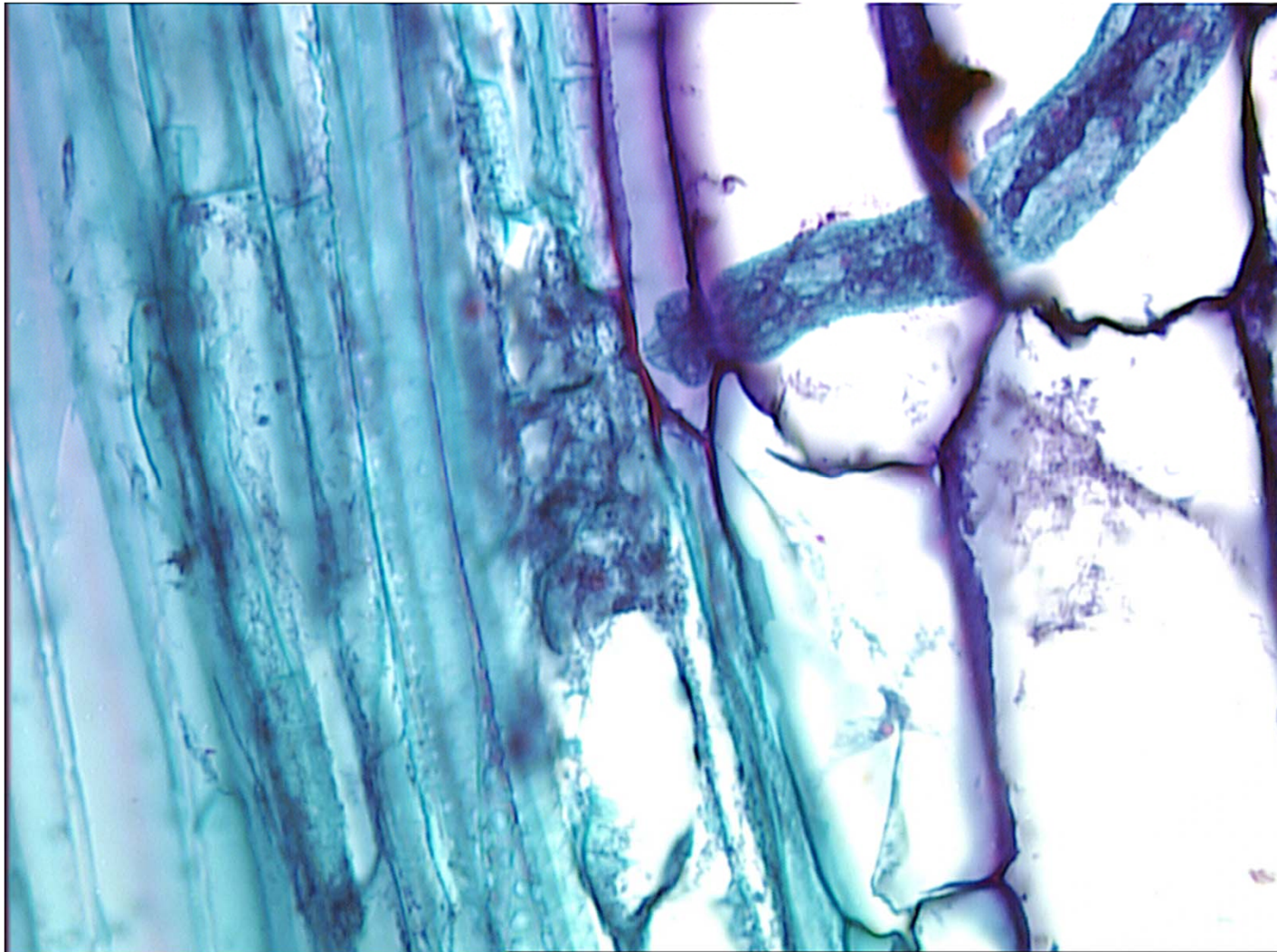


Identification of Cotton Genes Conferring Resistance against RKN

Application of procedures from other plant-nematode pathosystems to solve the problem



Klink et al. 2009

introduction

(1) outline of the cell biology approach to studying infection

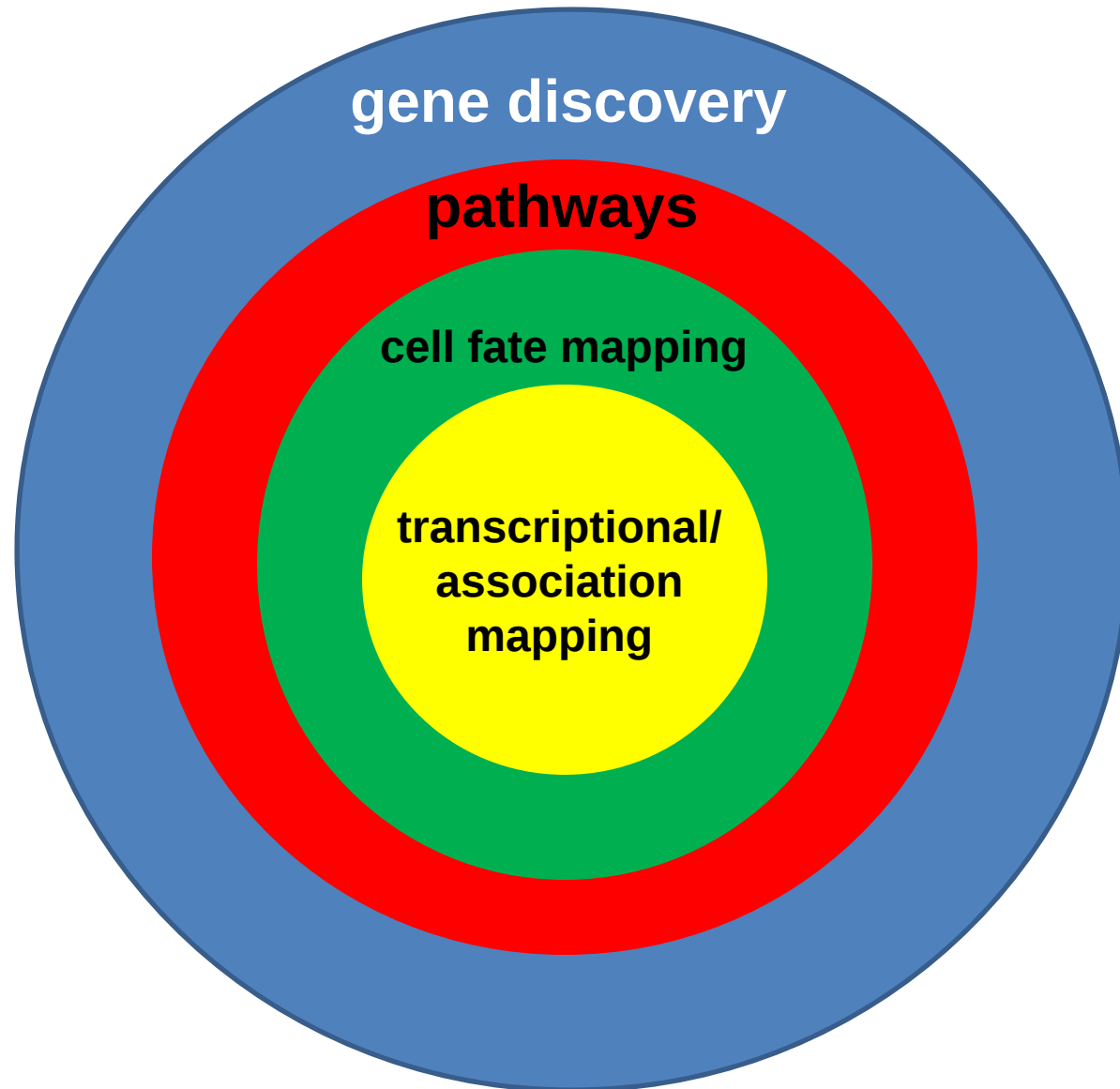
(2) gene discovery

(3) cell genotyping

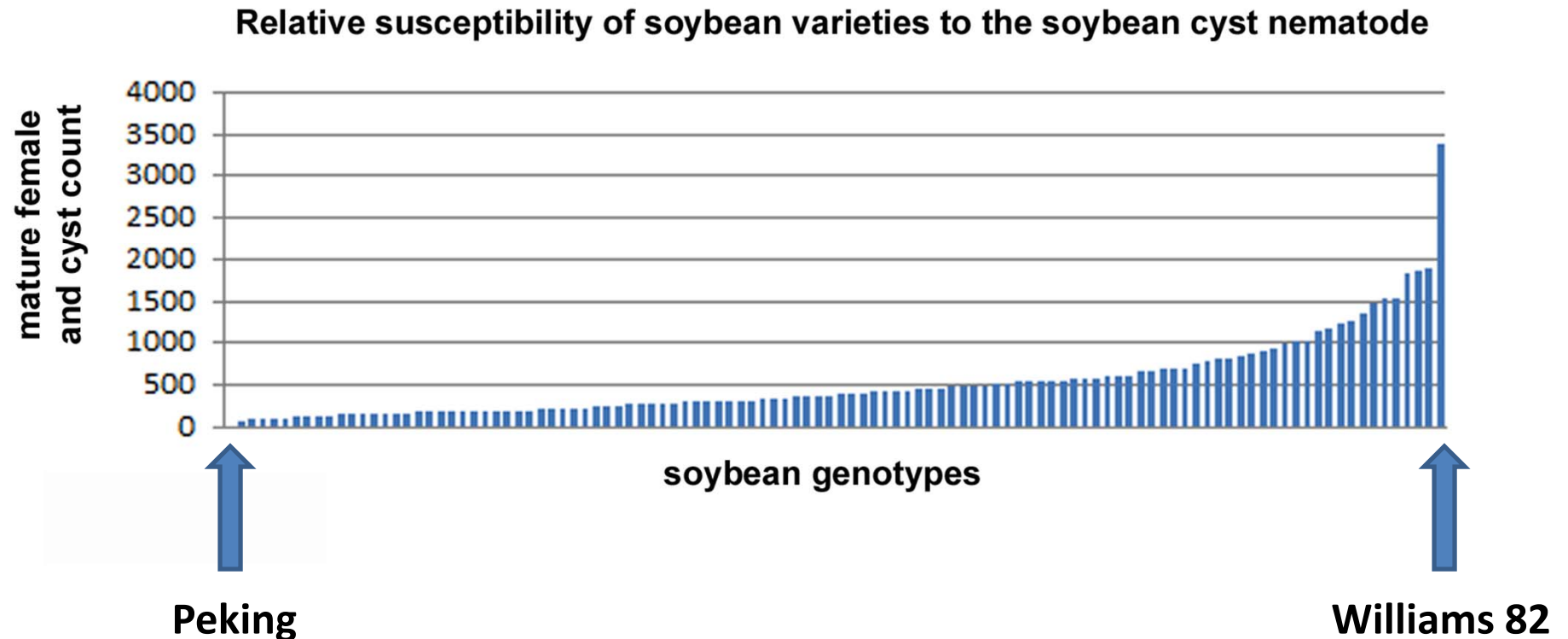
(4) functional studies

(5) development of the cotton pipeline

GOAL: Gene expression is organized in a manner to best identify genes that pertain to defense, to engineer resistance

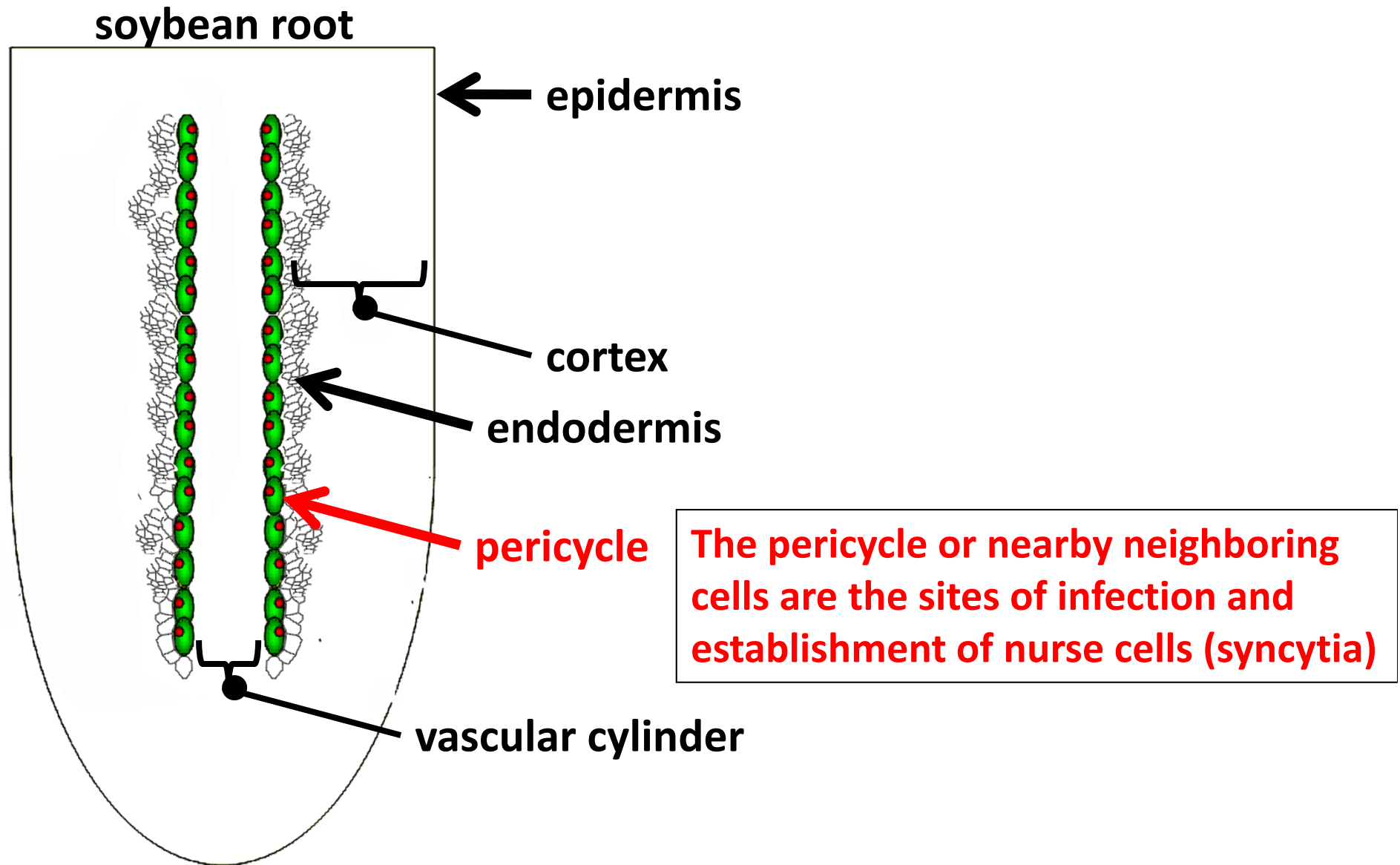


The range in the ability of soybean to resist infection suggests variability exists at the cellular level in the different genotypes as they cope with infection

