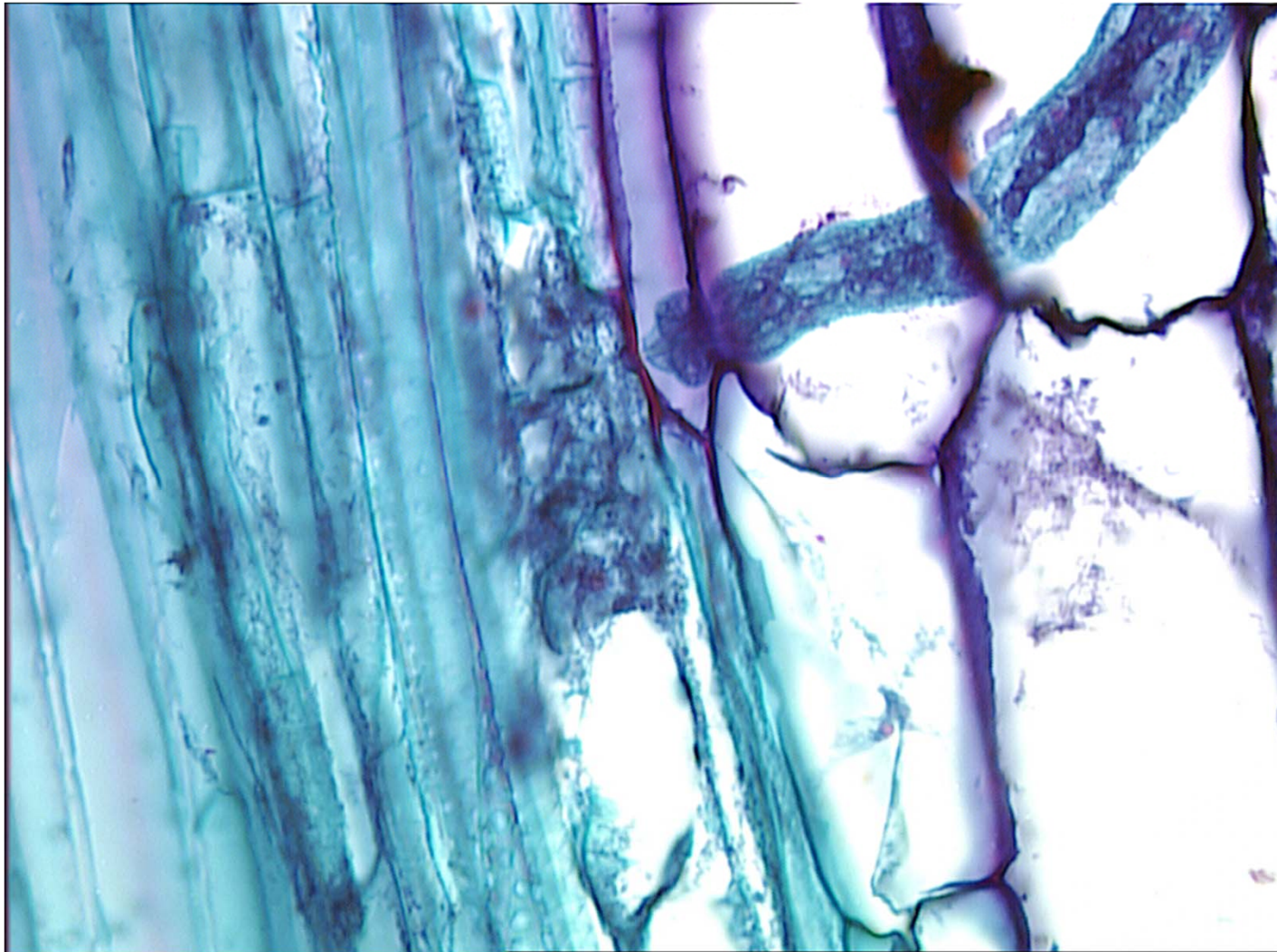


# **Identification of Cotton Genes Conferring Resistance against RKN**

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**

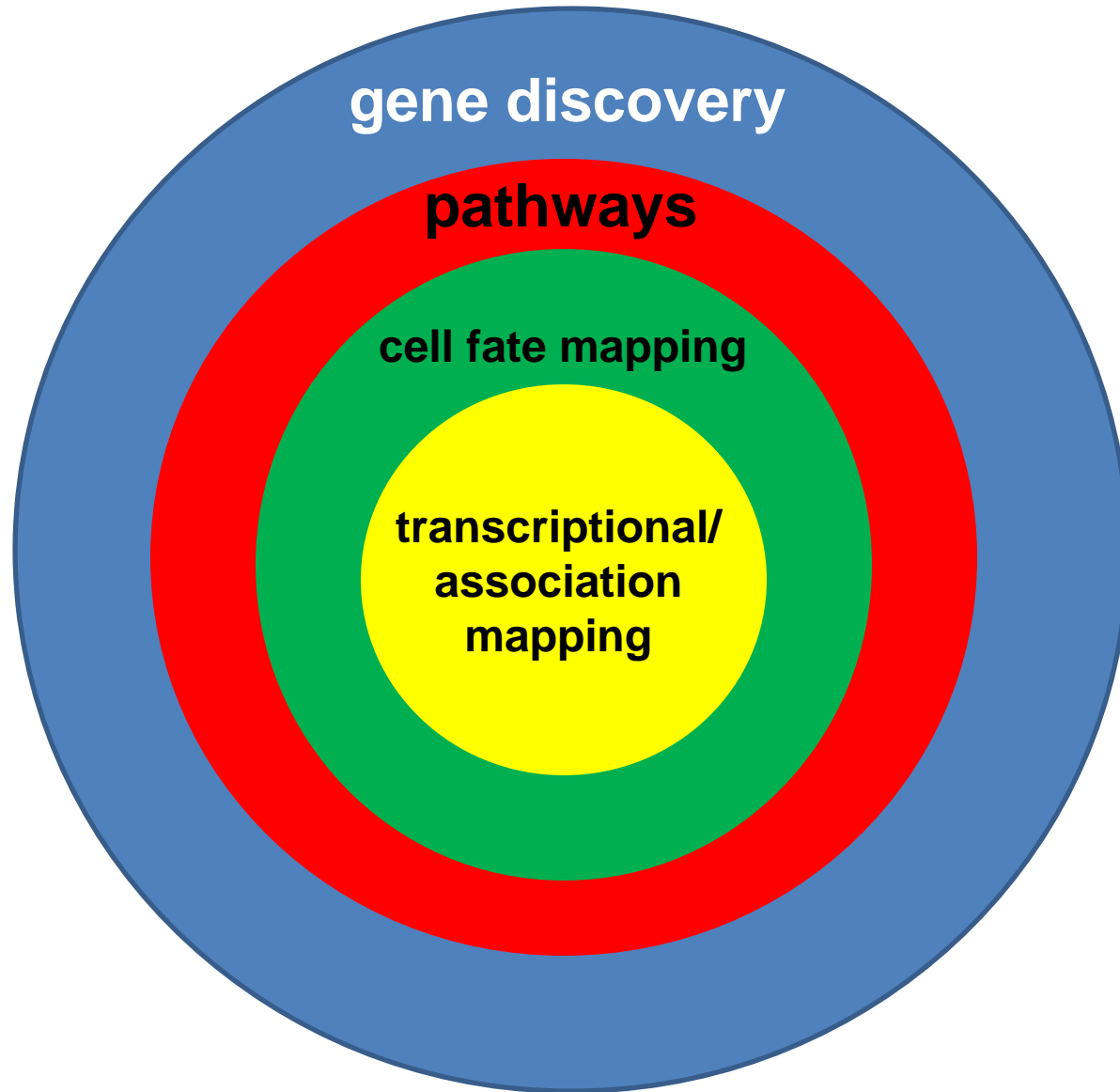
**(2) gene discovery**

**(3) cell genotyping**

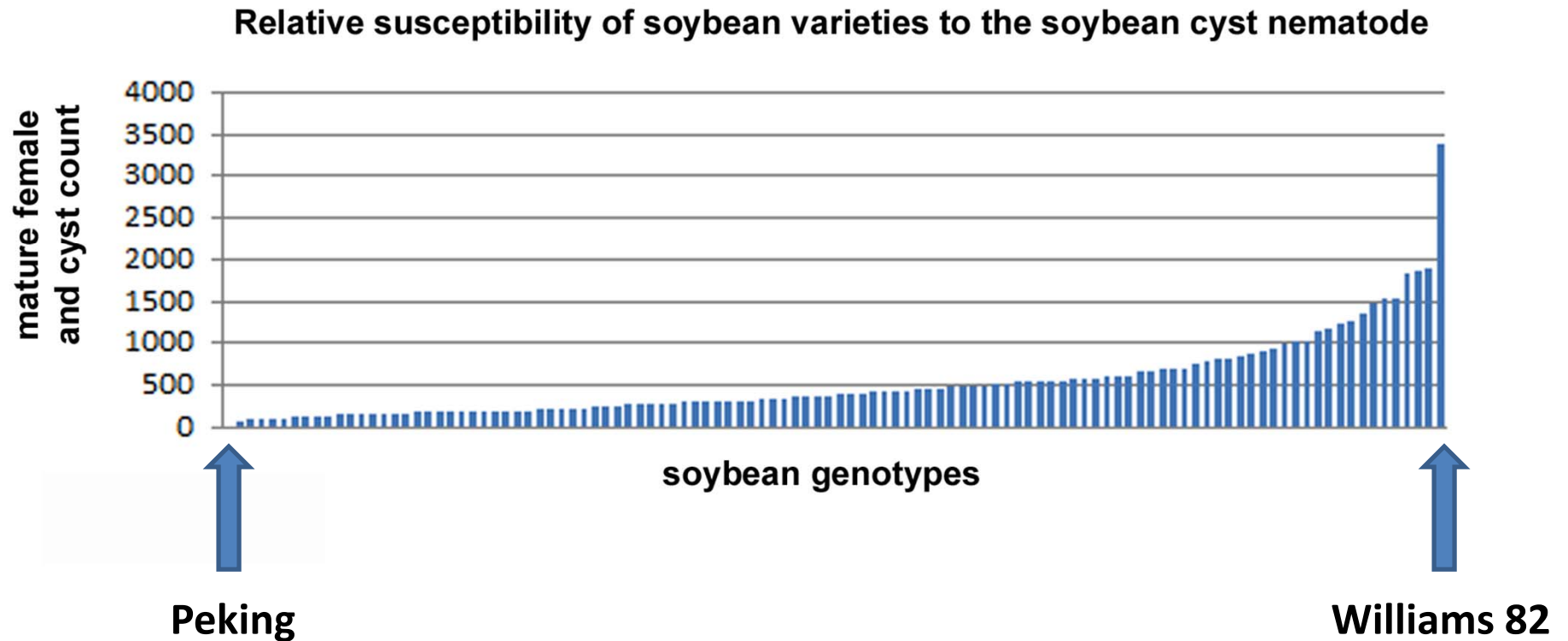
**(4) functional studies**

**(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**

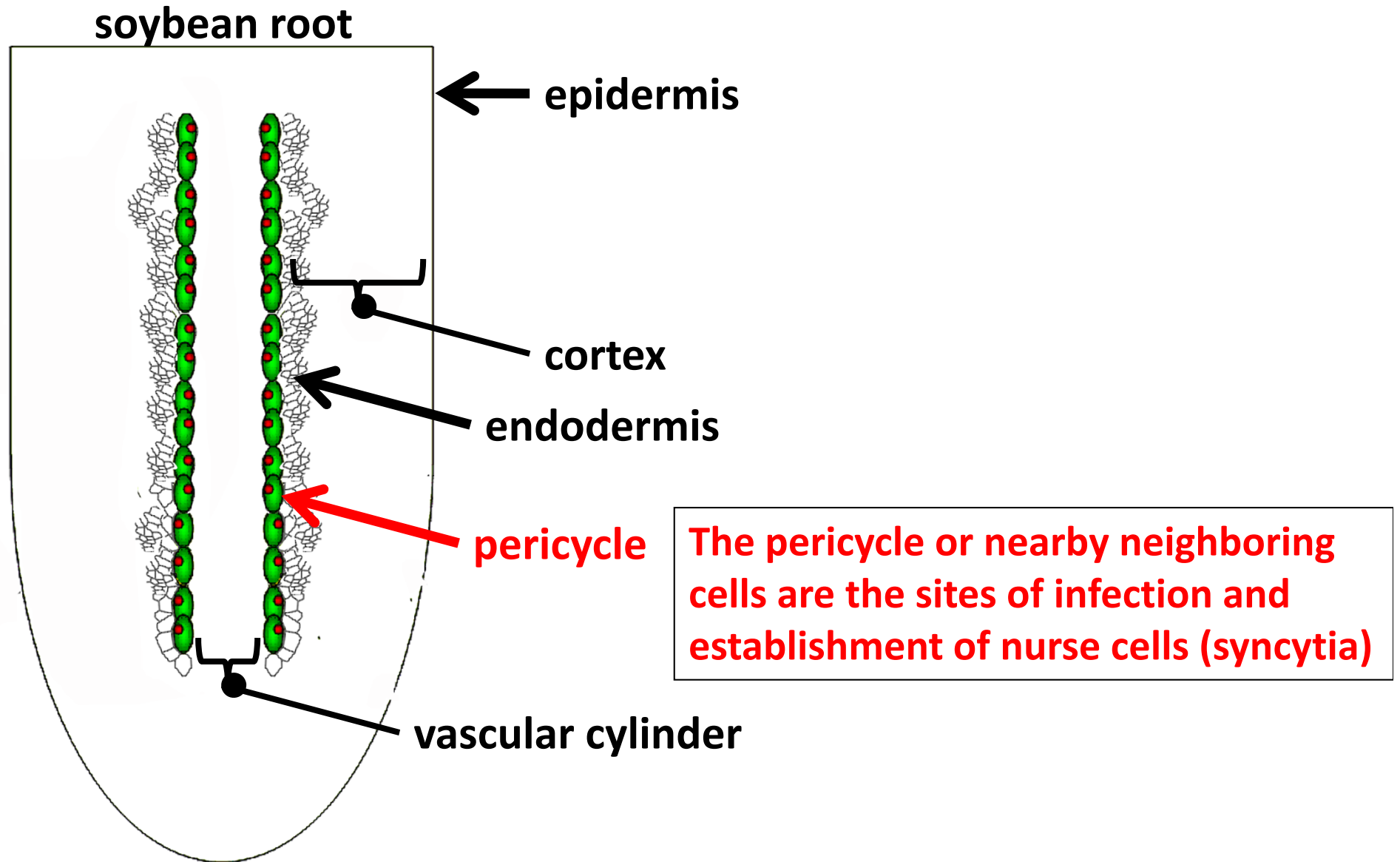


The range in the ability of soybean to resist infection suggests variability exists at the cellular level in the different genotypes as they cope with infection

