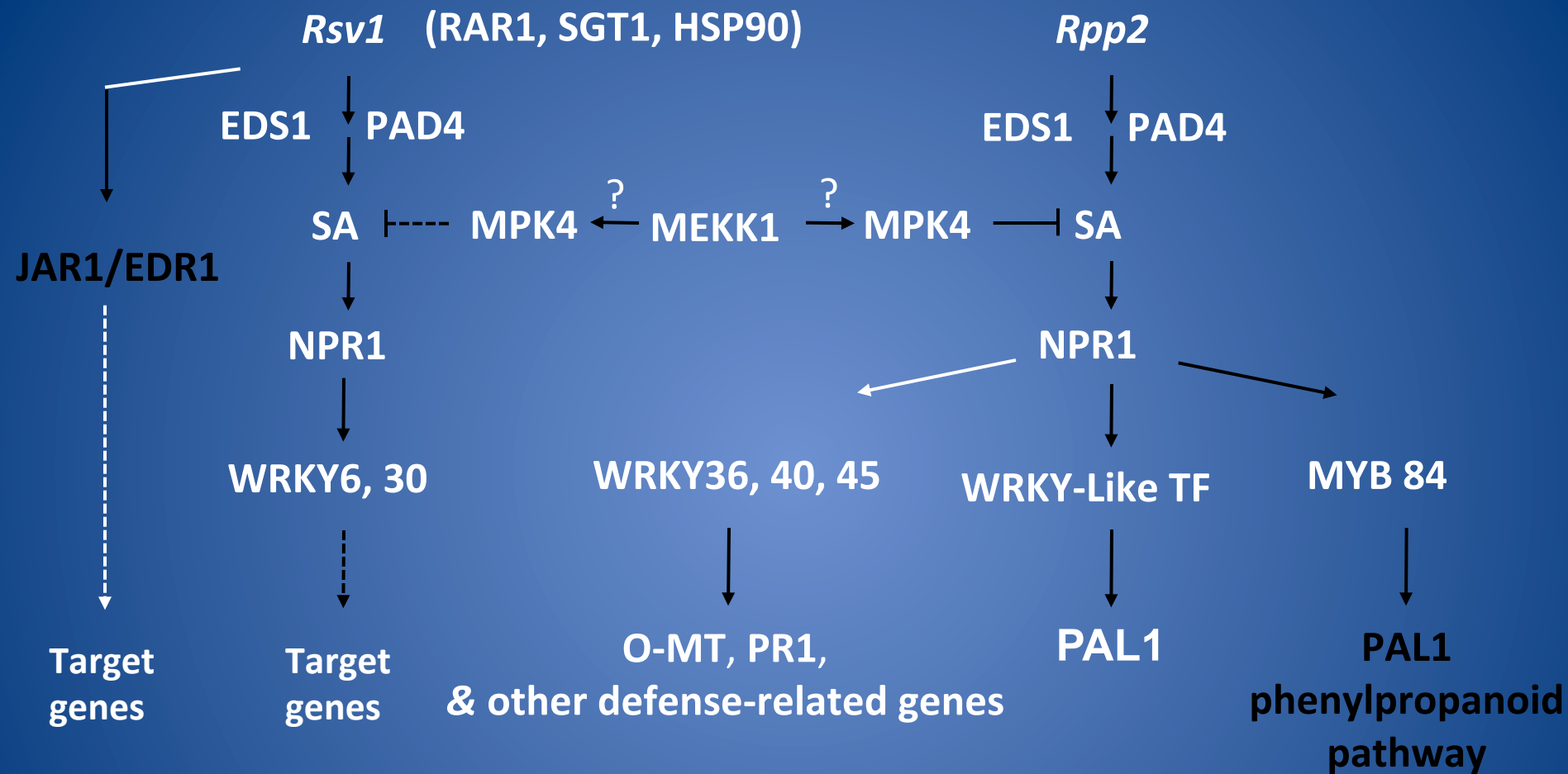
Verification of gene silencing



Rsv1 signaling network



Budding soybean defense gene network



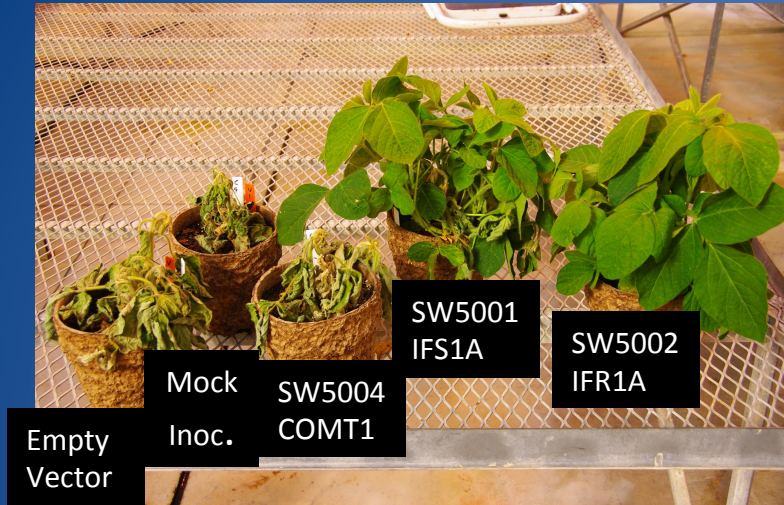
ER



HR

Other soybean pathosystems under exploration using VIGS

Sclerotinia – lignin pathway constructs



Courtesy Craig Grau

Sudden Death Syndrome

Resistant



Susceptible



Courtesy Leonor Leandro

Soybean cyst nematode



Courtesy Thomas Baum

Cloning of gene at *Rhg4* locus for resistance to SCN encoding a serine hydroxymethyltransferase

Liu et al., *Nature* 492:256-260

Conclusions

- **BPMV VIGS is potentially effective gene silencing tool in a variety of tissues**
- **Library of BPMV VIGS clones:
<http://www.soybase.org/SoyVIGS/Welcome.html>**
- **11 genes required for *Rpp2* resistance to Asian soybean rust**
- **8 genes required for *Rsv1* resistance to Soybean mosaic virus**
- **Other pathosystems are under exploration utilizing VIGS**

Collaborators



Plant Path. & Micro.

John Hill

Steve Whitham

Thomas Baum

Leonor Leandro

Chunquan (Chris) Zhang

Jianzhong (JZ) Liu

Parijat Juvale

Chunling Yang

Jaime Dittman

Al Eggenberger

Heidi Horstman

Silvia Cianzio

Univ. Wisconsin

Craig Grau

Univ. Minnesota

Dean Malvick

Univ. Missouri

Melissa Mitchum

Pramod Kandoth

FDWSU, Ft. Detrick, MD

Kerry Pedley

Ajay Pandey

Mandy Kendrick

Amy Ruck

Andrea Luquette

Reid Frederick

Kathy Schneider

CICG, Ames, IA

Michelle Graham

Randy Shoemaker

Rex Nelson



Southern Illinois University

Shiming Liu

Kahlid Meksem

Aziz Jamai



Londrina Brazil

Ricardo Abdelnoor

Fran Guimaraes

Danielle da Silva