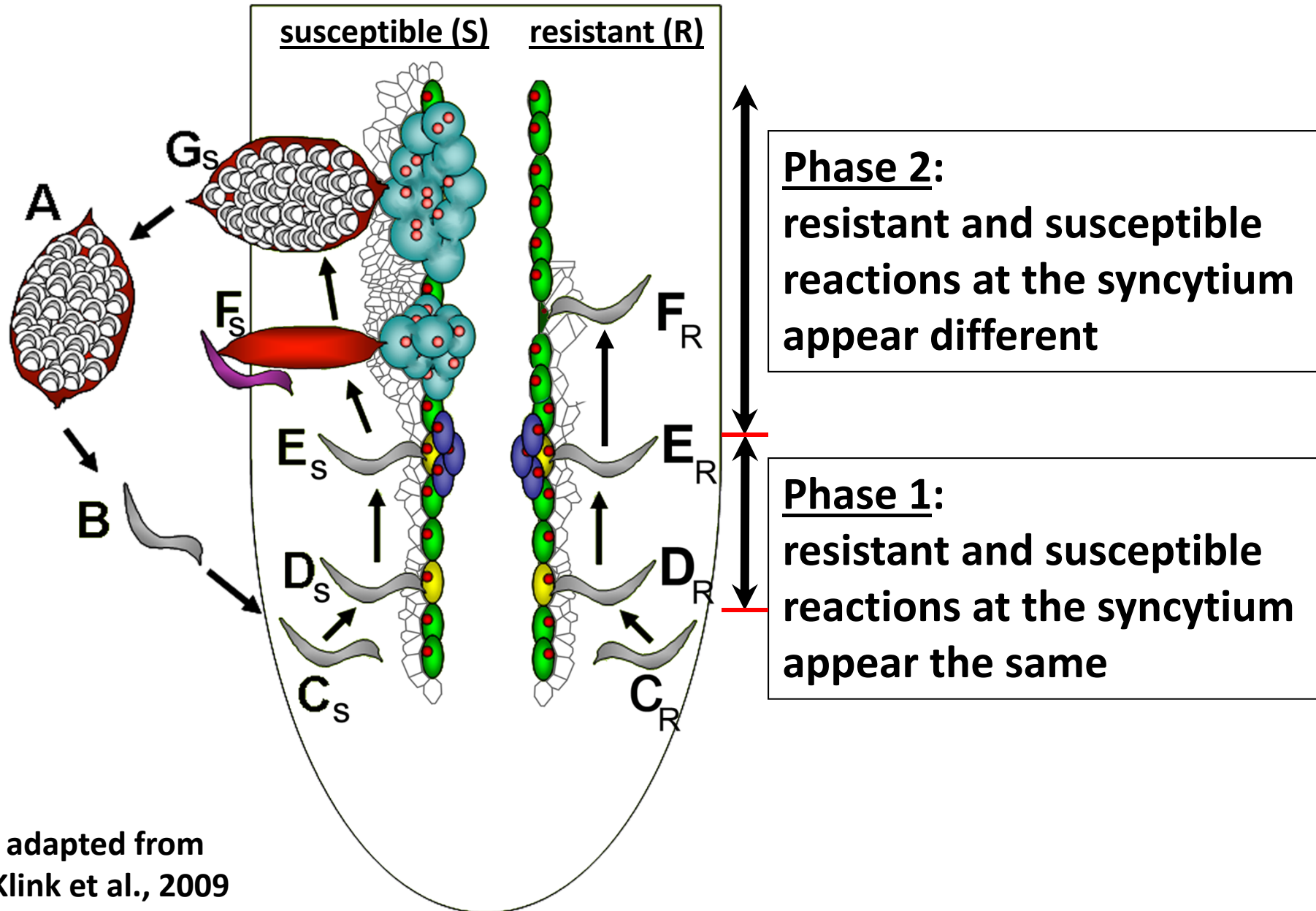


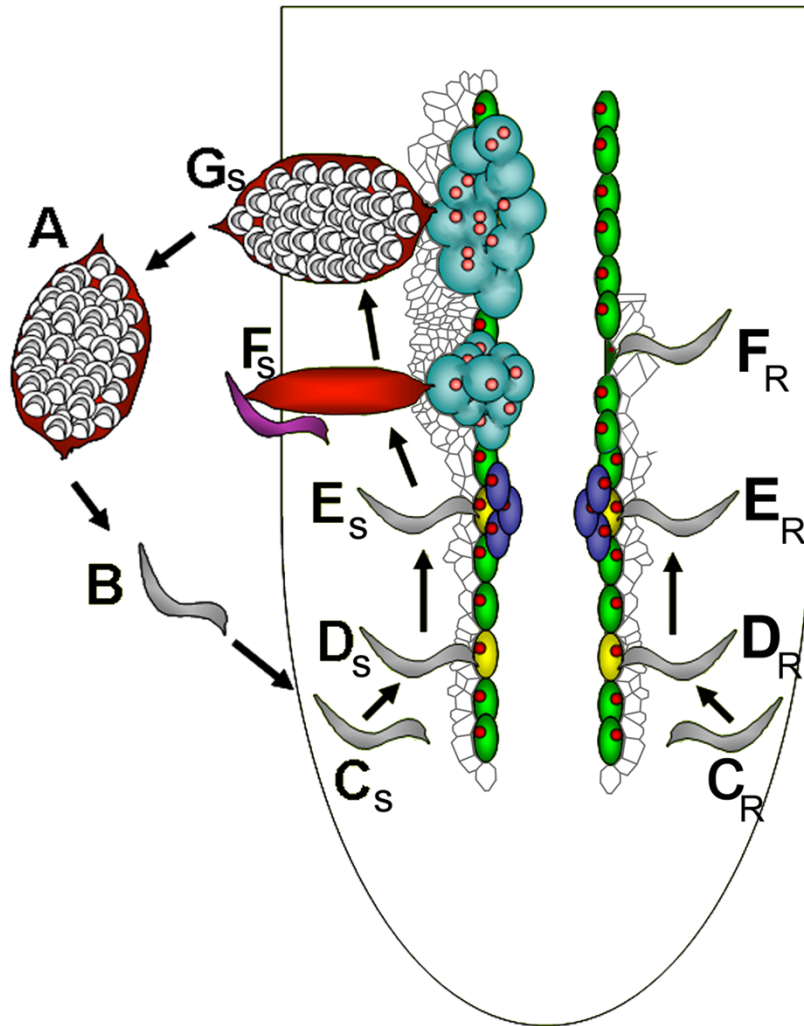
Klink et al. (2011a)

SCN infection is tightly linked to specific types of cells in the root



the resistant reaction undergoes two developmental phases





Phase 1: susceptible and resistant reactions

dissolution of cell walls

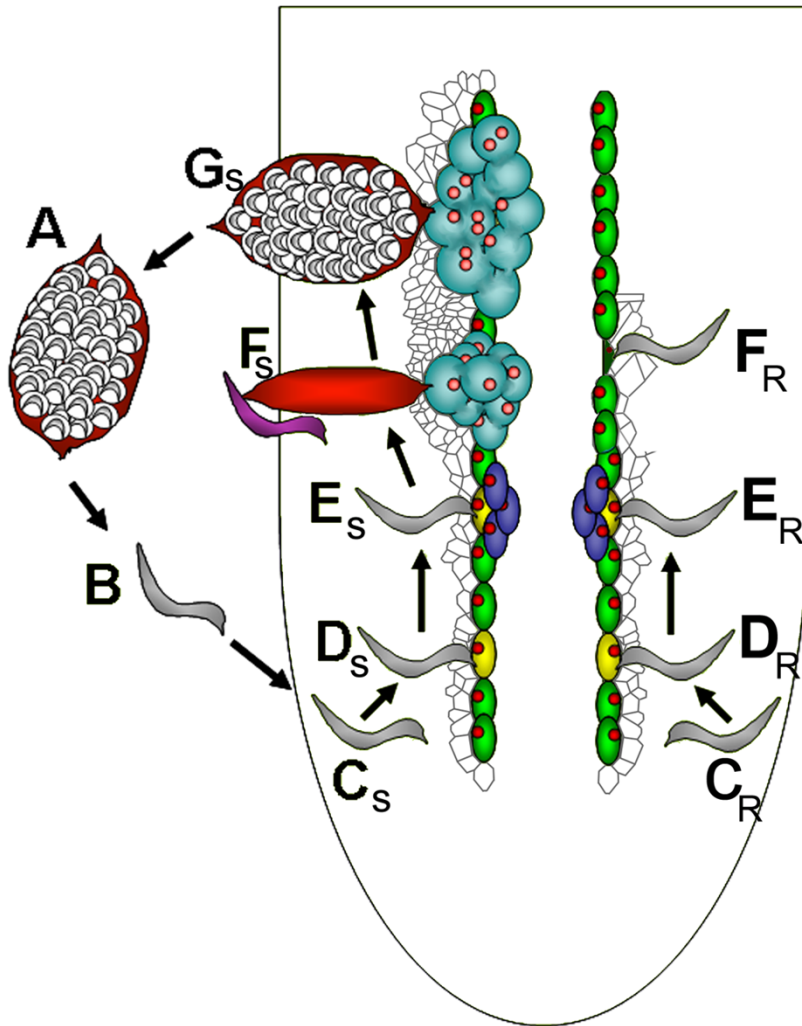
hypertrophy

an enlargement of nuclei

the development of dense cytoplasm

an increase in ER and ribosome content

Sources: Endo, 1964, 1965; Riggs et al. 1973; Kim et al. 1987; Kim and Riggs, 1992; Mahalingam and Skorpska, 1996



Phase 2: susceptible reaction

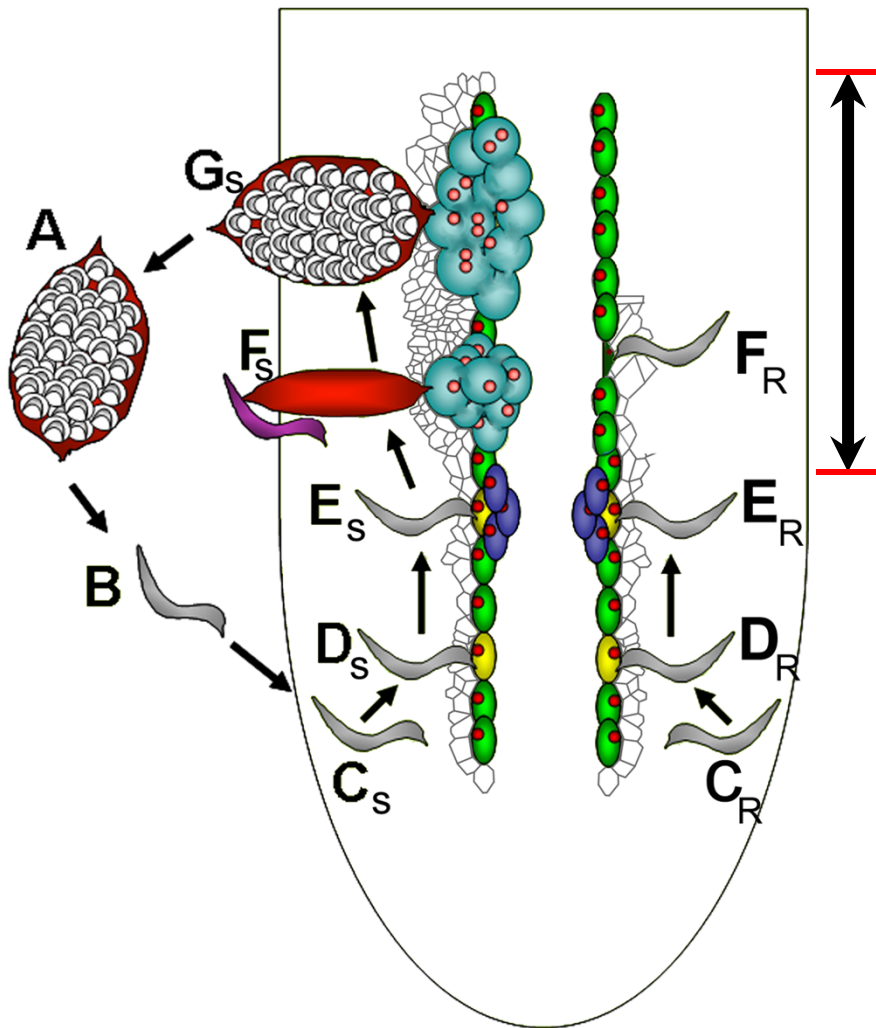
hypertrophy of nuclei and nucleoli

proliferation of cytoplasmic organelles

reduction and dissolution of the vacuole

cell expansion as it incorporates and fuses with adjacent cells

Sources: Endo and Veech 1970; Gipson et al 1971; Jones and Northcote, 1972; Riggs et al. 1973; Jones, 1981