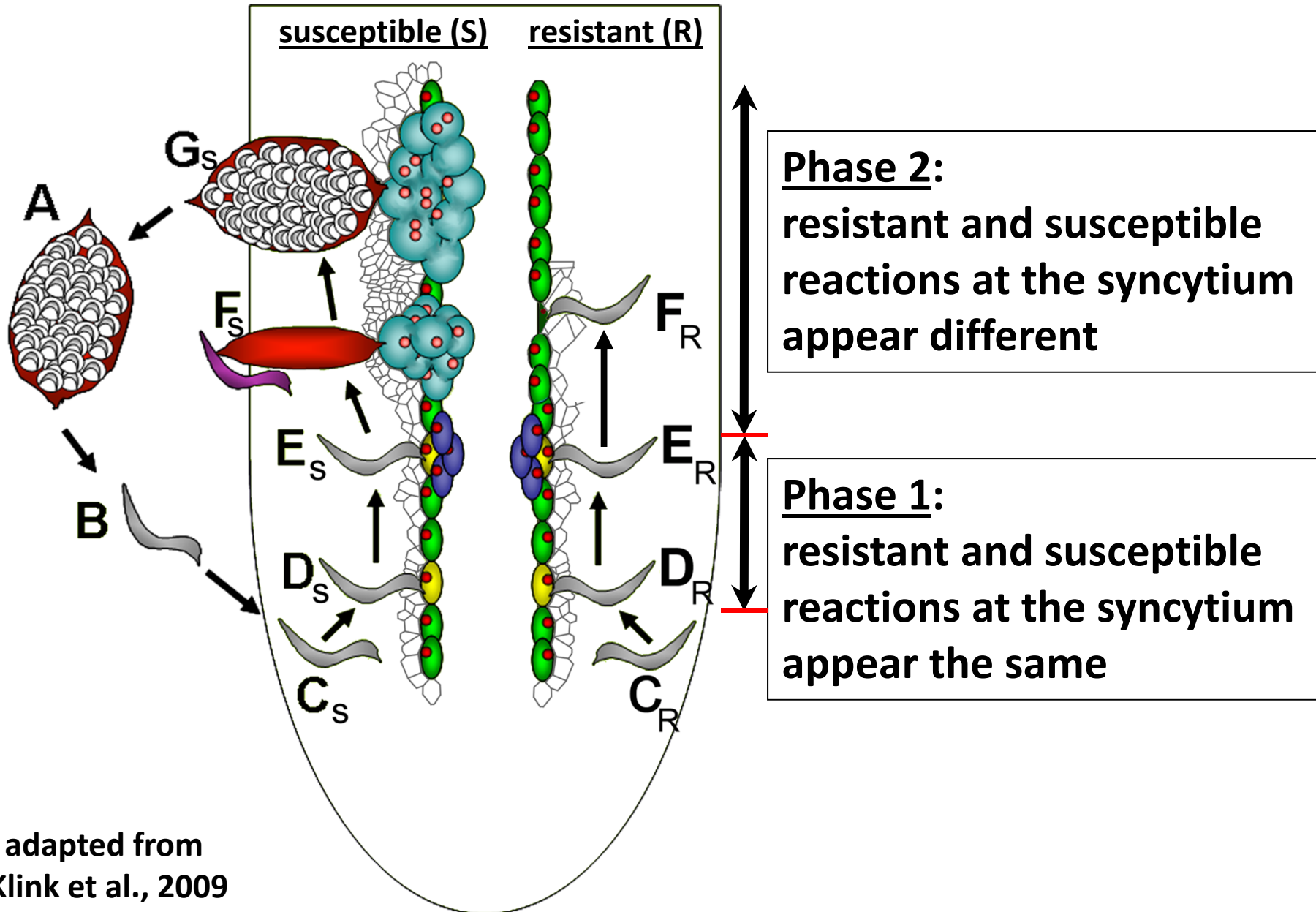


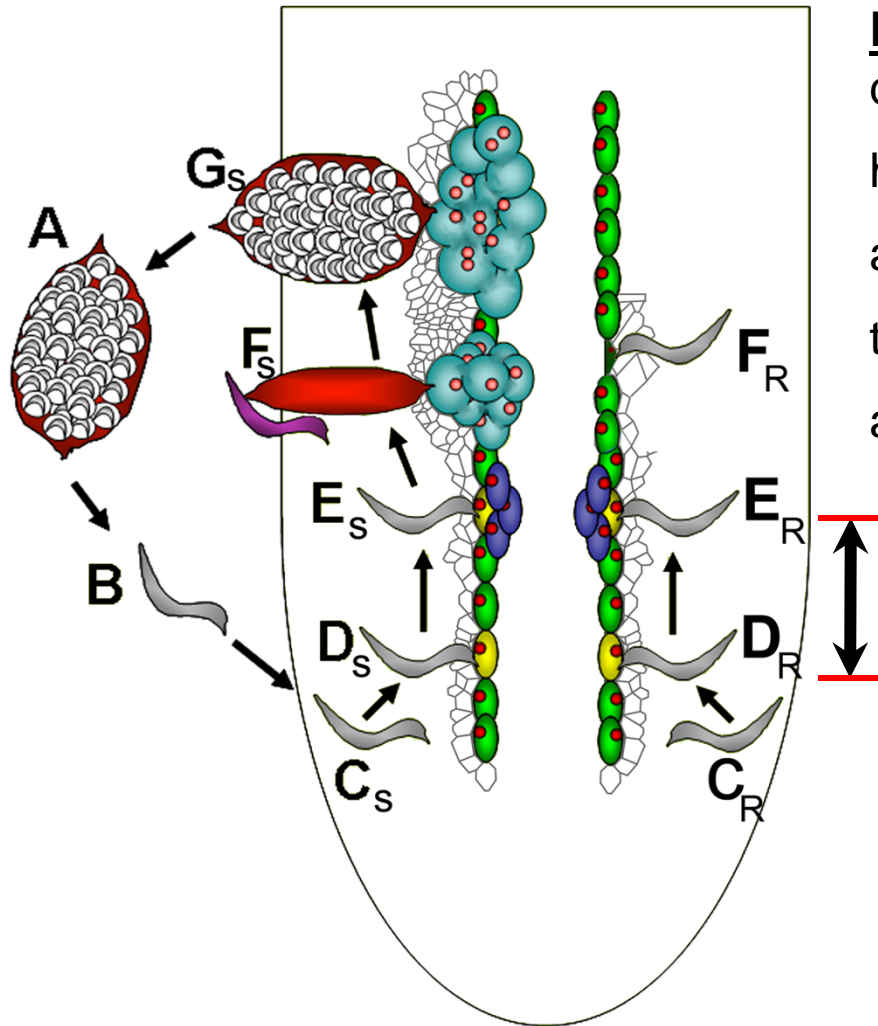


# SCN infection is tightly linked to specific types of cells in the root



# the resistant reaction undergoes two developmental phases





### Phase 1: susceptible and resistant reactions

dissolution of cell walls

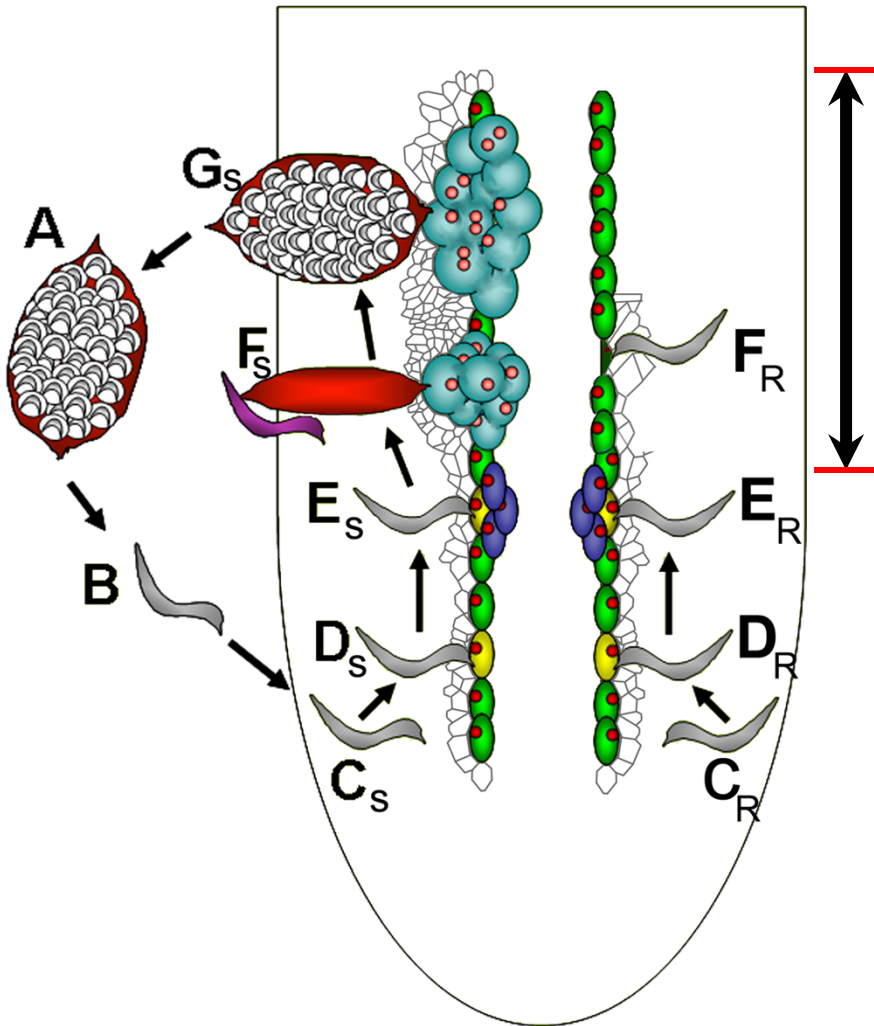
hypertrophy

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

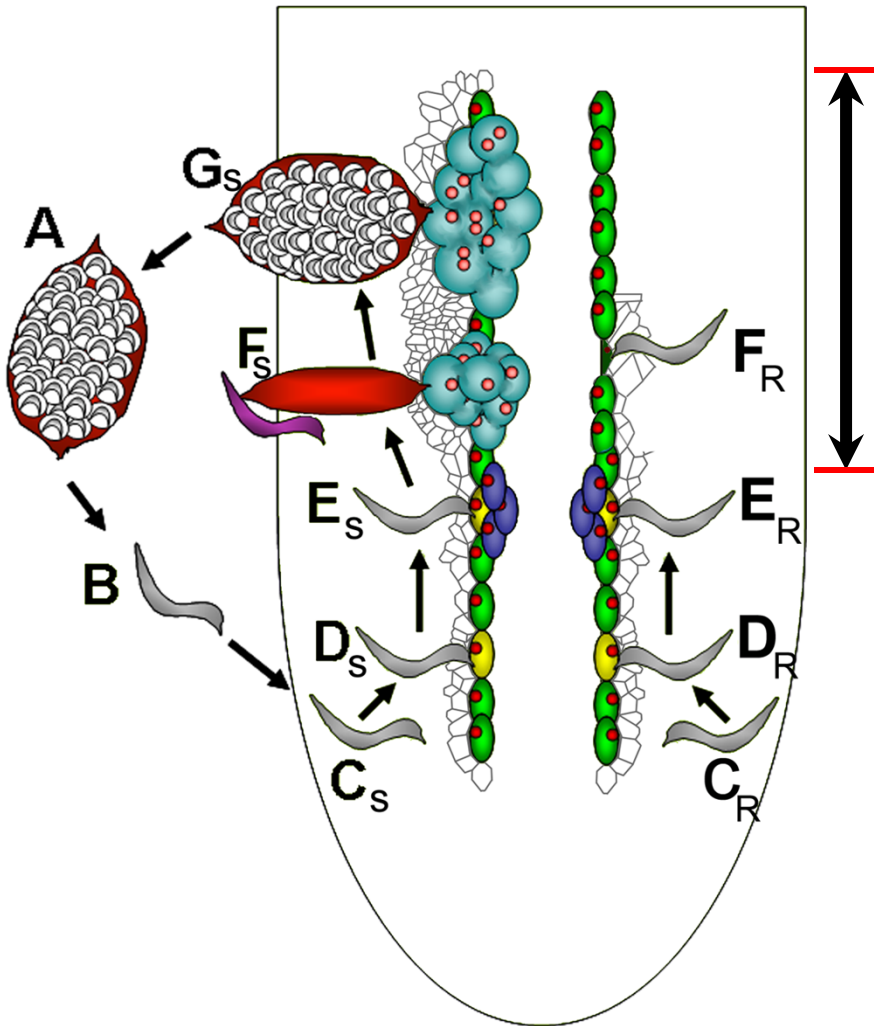
hypertrophy of nuclei and nucleoli

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

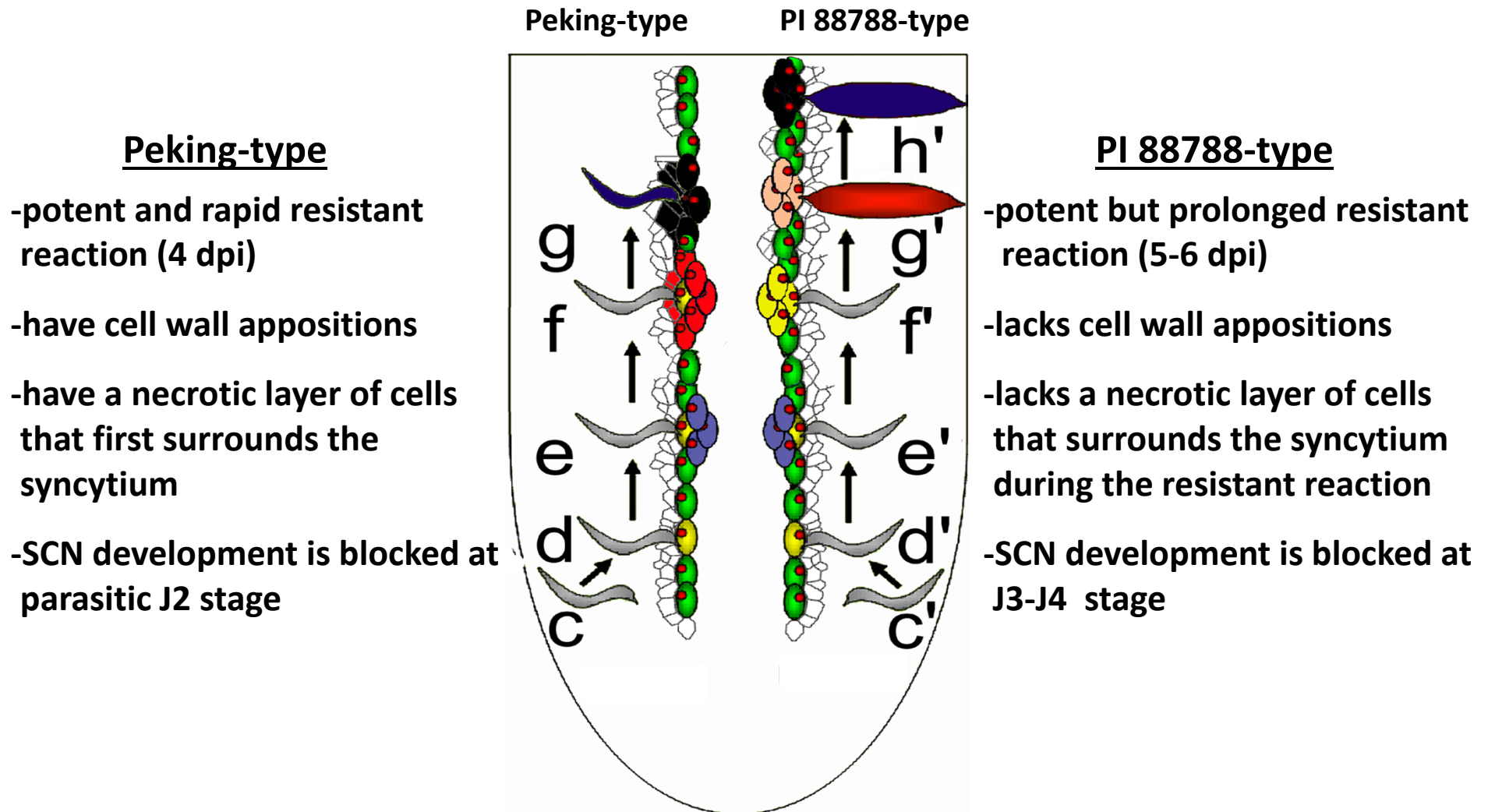


## Phase 2: resistant reaction

- 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)