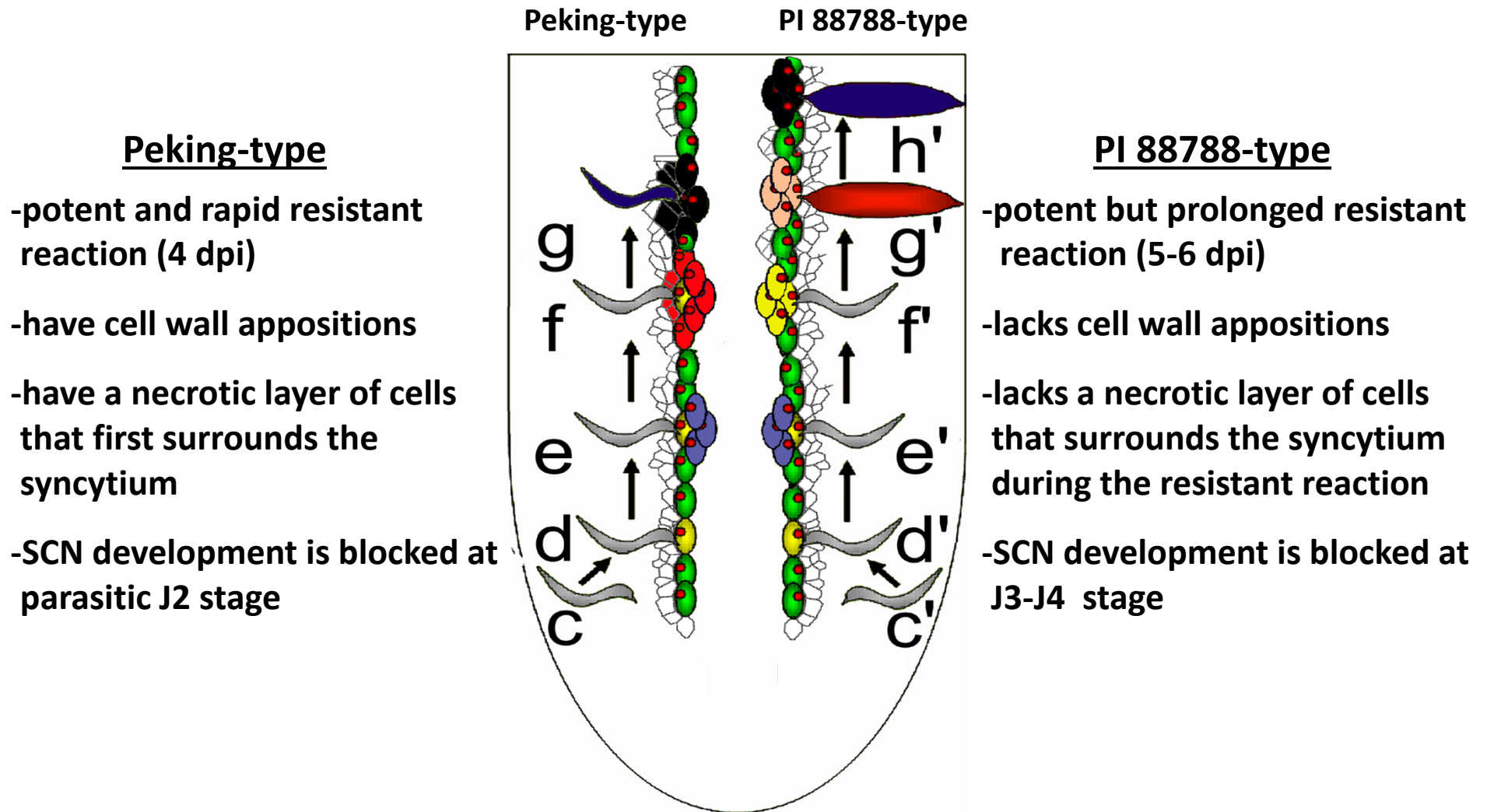


Phase 2: resistant reaction

- depends on the genotype of soybean

Sources: Endo and Veech 1970; Gipson et al 1971; Jones and Northcote, 1972; Riggs et al. 1973; Jones, 1981

From a series of cytological and ultrastructural studies, the resistant reaction was classified into two major types

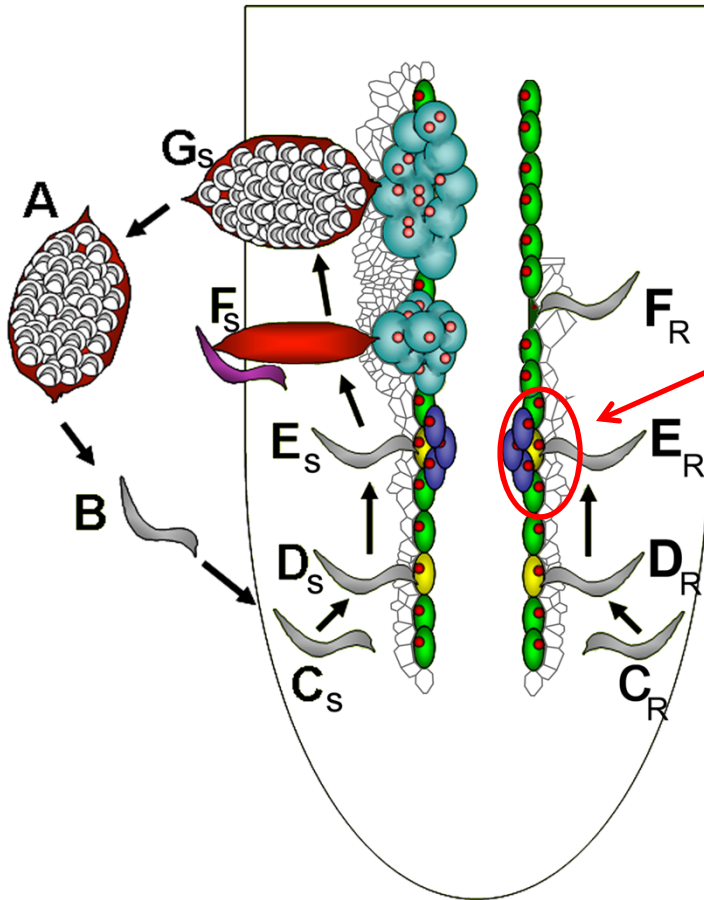


Sources: Ross, 1958; Endo 1964, 1965, 1991; Endo and Veech 1970; Gipson et al 1971; Riggs et al 1973; Kim et al, 1987; Kim and Riggs 1992; Mahalingam and Skorpska, 1996; Colgrove and Niblack et al. 2008 (adapted from Matsye et al. 2011)

(2) gene discovery

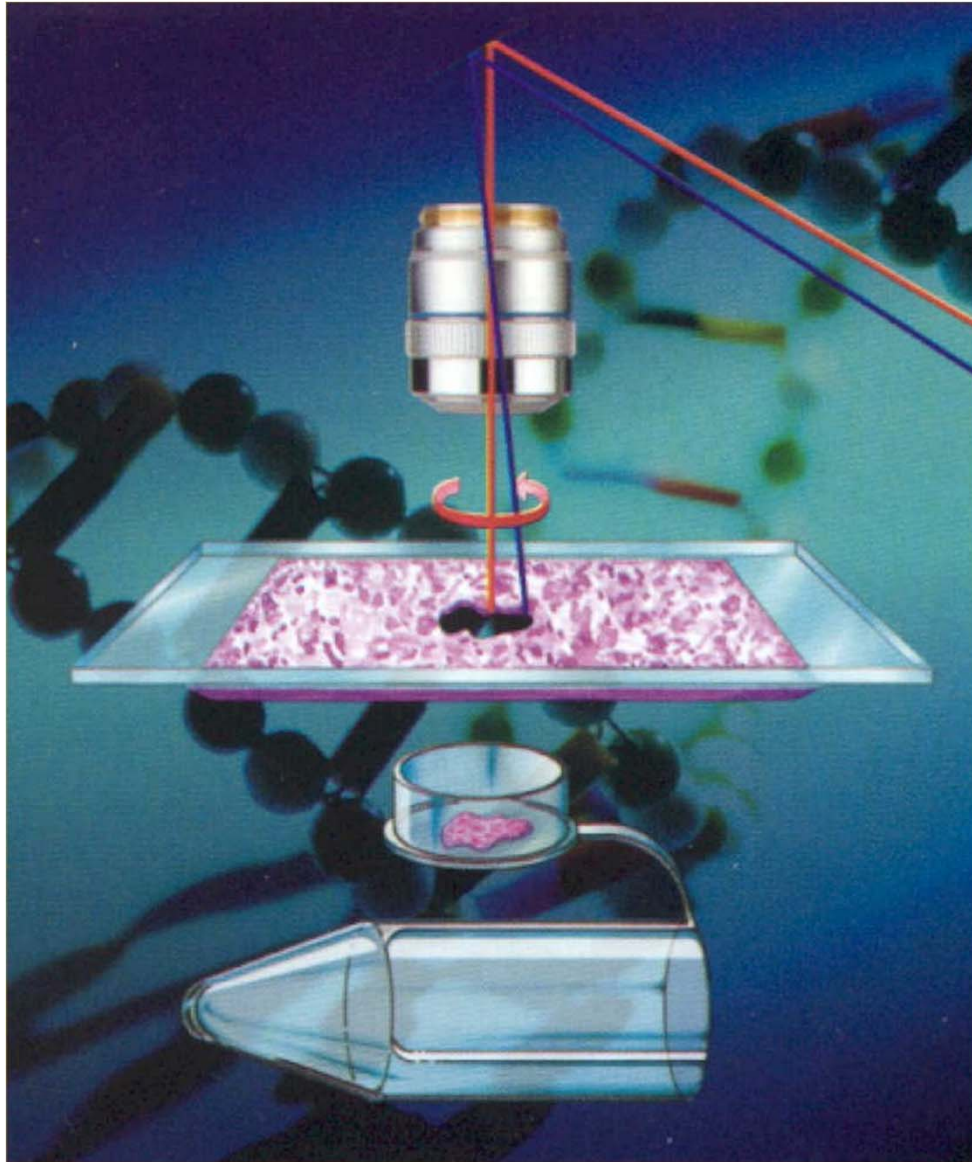
experimental approach: cell-type specific analyses

Why cell-type specific analyses?



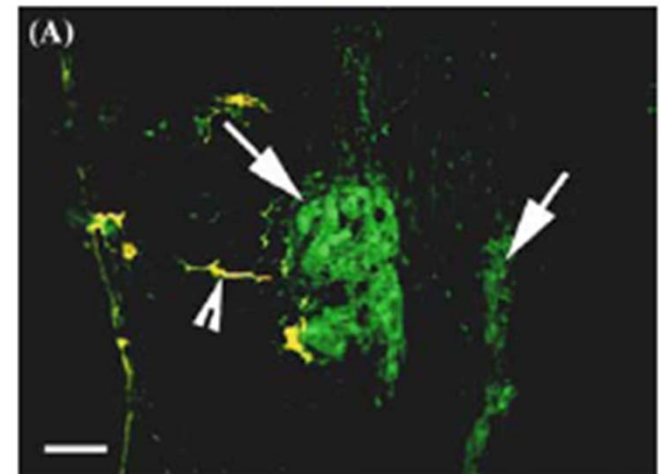
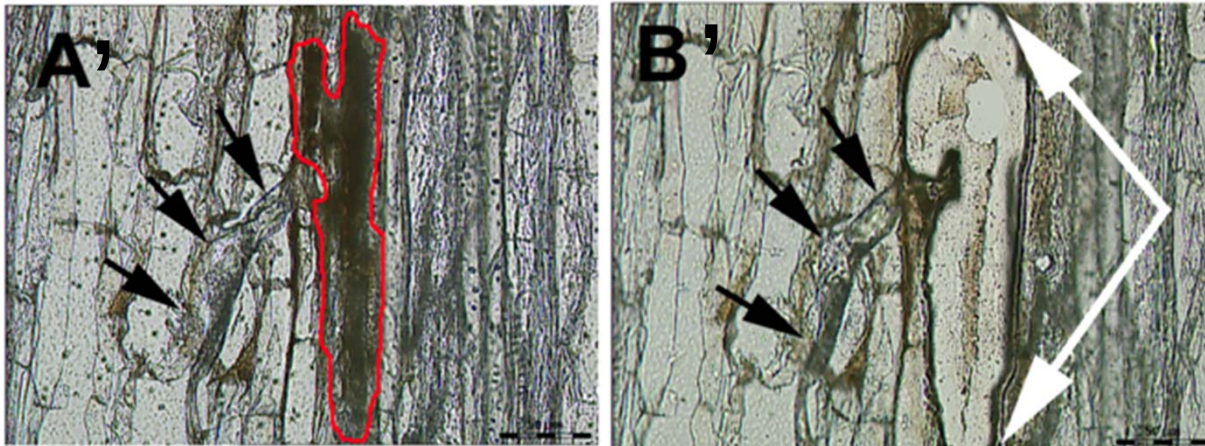
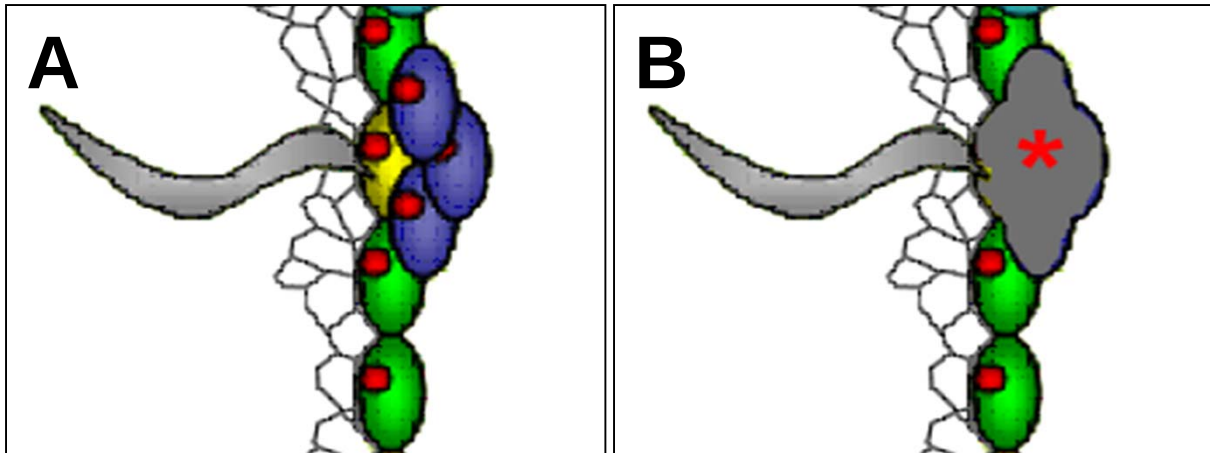
- (1) The feeding site (arrow) is the site of the resistant reaction
- (2) By collecting feeding sites, we would be concentrating the cell types undergoing the resistant or susceptible reaction
- (3) By concentrating the feeding sites, we would maximize the chances of identifying gene expression patterns that pertain to the resistant or susceptible reaction

challenge: identifying a technology to isolate syncytia



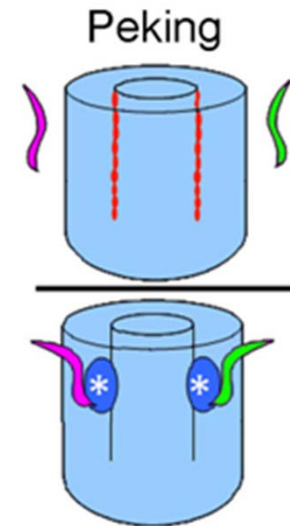
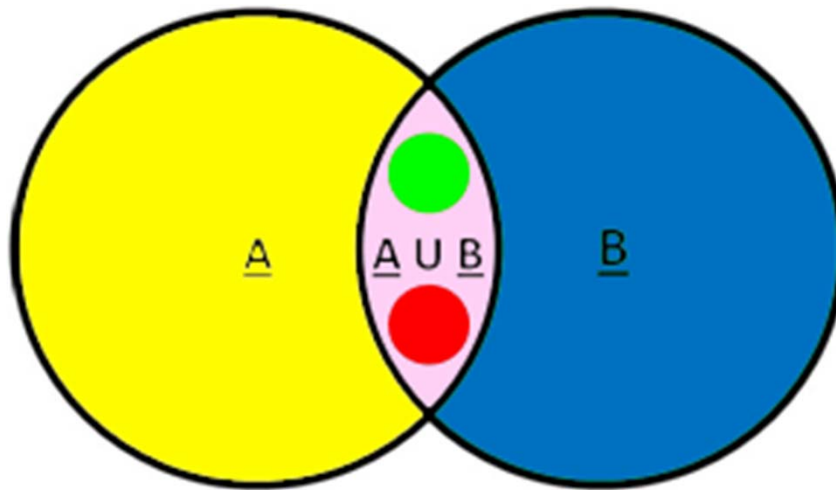
**Laser microdissection
(LM)**

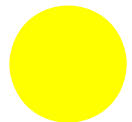
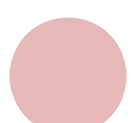
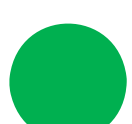
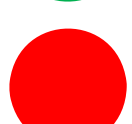
cell collection



Klink et al. 2005

gene expression studies



-  **Sample A:** present call-discarded in differential expression (DE) studies
-  **Sample B:** present call-discarded in differential expression studies
-  **A U B:** present call, considered in DE studies-not DE
-  **A U B:** present call, considered in DE studies-DE-induced
-  **A U B:** present call, considered in DE studies-DE-suppressed

Analysis approaches

(1) Intergenotype studies:

What genes are either uniquely expressed or expressed in common between the Peking and PI 88788 forms of the resistant reaction?