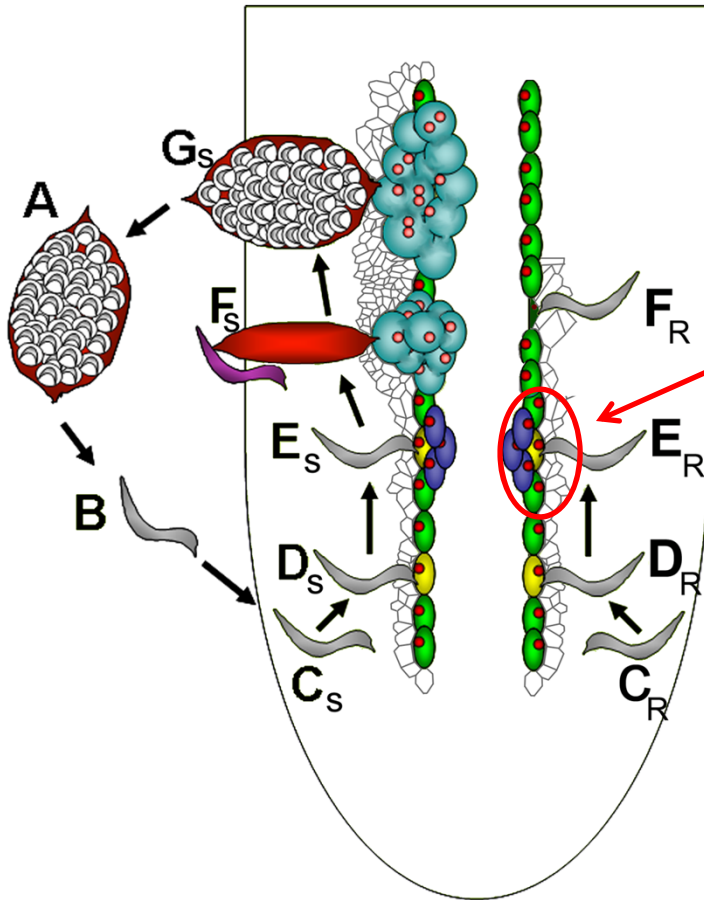


## **(2) gene discovery**

# experimental approach: cell-type specific analyses

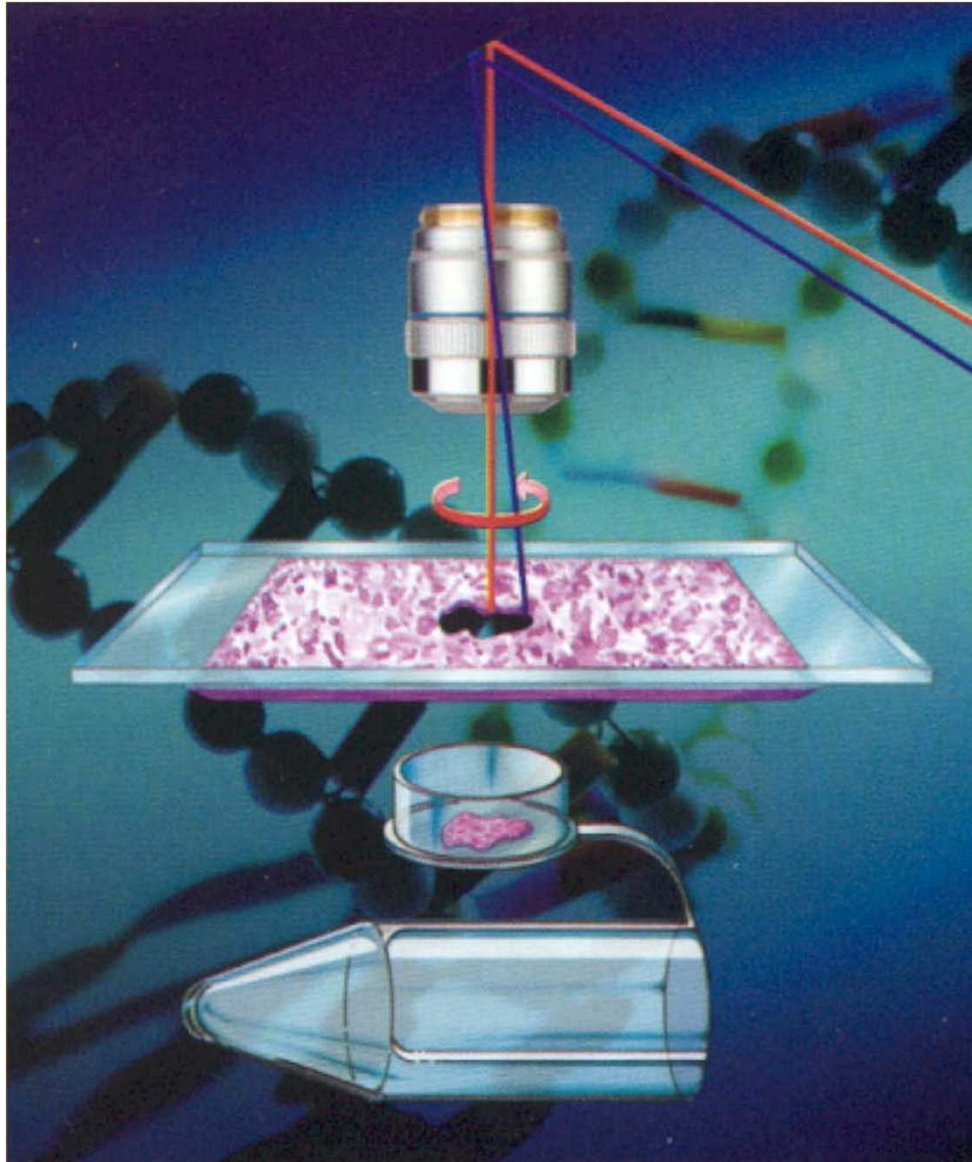
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## Why cell-type specific analyses?



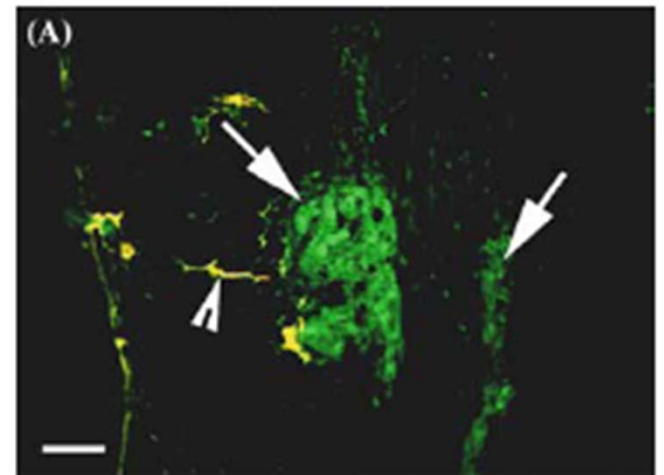
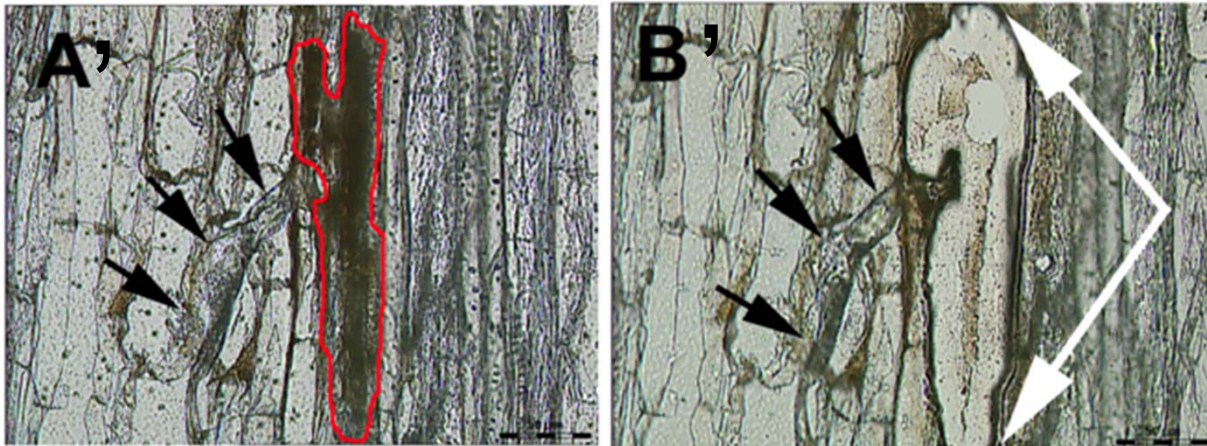
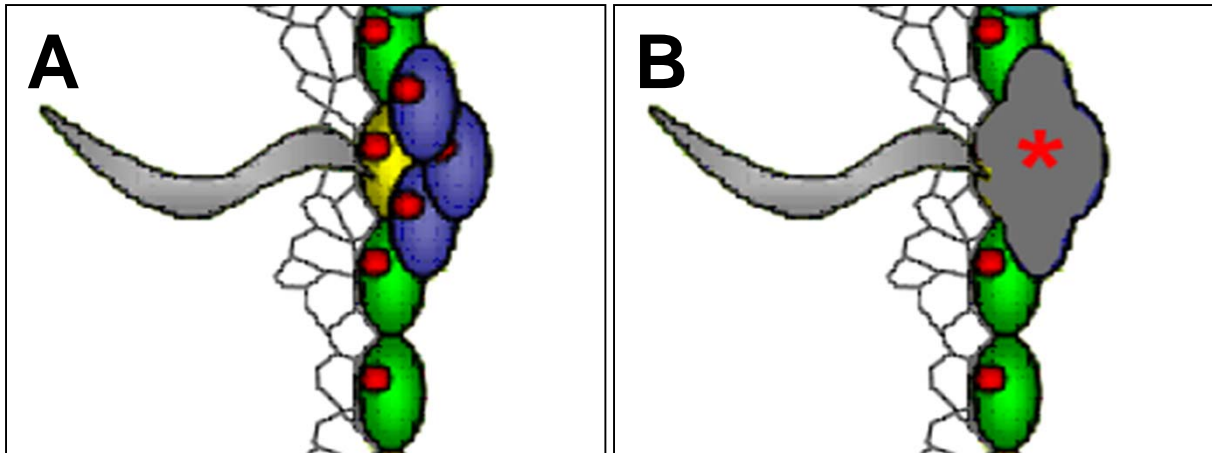
- (1) The feeding site (arrow) is the site of the resistant reaction
- (2) By collecting feeding sites, we would be concentrating the cell types undergoing the resistant or susceptible reaction
- (3) By concentrating the feeding sites, we would maximize the chances of identifying gene expression patterns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

# challenge: identifying a technology to isolate syncytia



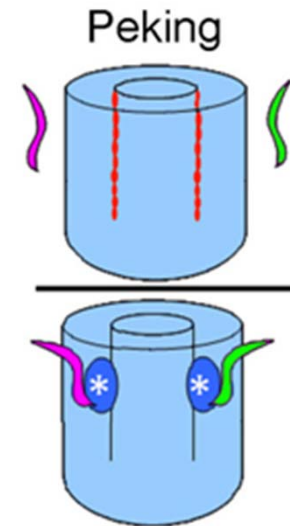
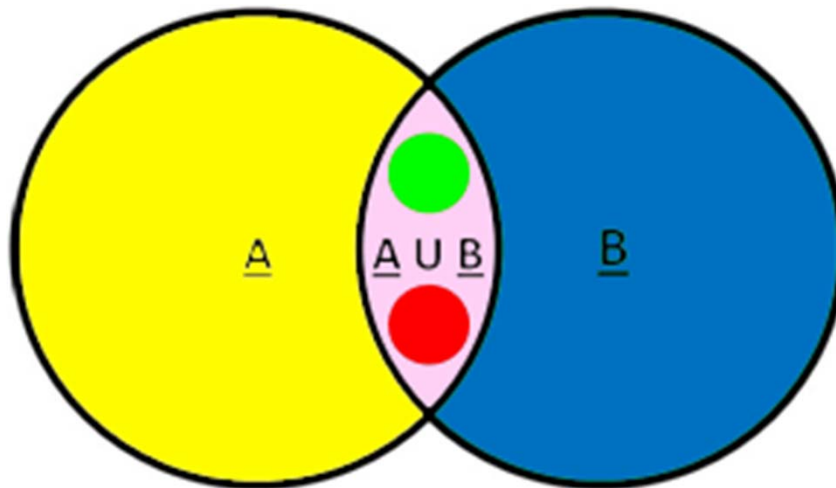
**Laser microdissection  
(LM)**

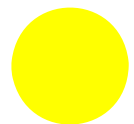

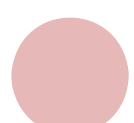

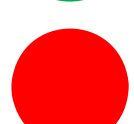
# cell collection



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?

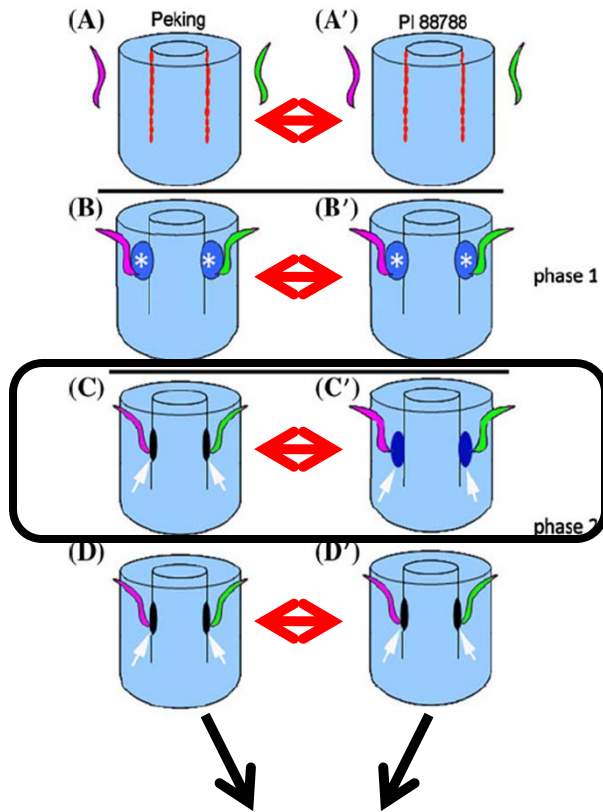
## **(3) Time series studies:**

What genes are uniquely expressed throughout the resistant reaction?

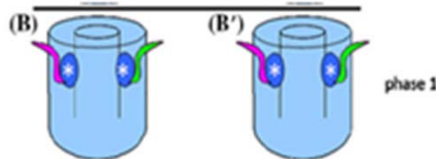


# (1) Intergenotype studies

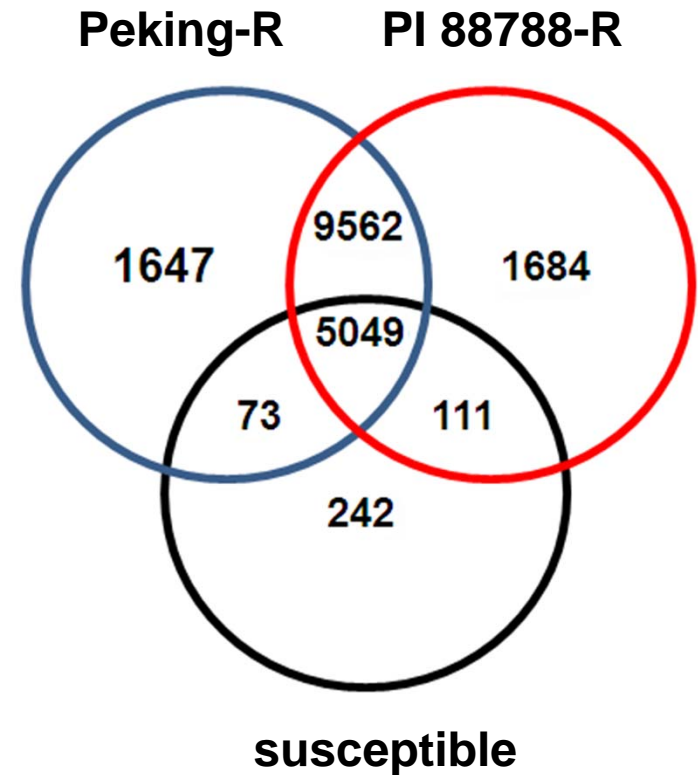
**resistant  
syncytia**



**susceptible  
syncytia**

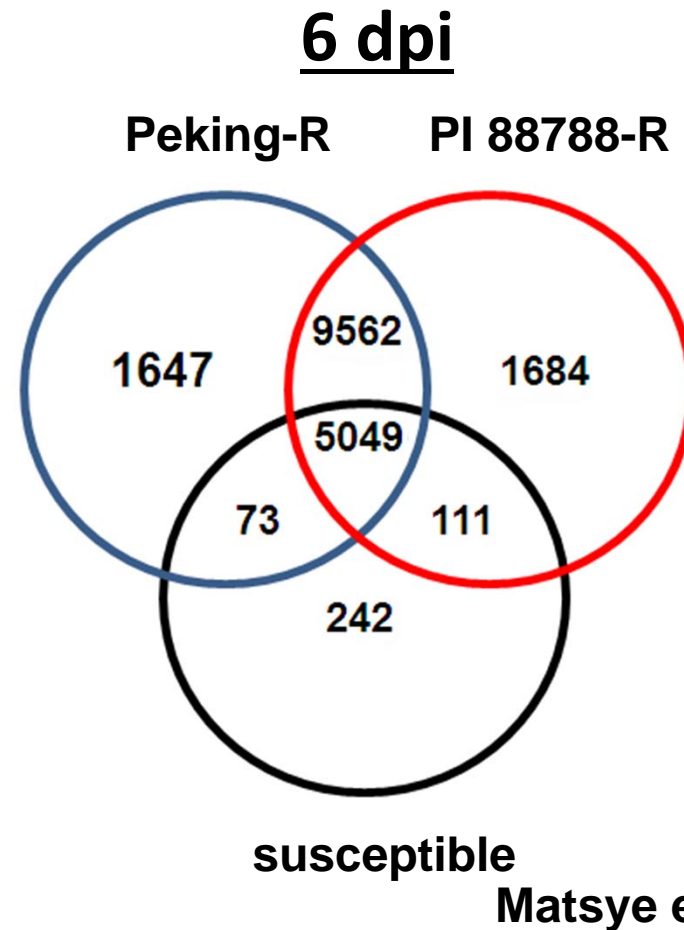
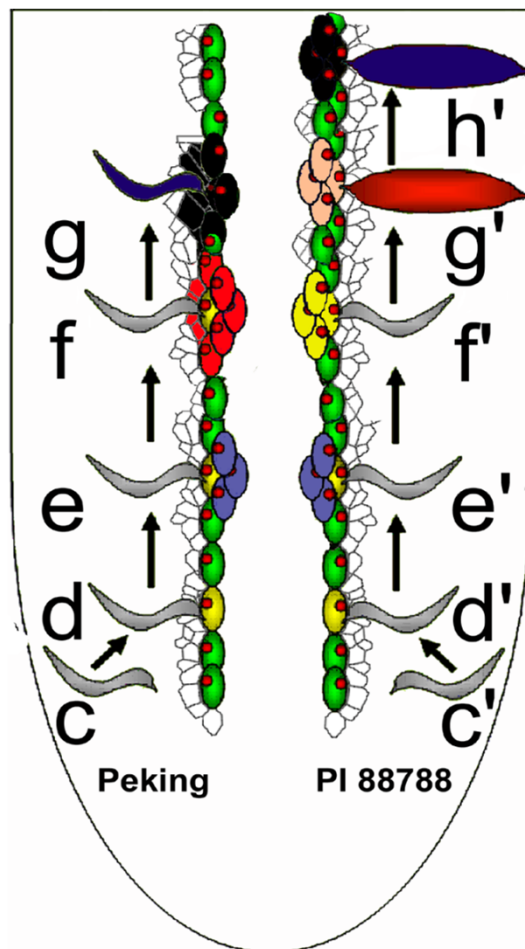


**6 dpi**



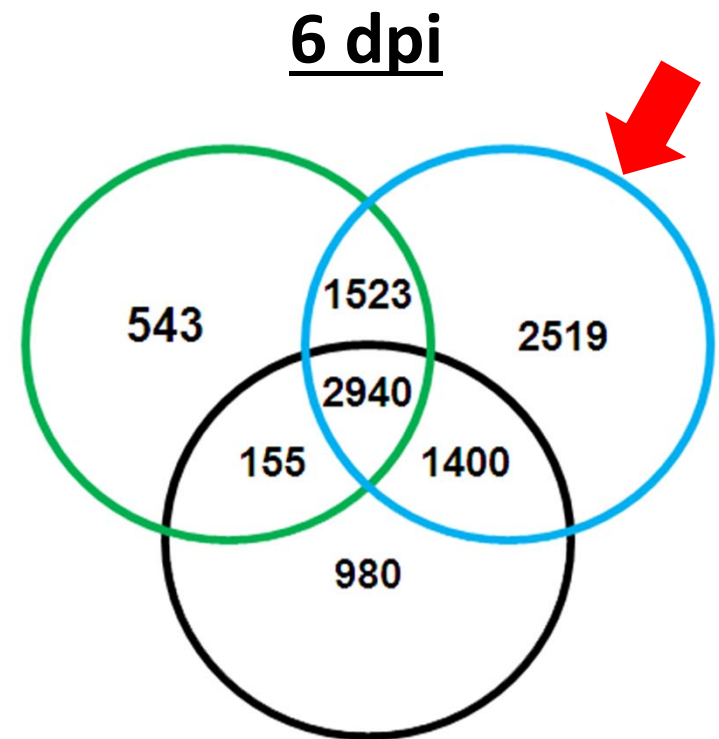
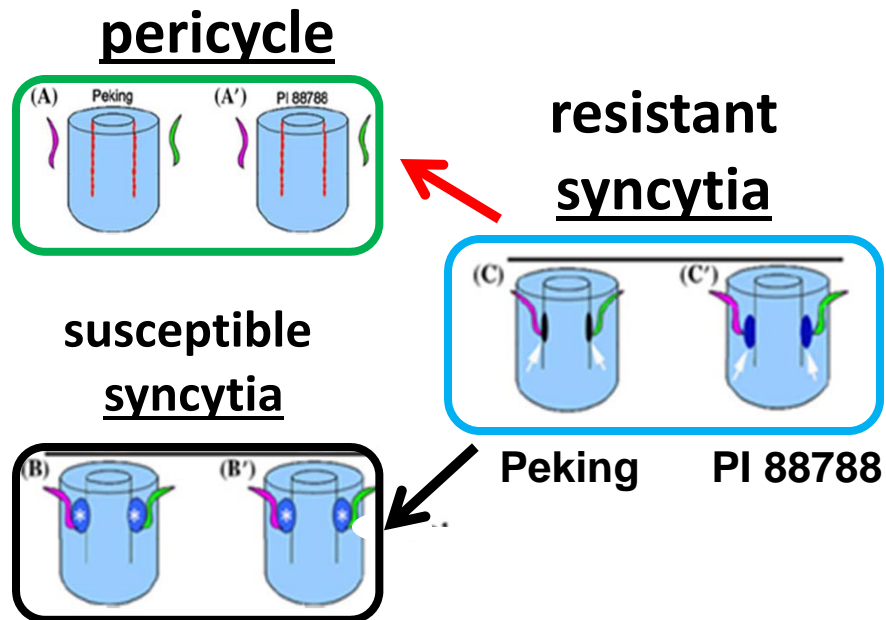
## Outcome of the intergenotype studies