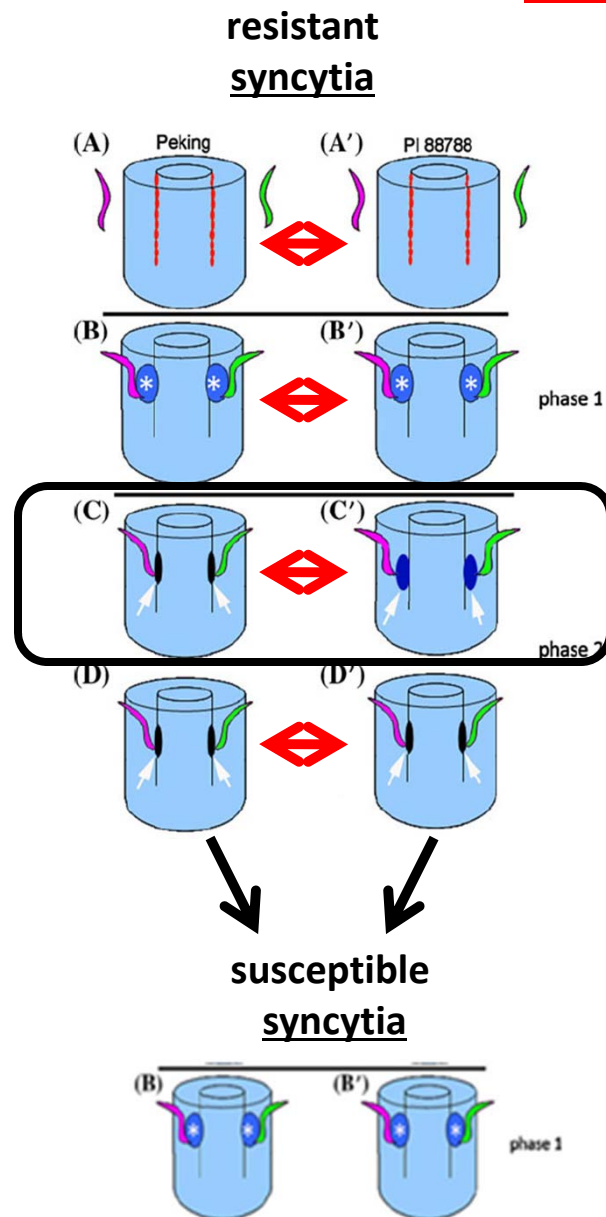
(2) Time point studies:

What genes are uniquely expressed during the resistant reaction at a specific time?

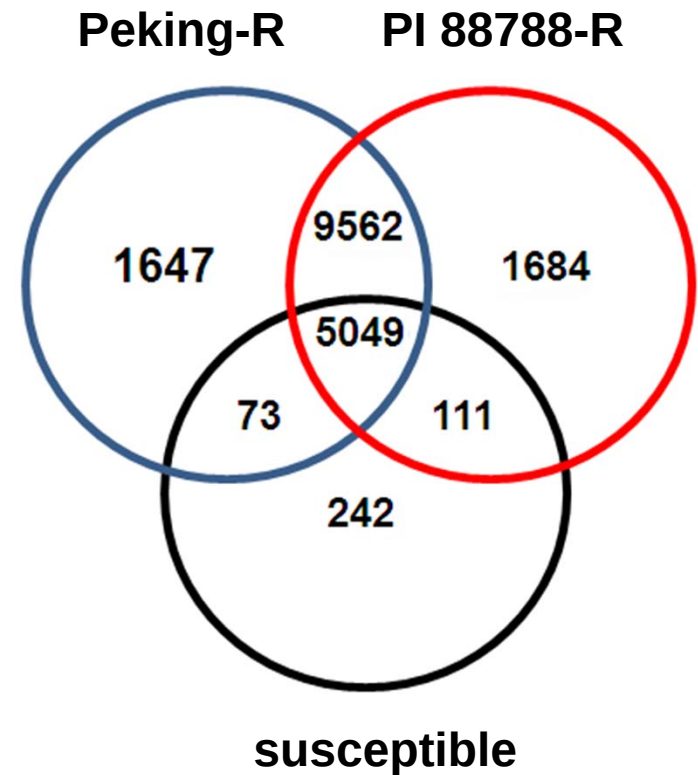
(3) Time series studies:

What genes are uniquely expressed throughout the resistant reaction?

(1) Intergenotype studies



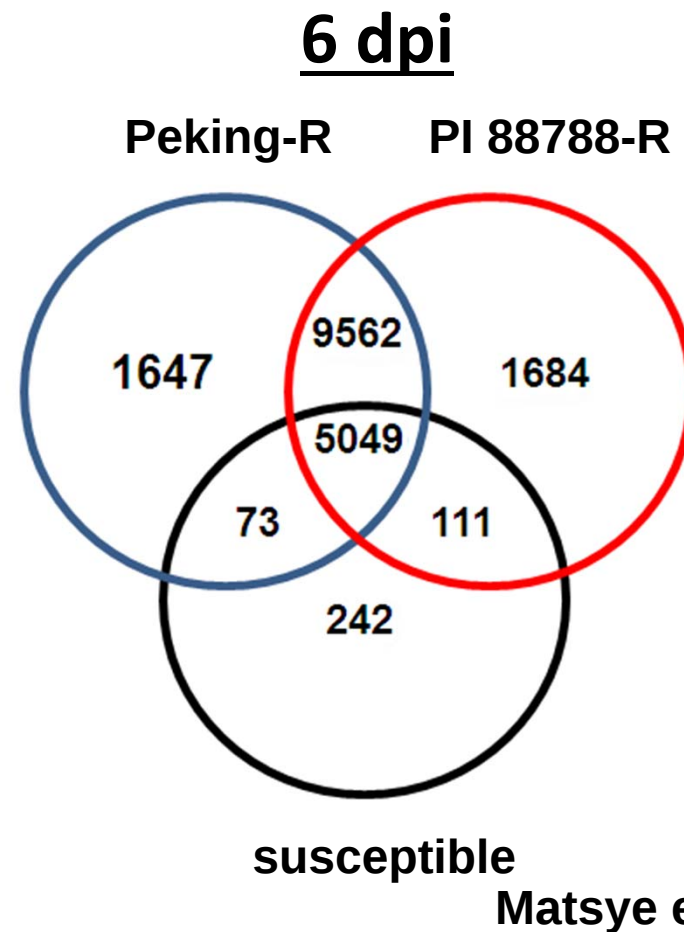
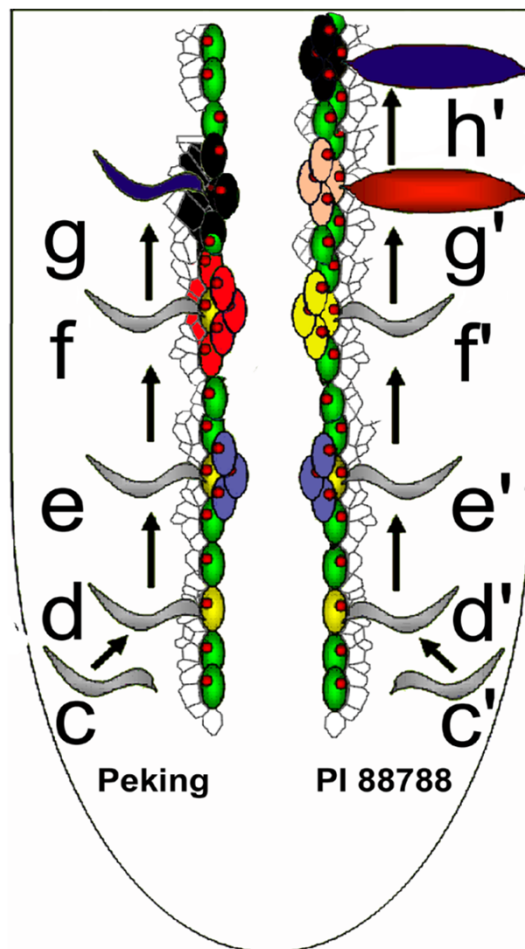
6 dpi



Matsye et al. 2011

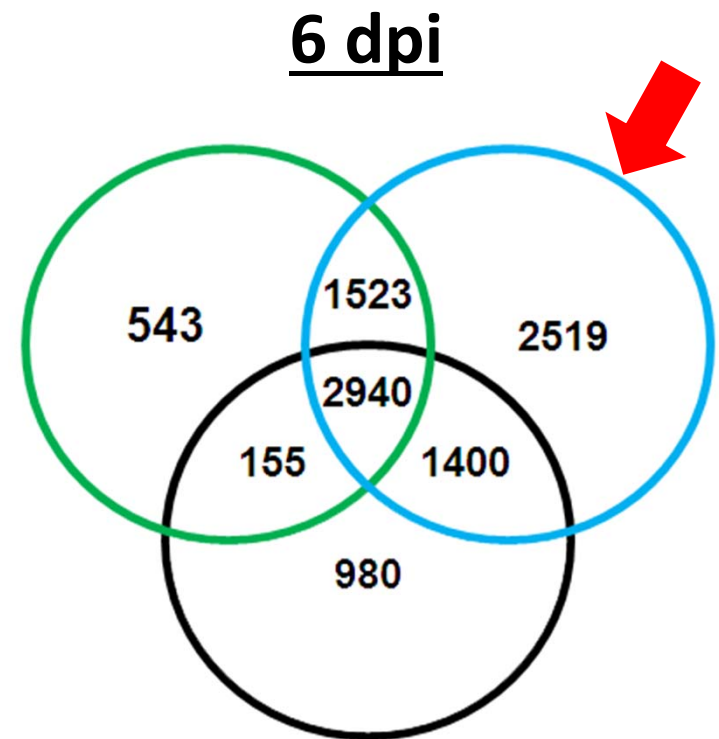
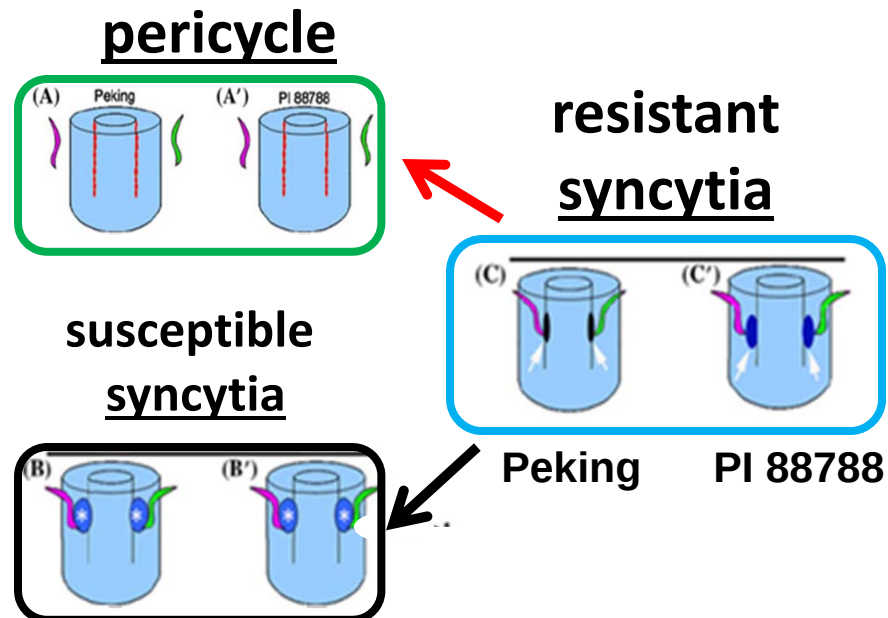
Outcome of the intergenotype studies

- The **Peking** and **PI 88788** genotypes share a common genetic program that is engaged during their defense responses.
- The **Peking** and **PI 88788** genotypes have gene expression that is occurring during their different forms of the defense response



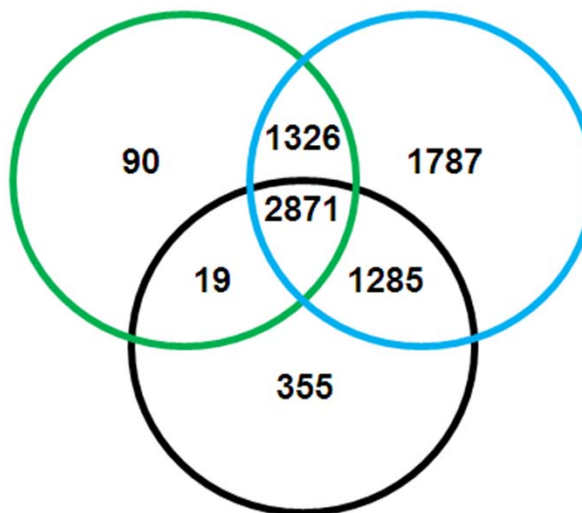
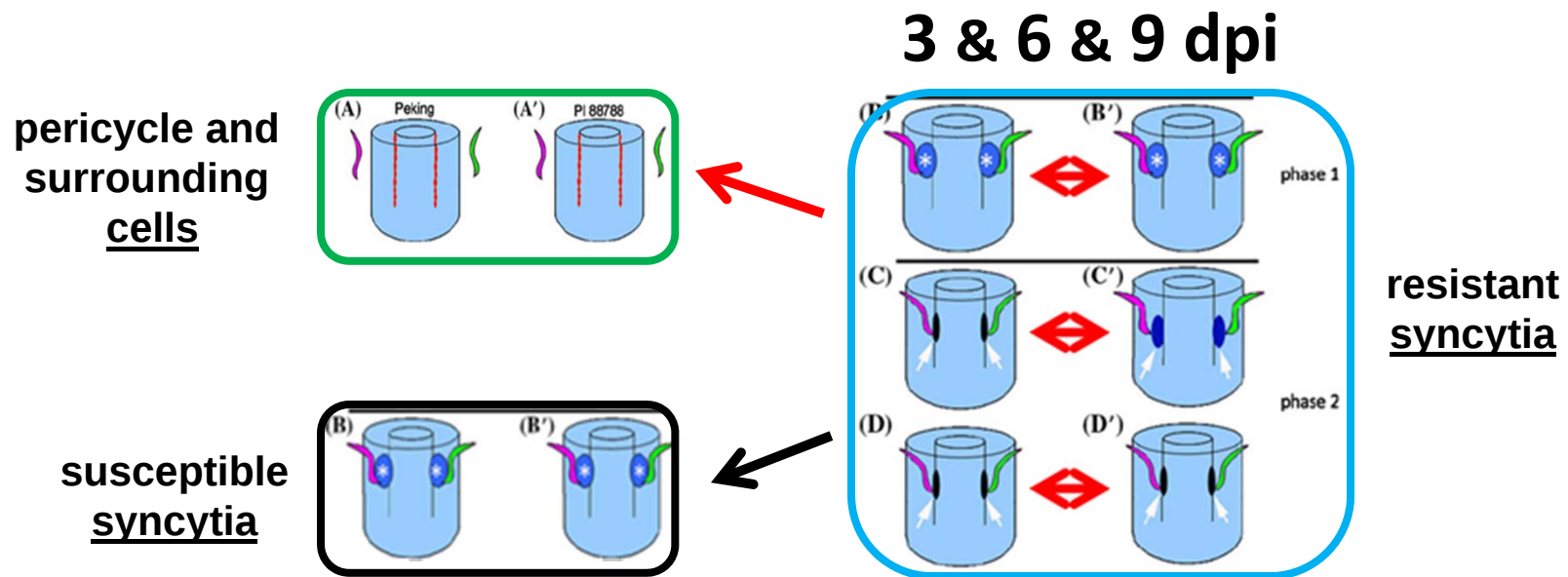
(2) Time point studies

Designed to identify genes that pertain to defense at a specific time point



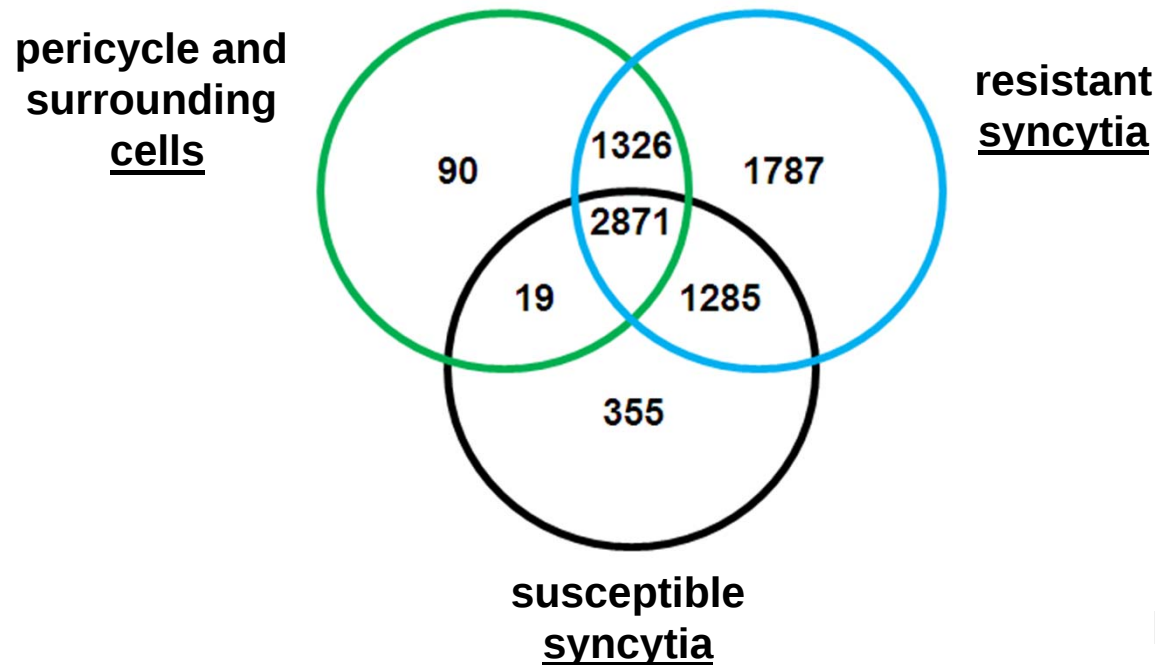
(3) Time series studies:

Designed to identify genes that always pertain to defense



Matsye et al. 2011

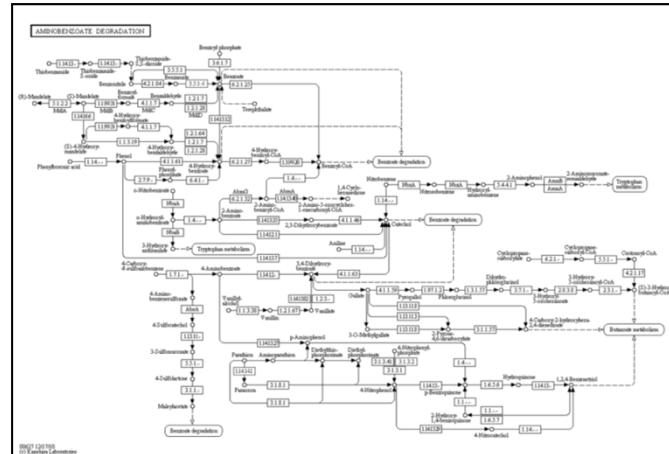
(3) Time series studies: 3 & 6 & 9 dpi



Matsye et al. 2011

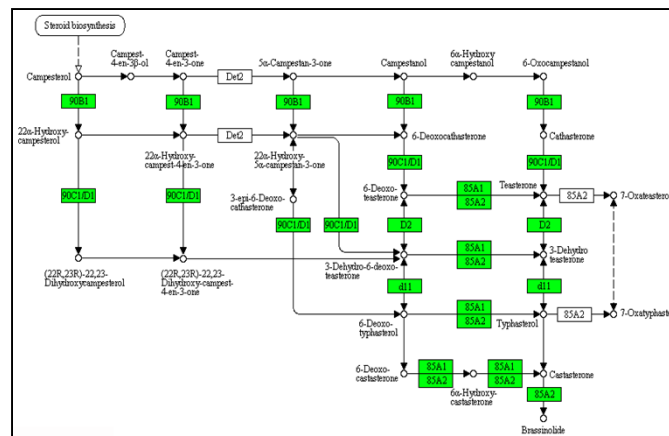
chromosomal location	Gene Annotation	Illumina: % of tags
Glyma13g06450.1	unknown	17.3967
Glyma17g07250.1	XYLOGLUCAN ENDOTRANSGLYCOSYLASE 6 (XTR6)	11.66824
Glyma14g07460.1	BOTRYTIS-INDUCED KINASE1 (BIK1)	9.966236
Glyma12g33530.1	fasciclin-like arabinogalactan protein 9.2	7.118084
Glyma03g35180.2	zinc finger (AN1-like) family protein	6.697751
Glyma08g03130.1	hua enhancer 2 (HEN2) RNA helicase IN	1.846705
Glyma05g05290.1	Bax inhibitor-like protein	1.529733
Glyma12g35990.1	Bax inhibitor-like protein	1.442451
Glyma02g11060.1	AP2/EREBP-Mediated Defense Pathway	1.120886
Glyma04g43160.1	ubiquitin-fold modifier 1-like (Ufm1)	1.070354
Glyma13g42330.1	LIPOXYGENASE 1 (LOX1)	0.243471

Kyoto Encyclopedia of Genes and Genomes (KEGG): (Goto et al. 1996)
 designed to computerize the current knowledge of metabolic and
 regulatory pathways. KEGG includes a plant database (Masoudi-
 Nejad et al. 2008)



**aminobenzoate
 degradation
 pathway**

Pathway Analysis and Integrated Coloring of Experiments (PAICE):
 (Hoseini et al. unpublished; Matsye et al. 2011).



**steroid
 biosynthesis
 pathway**

Specific biochemical pathways are active in specific cell types during the defense response

PAICE Pathway	3 dpi	6 dpi	9 dpi	PAICE Pathway	3 dpi	6 dpi	9 dpi
Alanine, aspartate and glutamate metabolism				Cysteine and methionine metabolism			

pericycle and
surrounding
cells

syncytia-R

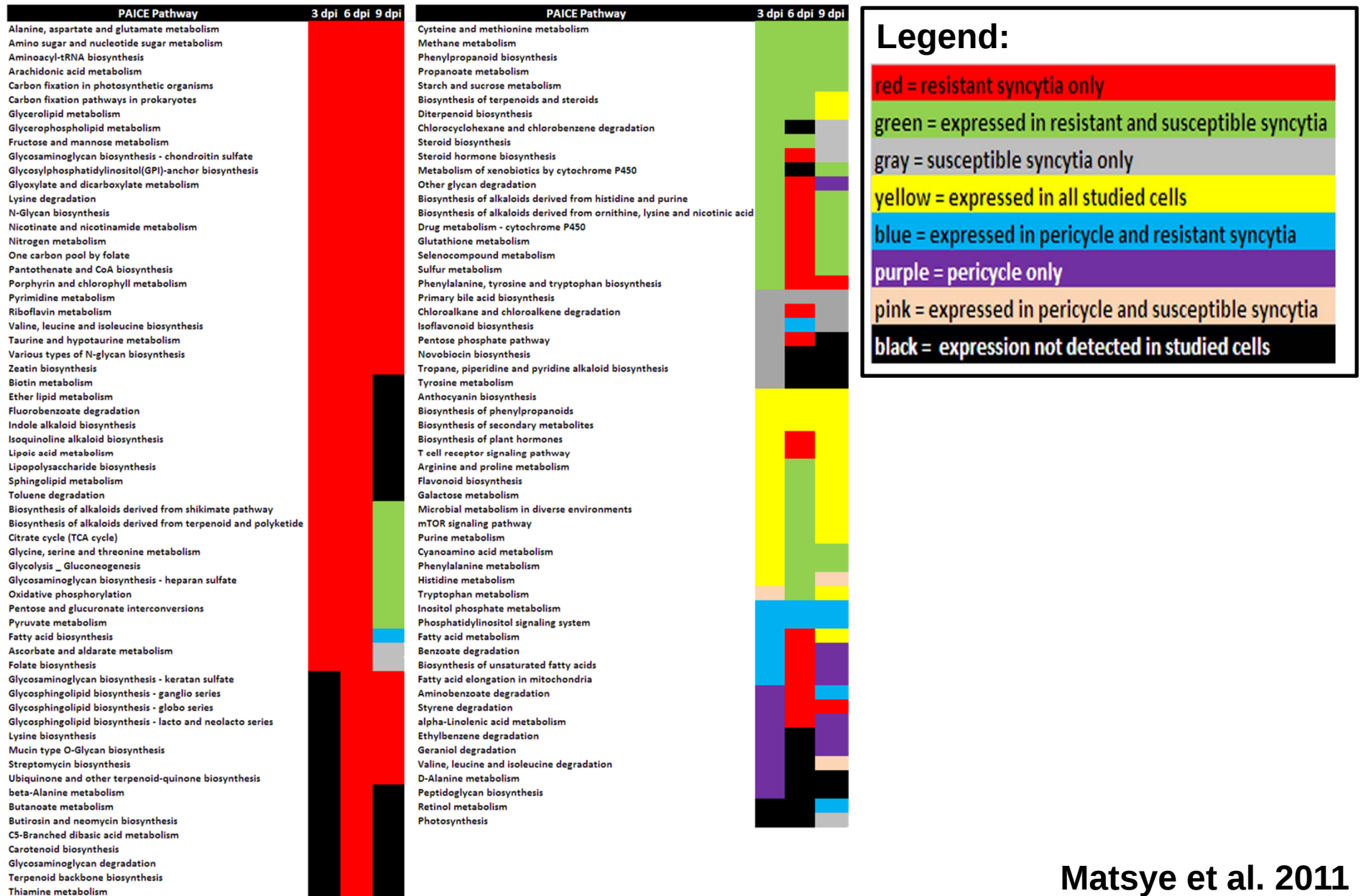


susceptible

Legend:

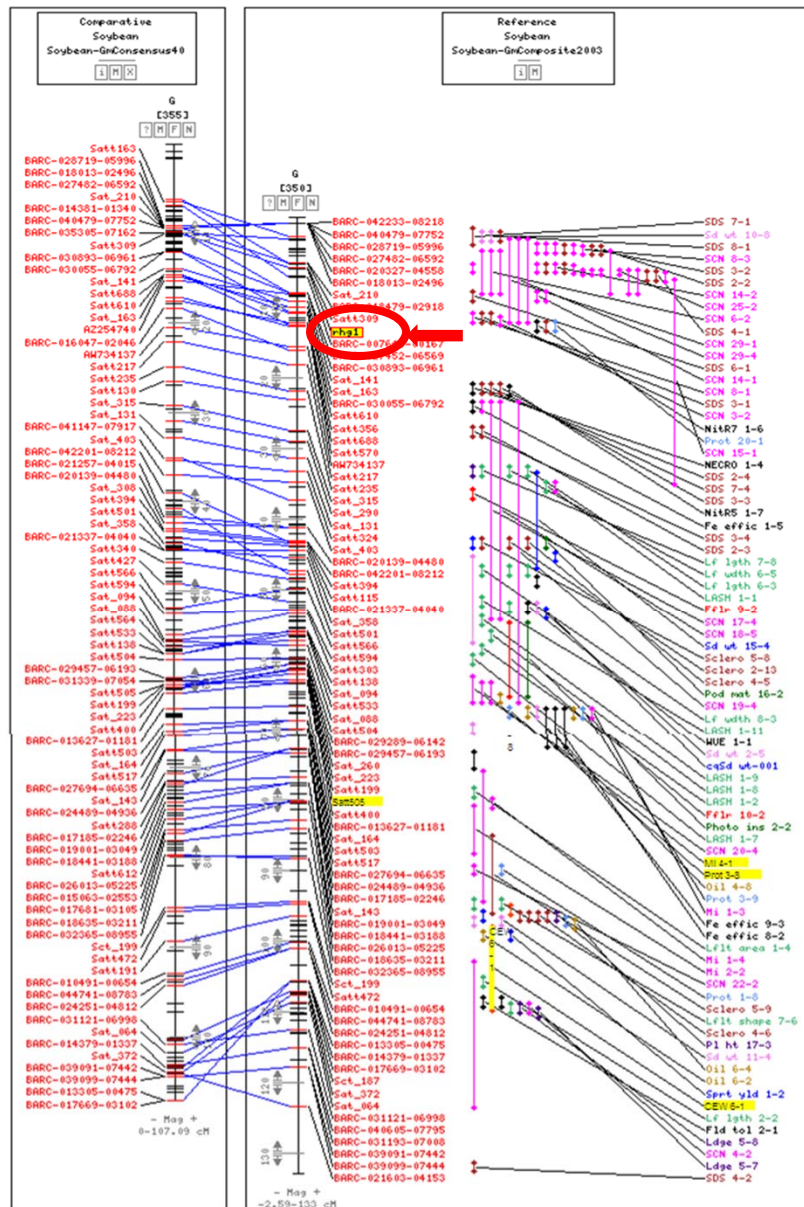
red = resistant syncytia only
green = expressed in resistant and susceptible syncytia
gray = susceptible syncytia only
yellow = expressed in all studied cells
blue = expressed in pericycle and resistant syncytia
purple = pericycle only
pink = expressed in pericycle and susceptible syncytia
black = expression not detected in studied cells

metabolic activity of SCN infection

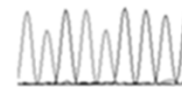


Matsye et al. 2011

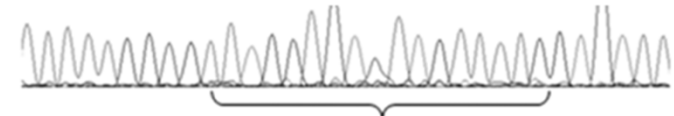
(3) cell genotyping



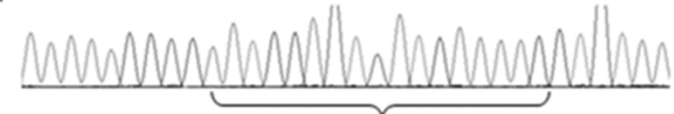
identification of genetic elements



Peking cDNA



PI 437654 cDNA



Matsye et al. submitted

Gene expression occurring at the *rhg1-b* locus in both Peking and PI 88788 as compared to pericycle

	(3 dpi)			(6 dpi)			(9 dpi)			(3/6/9 dpi)		
	<u>P</u>	<u>C</u>	<u>S</u>	<u>P</u>	<u>C</u>	<u>S</u>	<u>P</u>	<u>C</u>	<u>S</u>	<u>P</u>	<u>C</u>	<u>S</u>
trehalose-6 phosphate												
myo-inositol-3-phosphate synthase (MIPS)												
N/A												
short chain dehydrogenase/reductase												
conserved protein												
GAMMA-GLUTAMYL TRANSPEPTIDASE 1												
amino acid transporter	○	○	●	○	○	●	○	○	●	○	○	●
alpha-soluble NSF attachment protein	○	○	●	○	○	●	○	○	●	○	○	●
conserved protein												
ATP-dependent rRNA helicase spb4												
hydrolase												
ferritin												
N/A												
eukaryotic translation initiation factor 2												
pollen Ole e 1 allergen and extensin family												
conserved protein												
ribosomal protein L10a												
N/A												

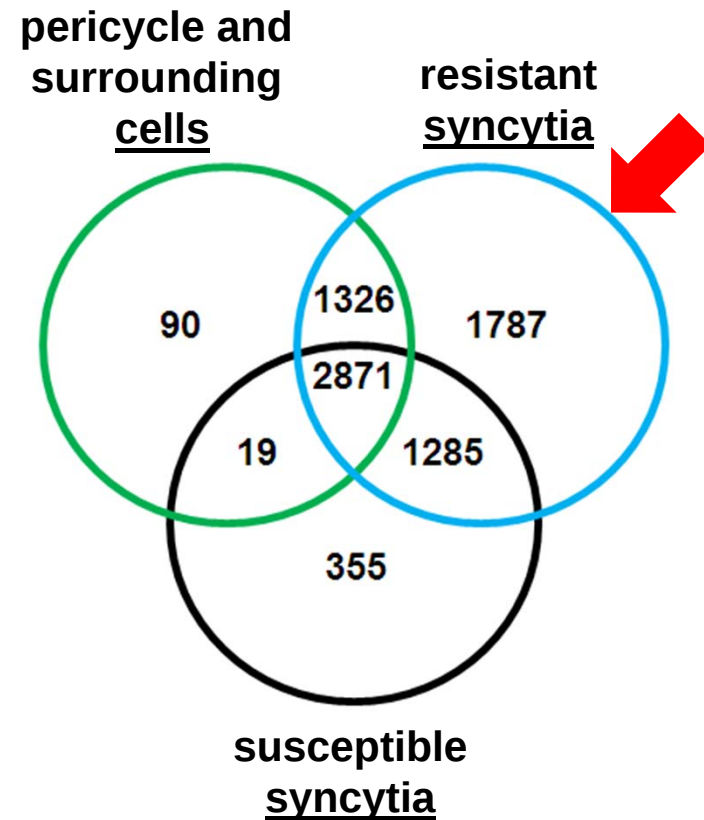
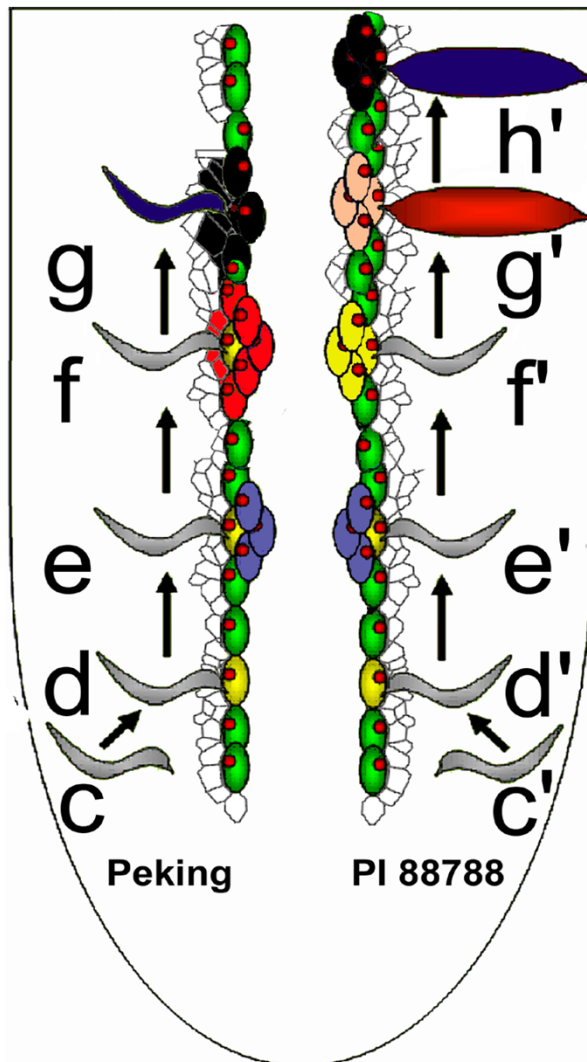
LEGEND	
○	not present
●	syncytium
●	pericycle
●	common
●	resistant syncytium (only)

Expression confirmed by Illumina® deep sequencing

Matsye et al. 2011

outcome of the time point studies

- The gene expression that is common to **Peking** and **PI 88788** is also occurring specifically during the defense response.
- Some of the genes are expressed throughout the defense response



(4) functional studies

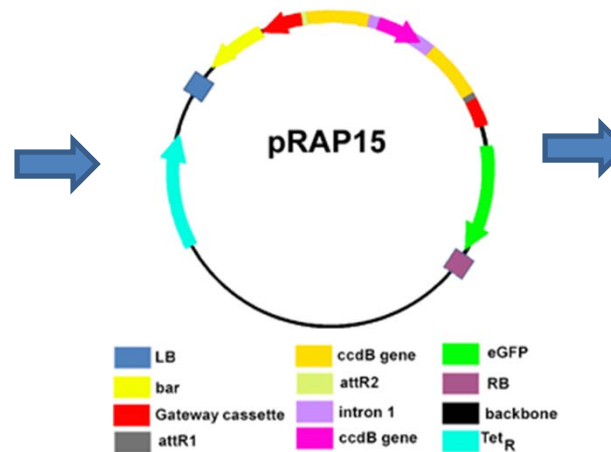


A. rhizogenes-mediated soybean transformation method

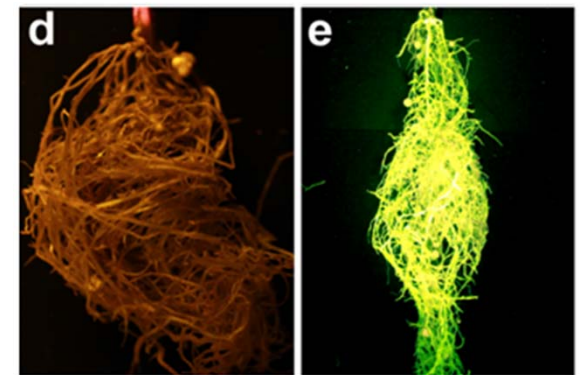
grow plants



develop expression vectors

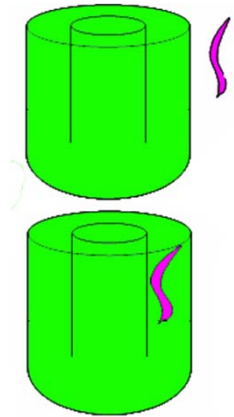


transgenic plants



susceptible soybean genotype that is genetically engineered is tested for resistance to SCN

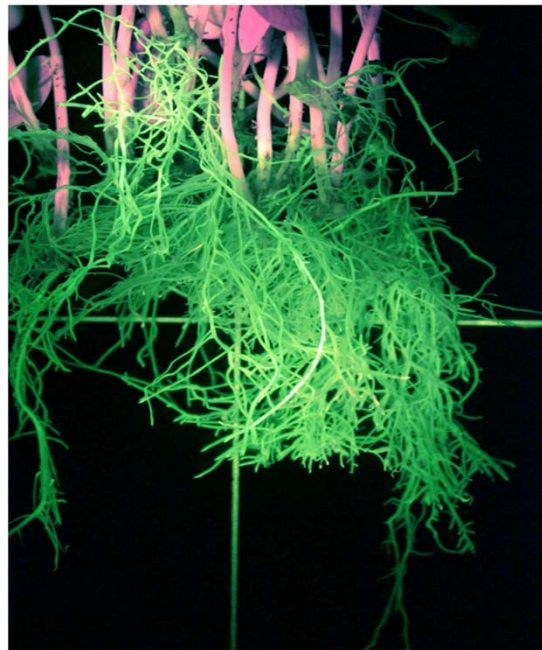
in planta
expression/
nematode
inoculation