- 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



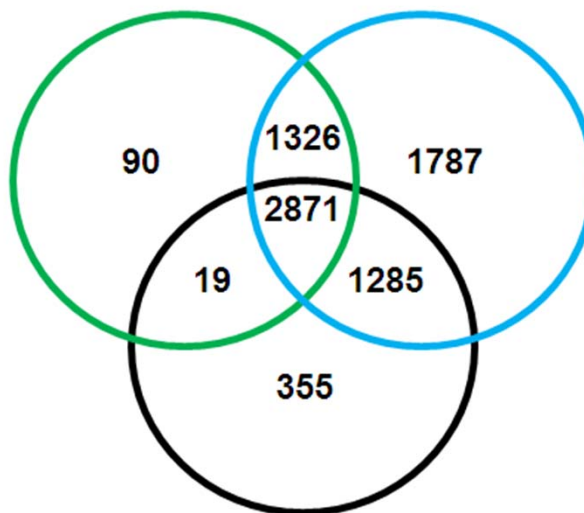
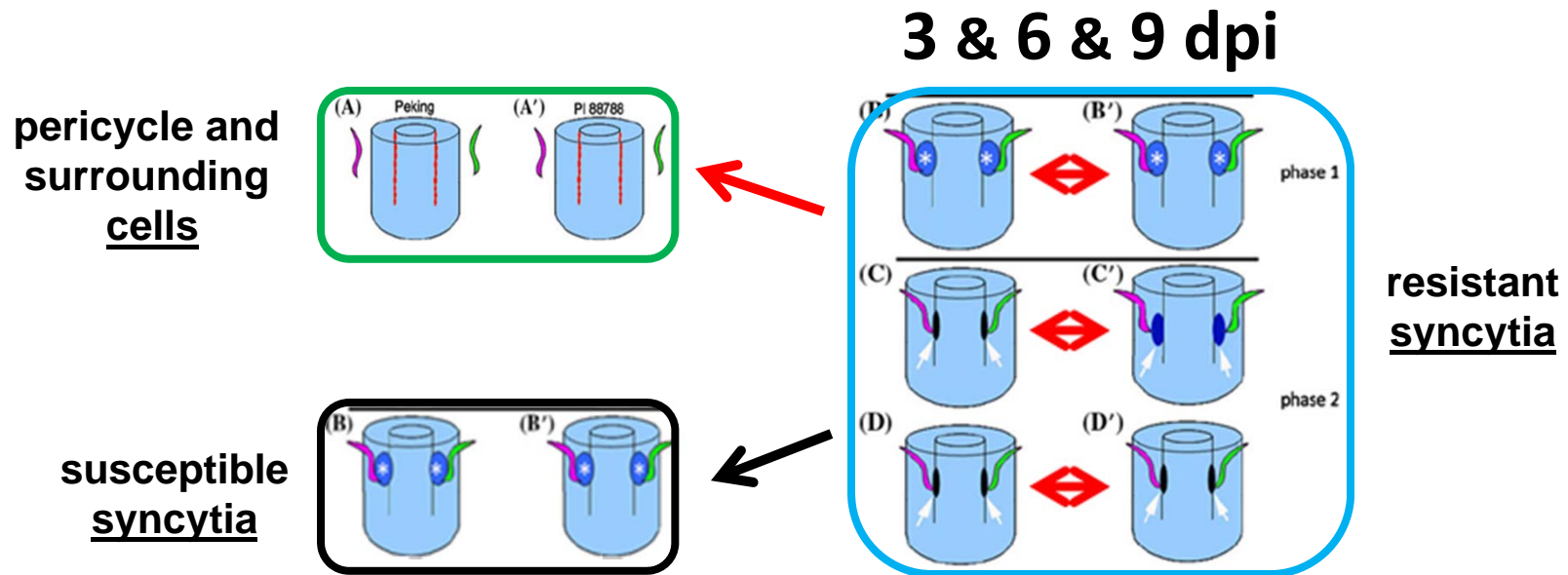
## (2) Time point studies

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

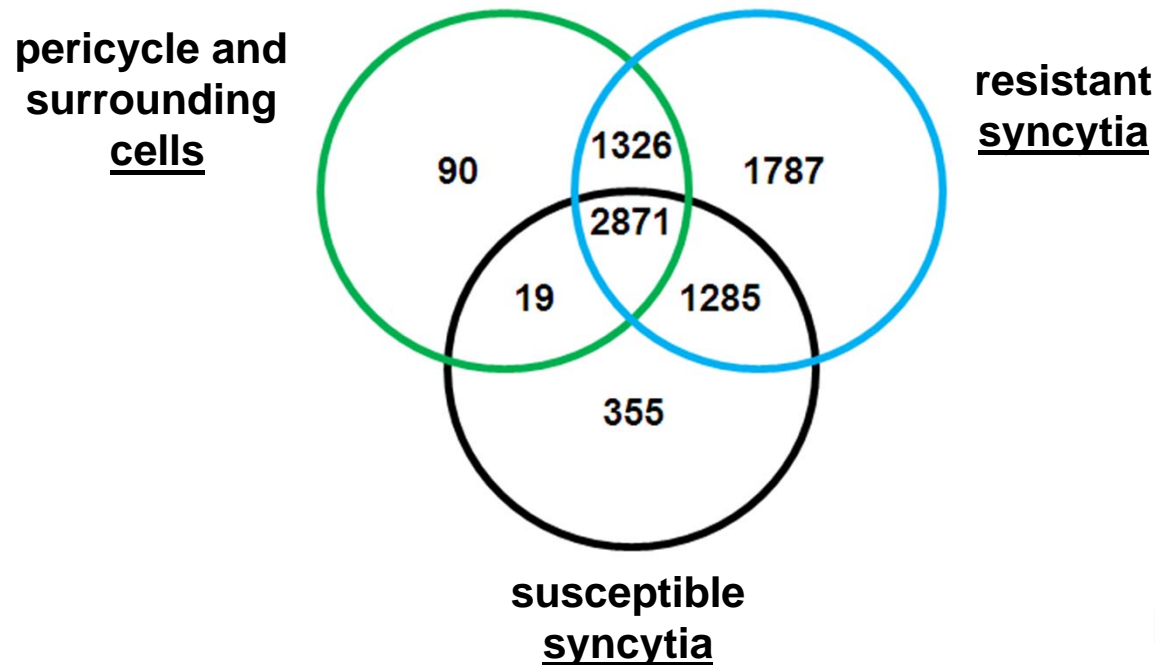


### (3) Time series studies:

Designed to identify genes that always pertain to defense

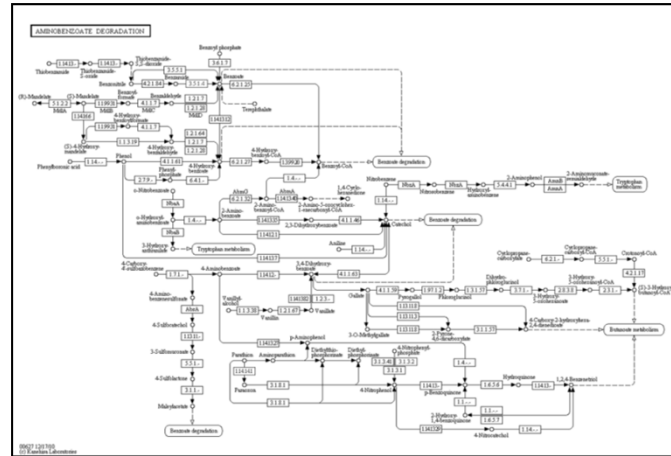


### (3) Time series studies: 3 & 6 & 9 dpi



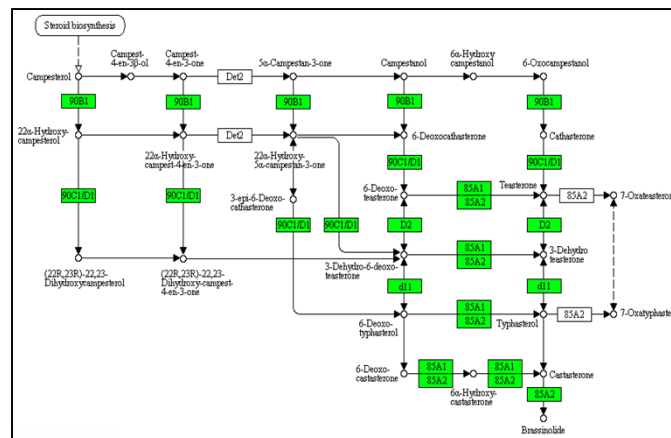
| chromosomal location | Gene Annotation                            | Illumina: % of tags |
|----------------------|--|---------------------|
| Glyma13g06450.1      | unknown                                    | 17.3967             |
| Glyma17g07250.1      | XYLOGLUCAN ENDOTRANSGLYCOSYLASE 6 (XTR6)   | 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