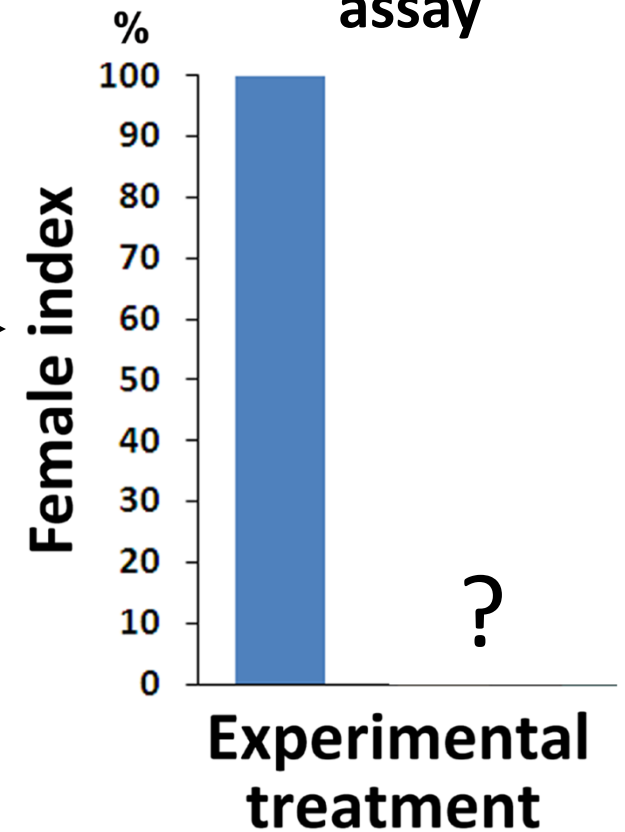
nematode
feeding



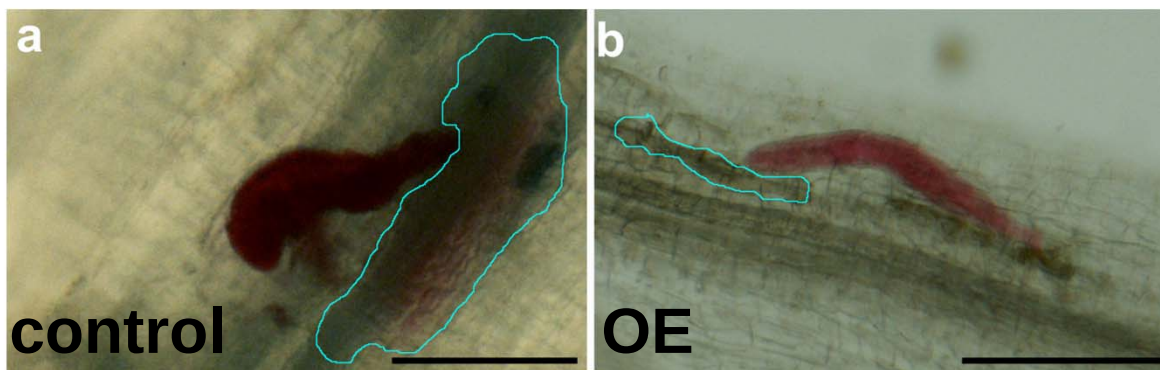
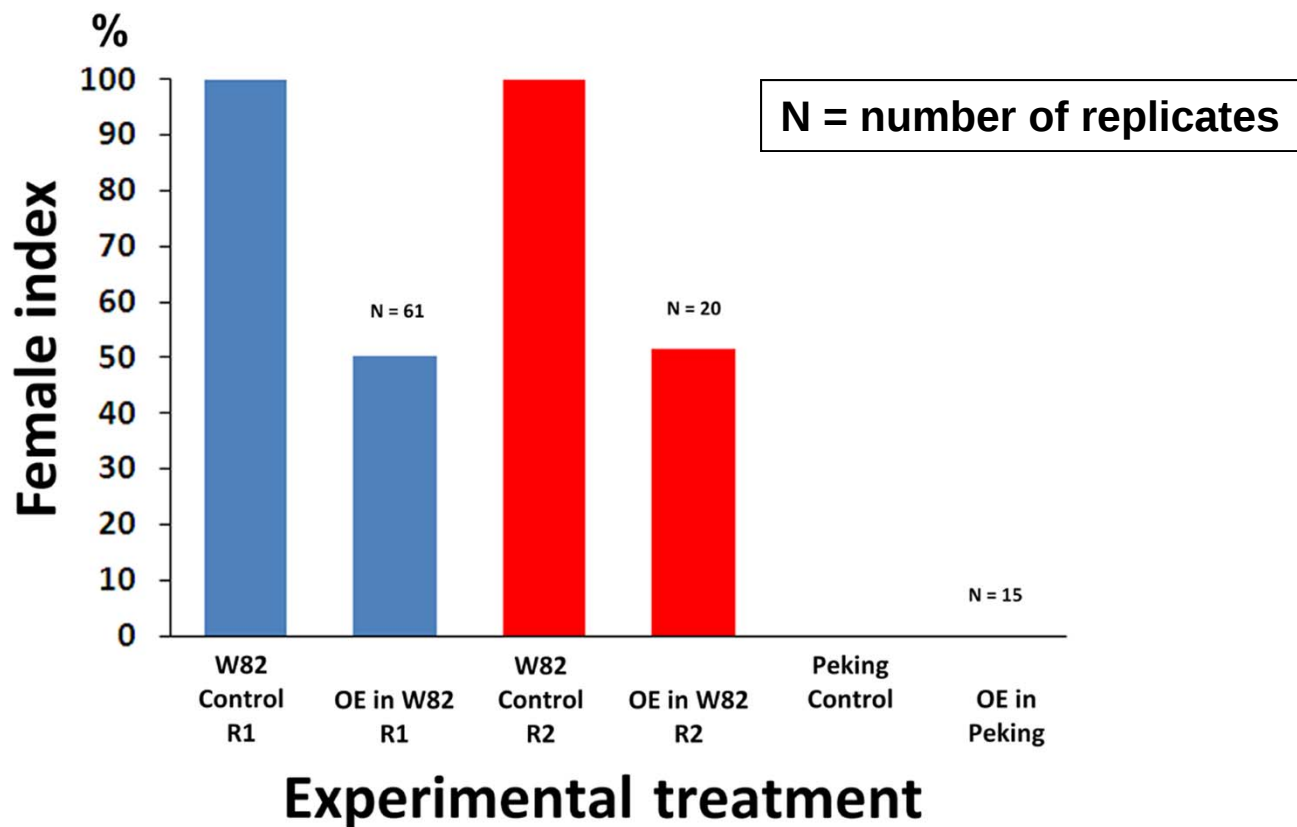
infect for 30 days



nematode
assay



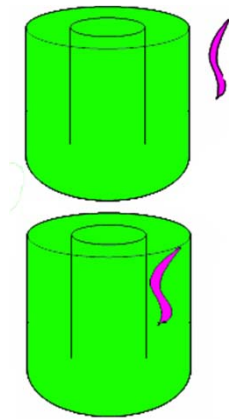
Susceptible soybean genotype that is genetically engineered can suppress infection by SCN



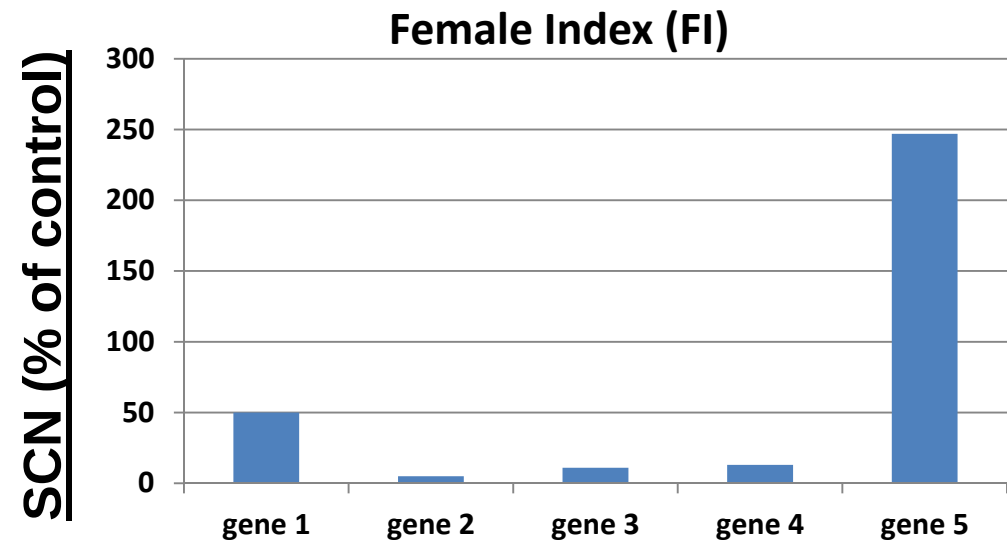
Matsye et al.
submitted

reverse genetic screens are revealing that it is possible to make a susceptible soybean genotype resistant to SCN

in planta
expression/
nematode
inoculation



nematode
feeding



(5) cotton pipeline

***A. rhizogenes*-mediated cotton transformation method:**
(1) collecting cotton



***A. rhizogenes*-mediated cotton transformation method:**
(2) trimming cotton roots



***A. rhizogenes*-mediated cotton transformation method:**
(3) placing cotton in with *A. rhizogenes*



***A. rhizogenes*-mediated cotton transformation method:**
(4) cocultivating cotton with *A. rhizogenes*



***A. rhizogenes*-mediated cotton transformation method:**
(5) vacume infiltration of cotton with *A. rhizogenes*



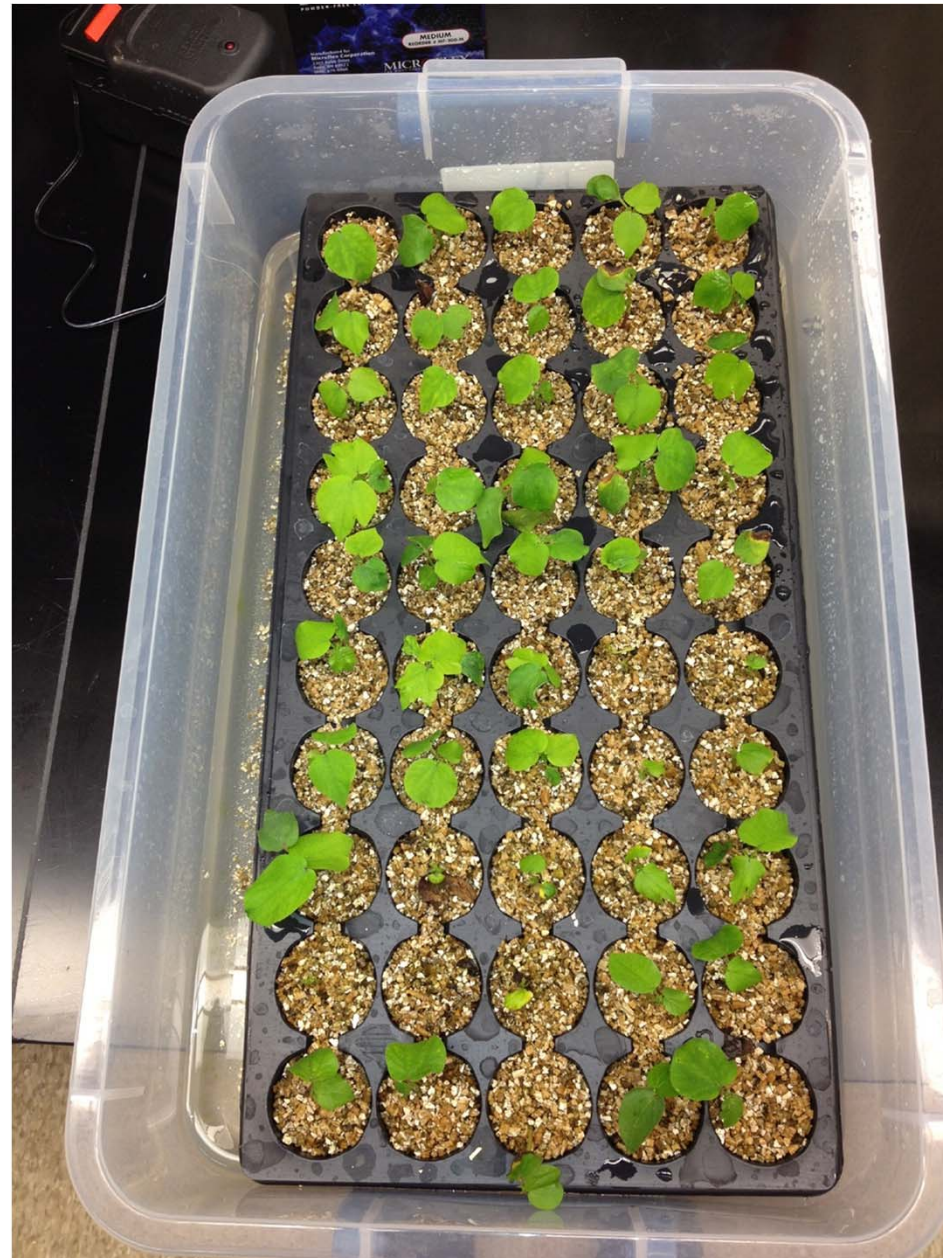
A. rhizogenes-mediated cotton transformation method:
(6) Incubate cotton at 28 degrees C.



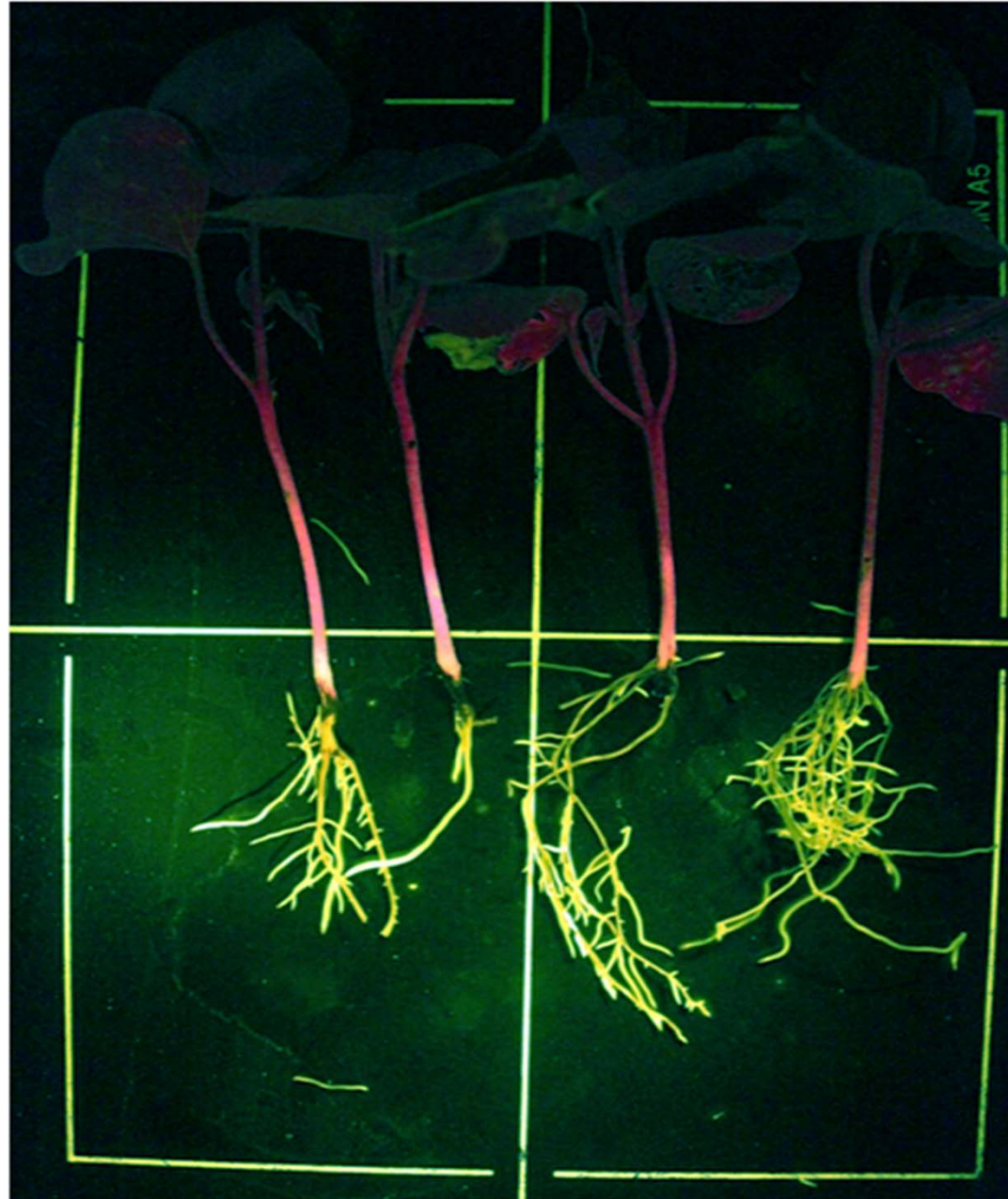
***A. rhizogenes*-mediated cotton transformation method:**
(7) culture cotton under lights



***A. rhizogenes*-mediated cotton transformation method:**
(8) cotton after two weeks

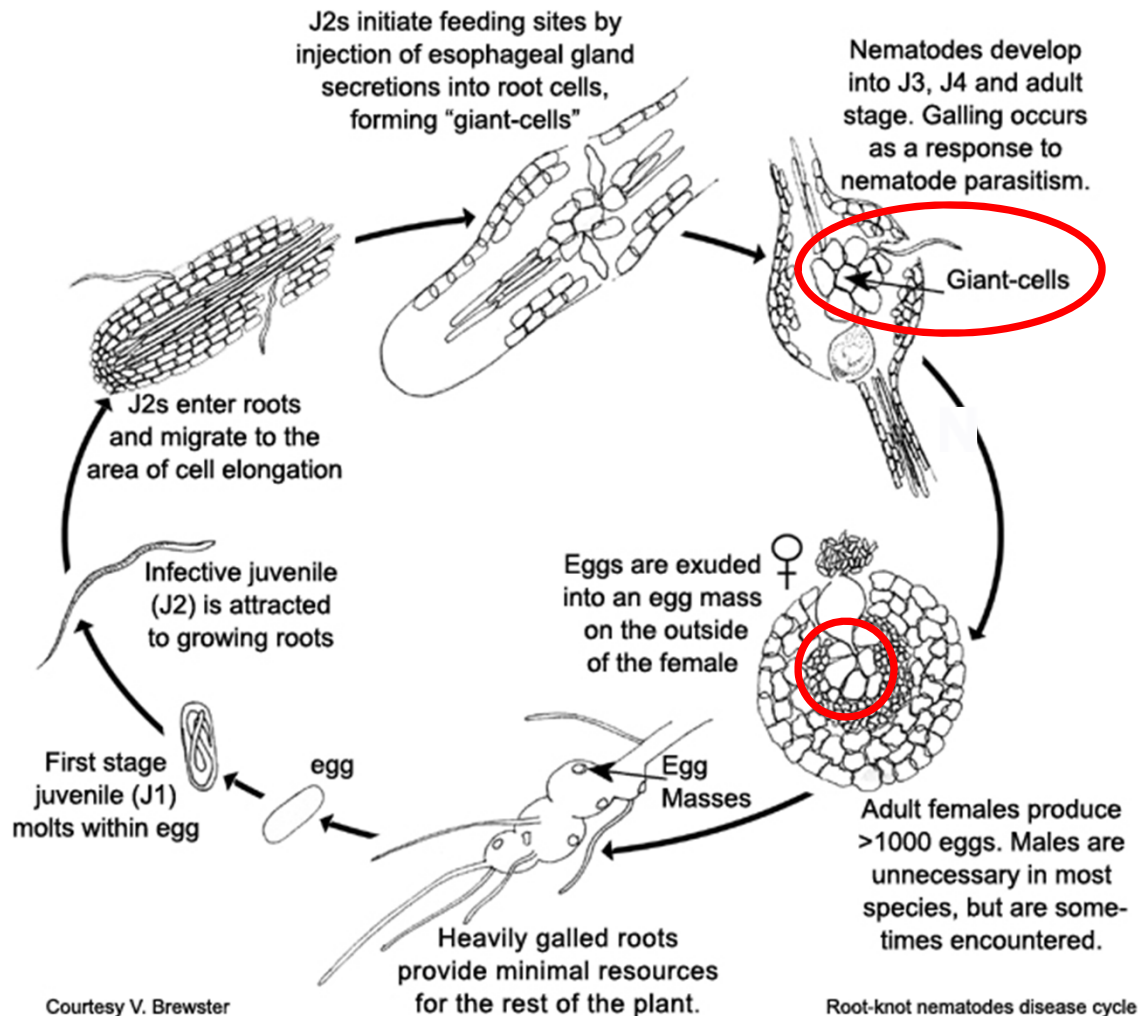


A. rhizogenes-mediated cotton transformation method:
(9) transgenic cotton

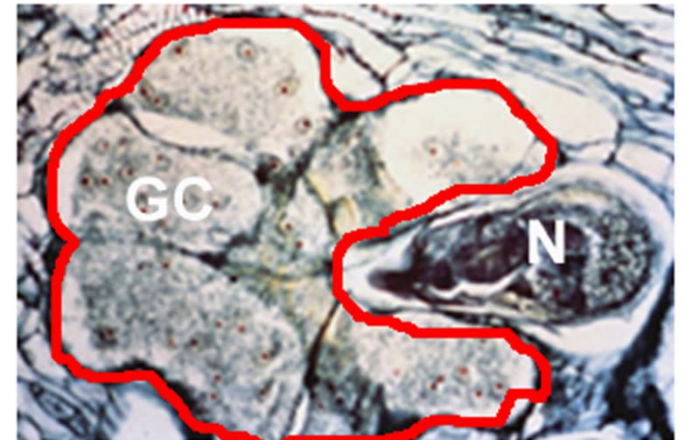


cotton pipeline: cell collection

Root knot nematode life cycle



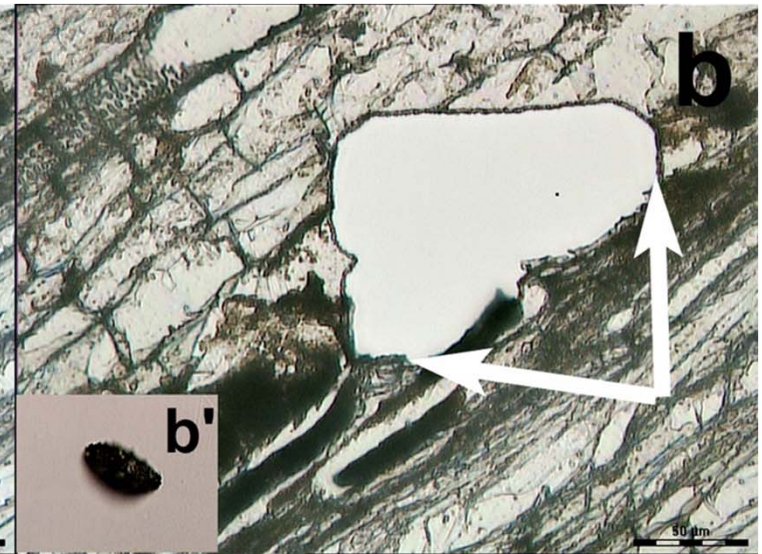
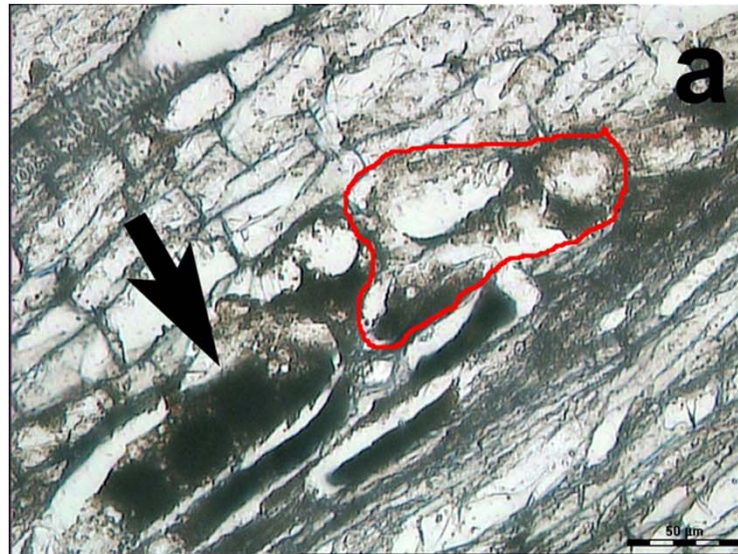
giant cells made by RKN



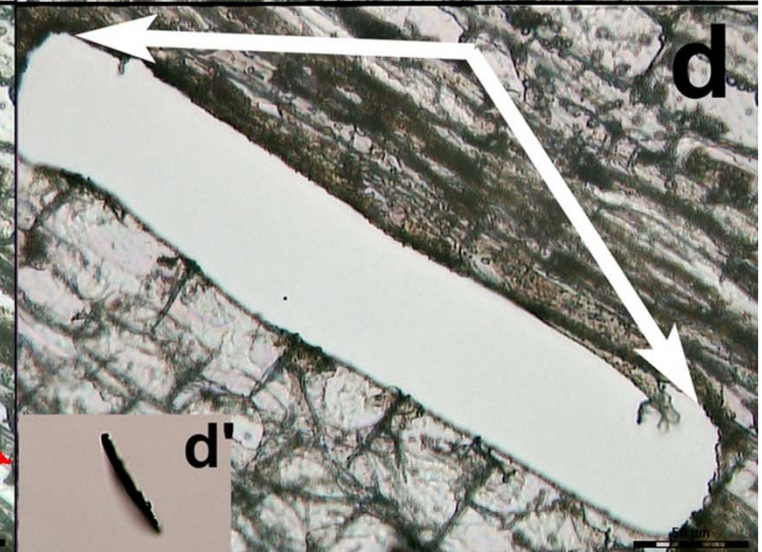
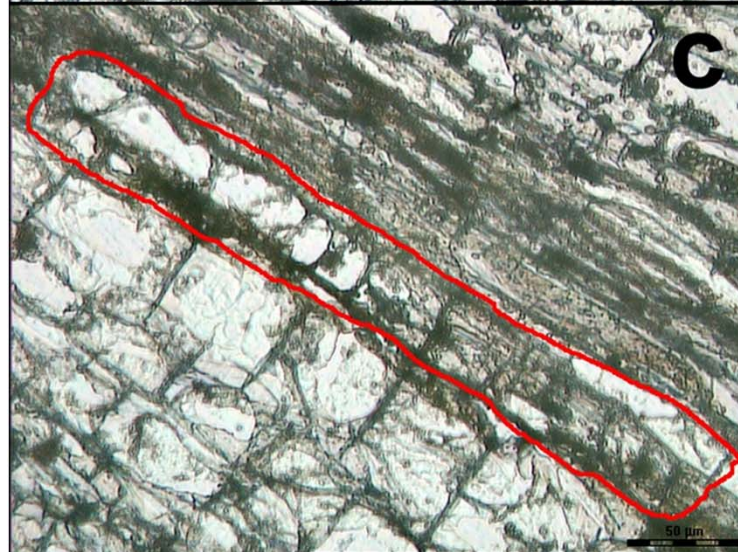
before LM

after LM

RKN-infected

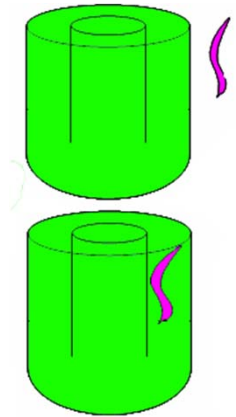


control cells



After gene expression studies, genetic engineering procedure is used to test gene function for resistance to RKN

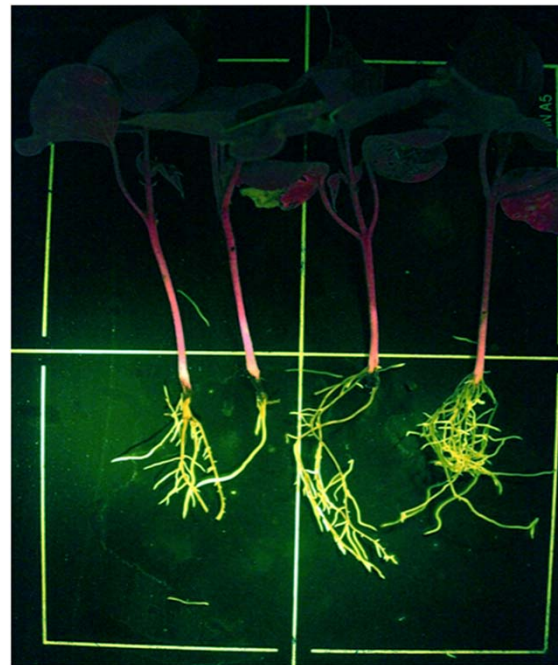
in planta
expression/
nematode
inoculation



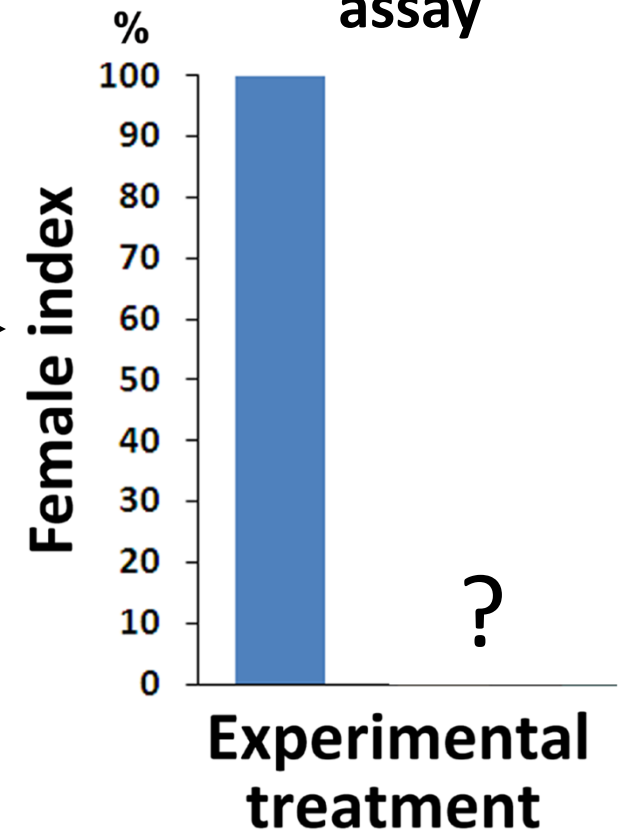
nematode
feeding



infect



nematode
assay



conclusions

The natural genetic variation in both plant and nematodes presents a major challenge for their control.

At the same time, the variation provides a substantial genetic toolbox because the genetic tools are in hand to manipulate it.

Annotation of gene expression occurring in the different cell types, makes it is possible to understand the mechanism of successful and failed parasitism.

That knowledge then can be used to engineer resistance that relates to the different plant genotypes of interest.



Left to right: Prateek Chaudhuary, Prachi Matsye, Kim Anderson, Ngoc Pham, Suchit Salian, Adrienne McMorris



Left to Right: Chris Jones, Kurt Showmaker, Christina Jones, Prachi Matsye, Patrick Garrett, Gary Lawrence, Brad Birch.