pericycle and surrounding cells      syncytia-R

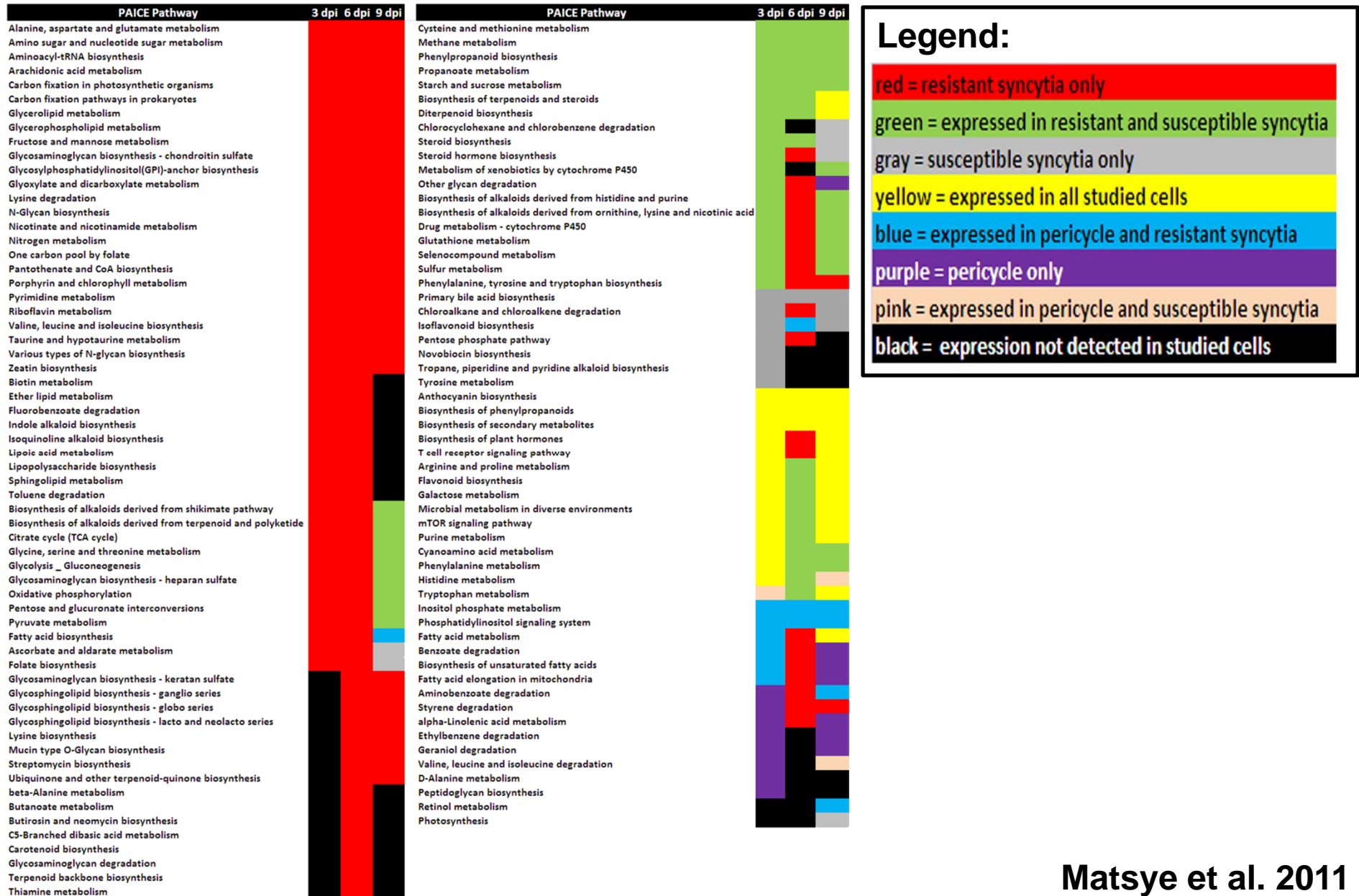


susceptible

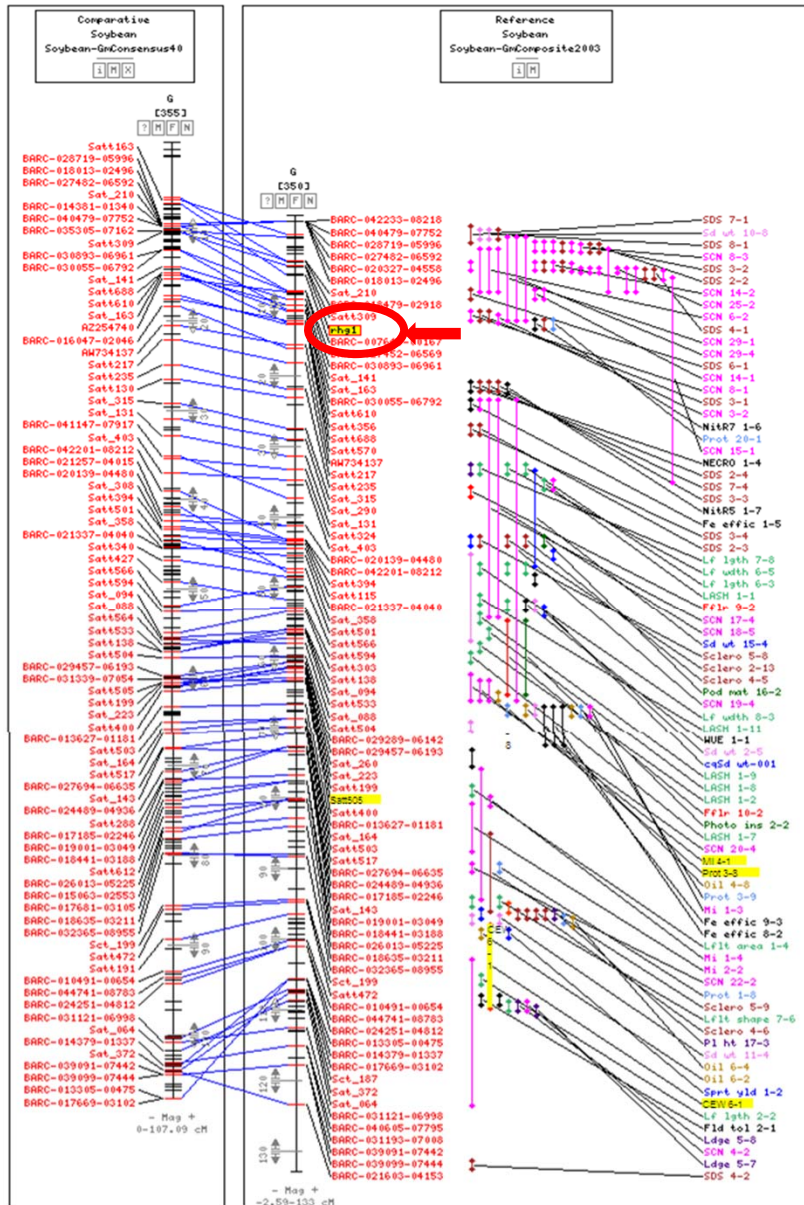
## Legend:

- 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

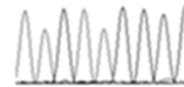


# (3) cell genotyping

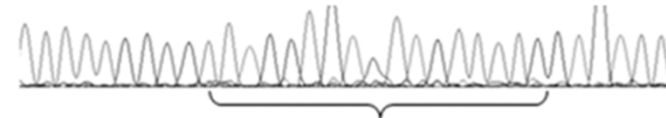


## identification of genetic elements

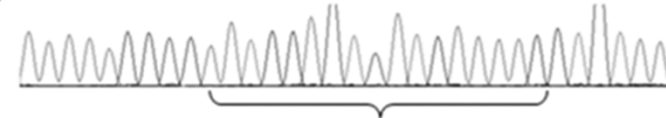
Williams 82 cDNA



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                           |          |          |          |          |          |          |          |          |          |             |          |          |
| <b>GAMMA-GLUTAMYL TRANSPEPTIDASE 1</b>      |          |          |          |          |          |          |          |          |          |             |          |          |
| amino acid transporter                      | ○        | ○        | ●        | ○        | ○        | ●        | ○        | ○        | ●        | ○           | ○        | ●        |
| alpha-soluble NSF attachment protein        | ○        | ○        | ●        | ○        | ○        | ●        | ○        | ○        | ●        | ○           | ○        | ●        |
| conserved protein                           |          |          |          |          |          |          |          |          |          |             |          |          |
| ATP-dependent rRNA helicase <i>spb4</i>     |          |          |          |          |          |          |          |          |          |             |          |          |
| hydrolase                                   |          |          |          |          |          |          |          |          |          |             |          |          |
| ferritin                                    |          |          |          |          |          |          |          |          |          |             |          |          |
| N/A   |          |          |          |          |          |          |          |          |          |             |          |          |
| eukaryotic translation initiation factor 2  |          |          |          |          |          |          |          |          |          |             |          |          |
| pollen Ole e 1 allergen and extensin family |          |          |          |          |          |          |          |          |          |             |          |          |
| conserved protein                           |          |          |          |          |          |          |          |          |          |             |          |          |
| ribosomal protein L10a                      |          |          |          |          |          |          |          |          |          |             |          |          |
| N/A   |          |          |          |          |          |          |          |          |          |             |          |          |

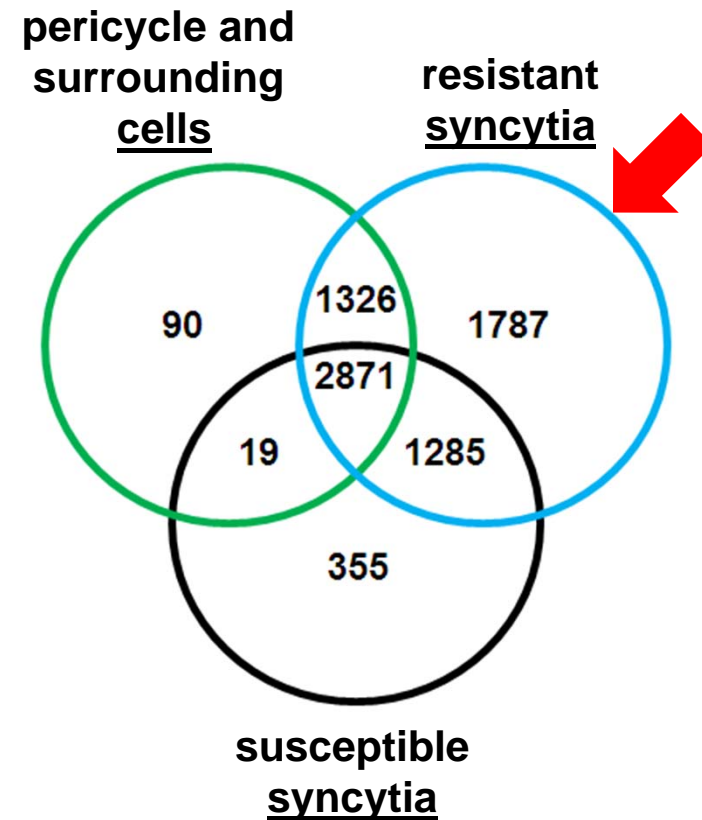
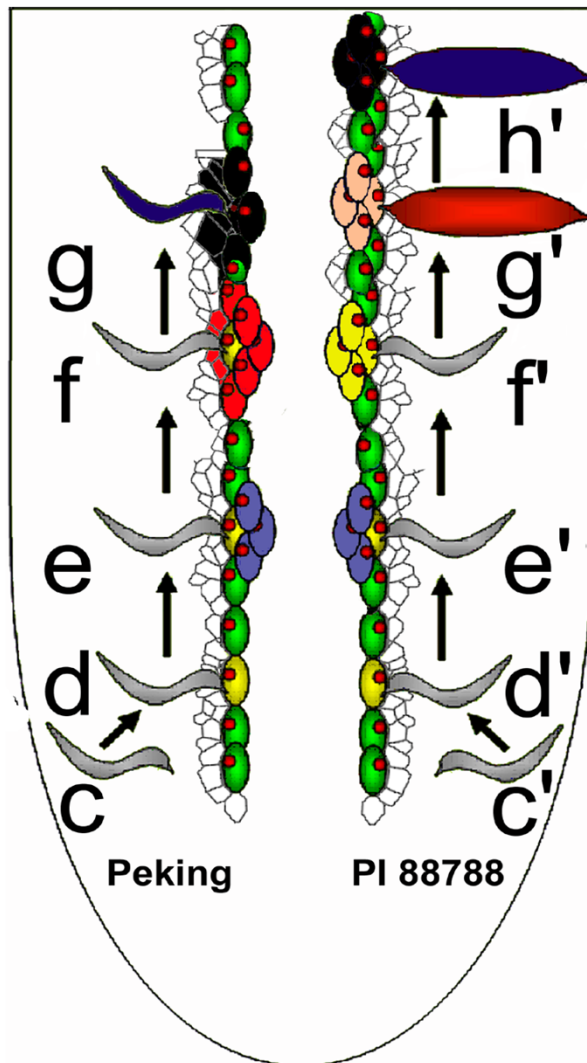
- LEGEND**
- not present
  - syncytium
  - pericycle
  - common
  - resistant syncytium (only)

Expression confirmed by Illumina® deep sequencing



## 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



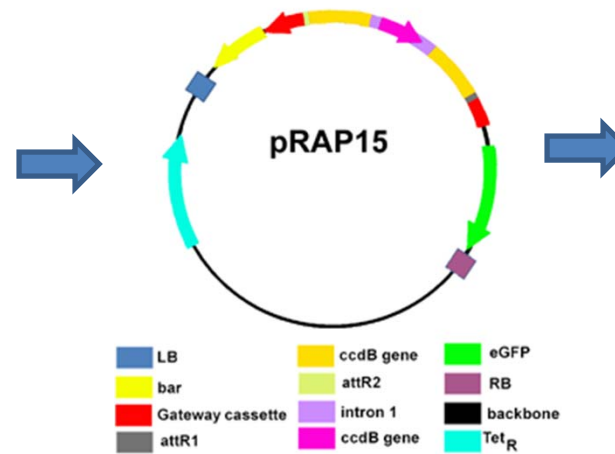


# *A. rhizogenes*-mediated soybean transformation method

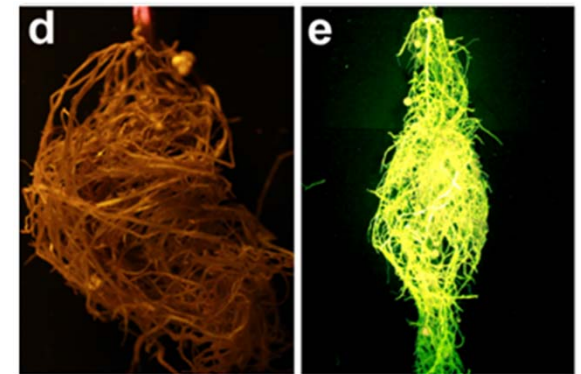
grow plants



develop expression vectors

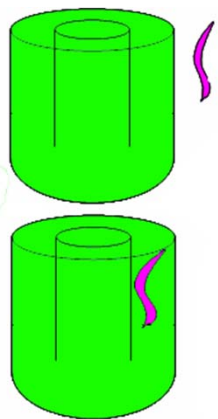


transgenic plants



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

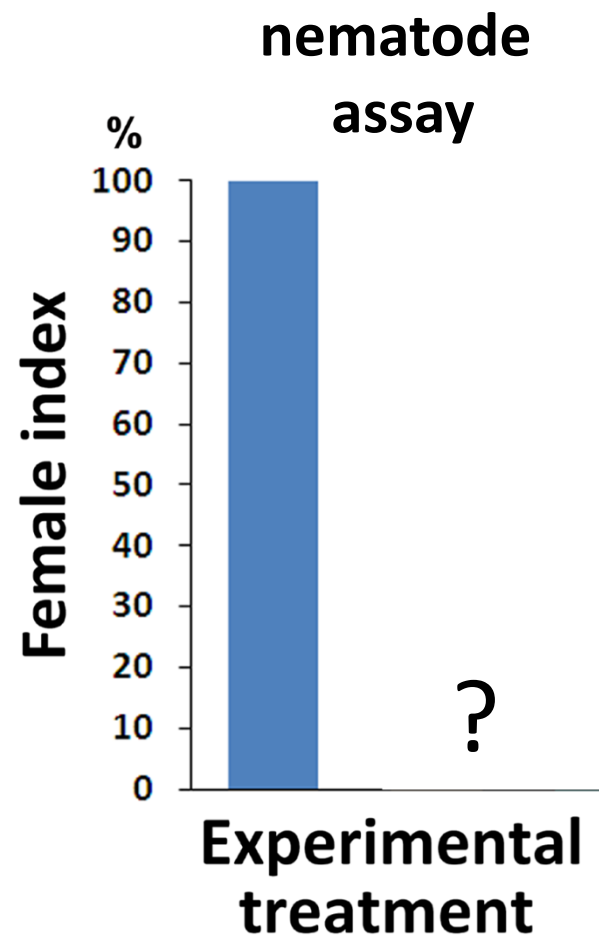
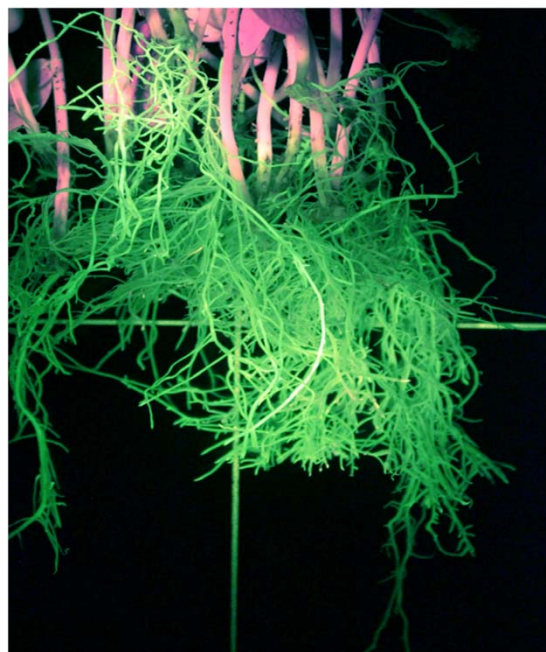
*in planta*  
expression/  
nematode  
inoculation



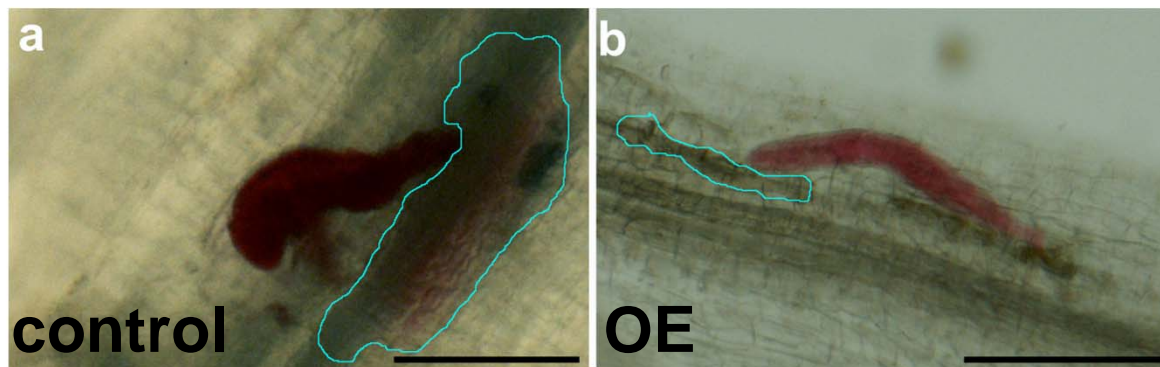
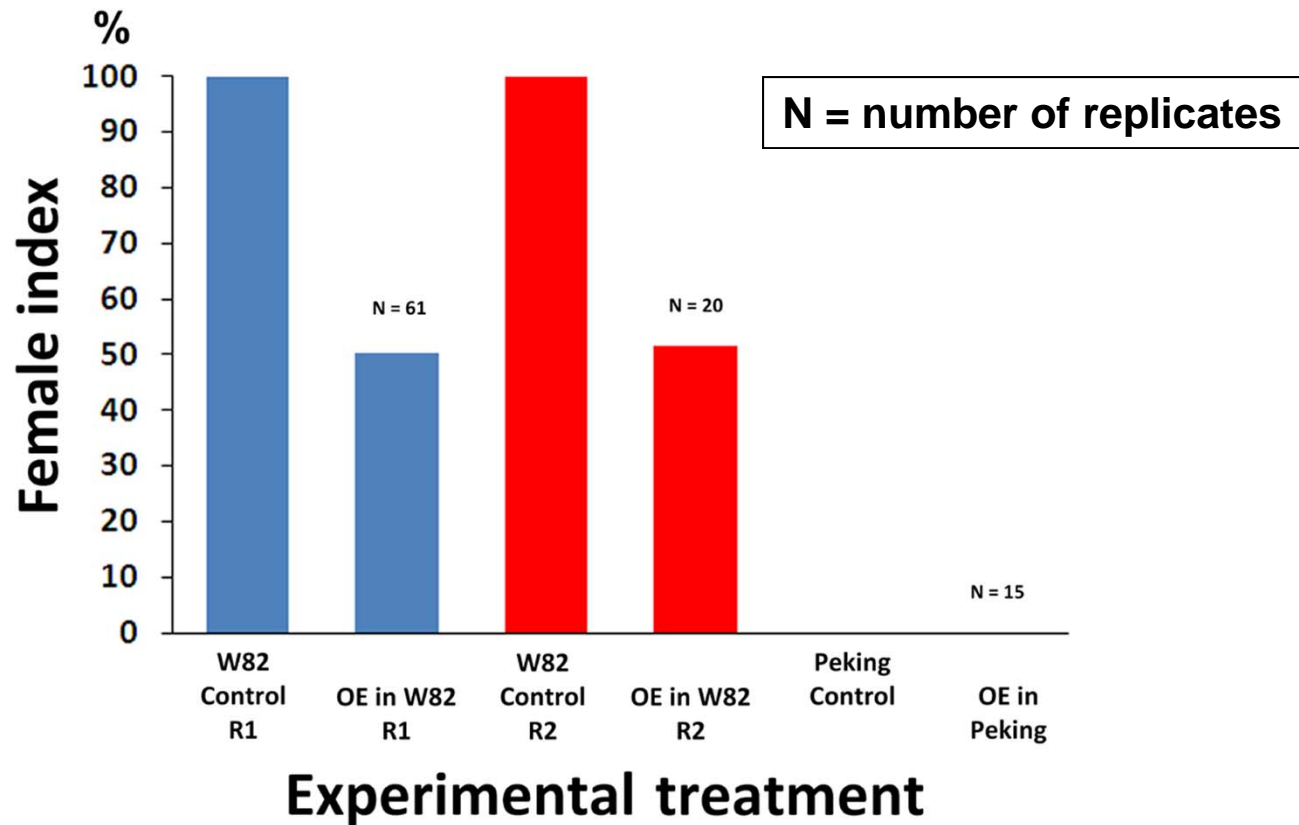
nematode  
feeding



infect for 30 days



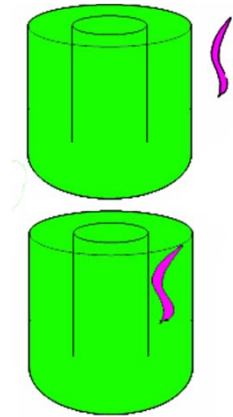
# Susceptible soybean genotype that is genetically engineered can suppress infection by SCN



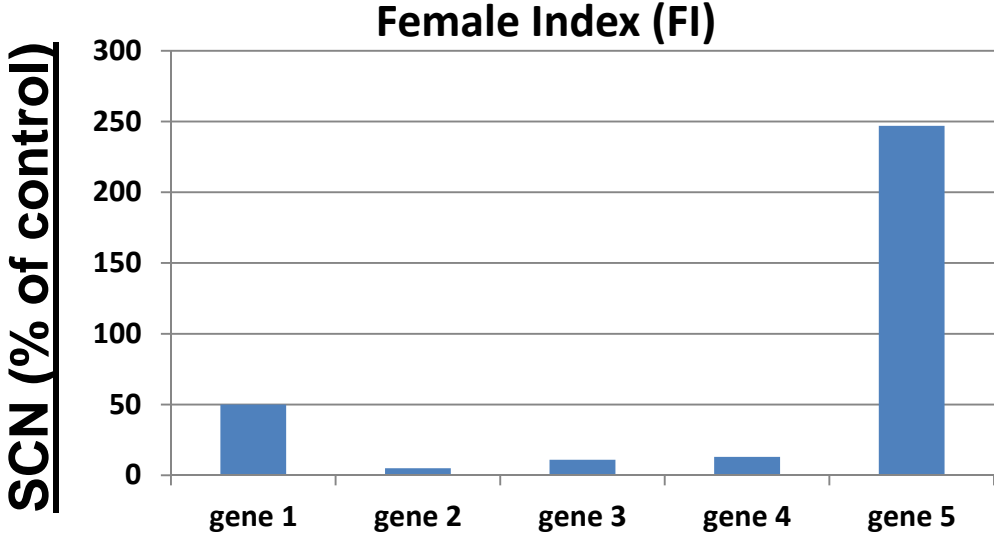
Matsye et al.  
submitted

# 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***





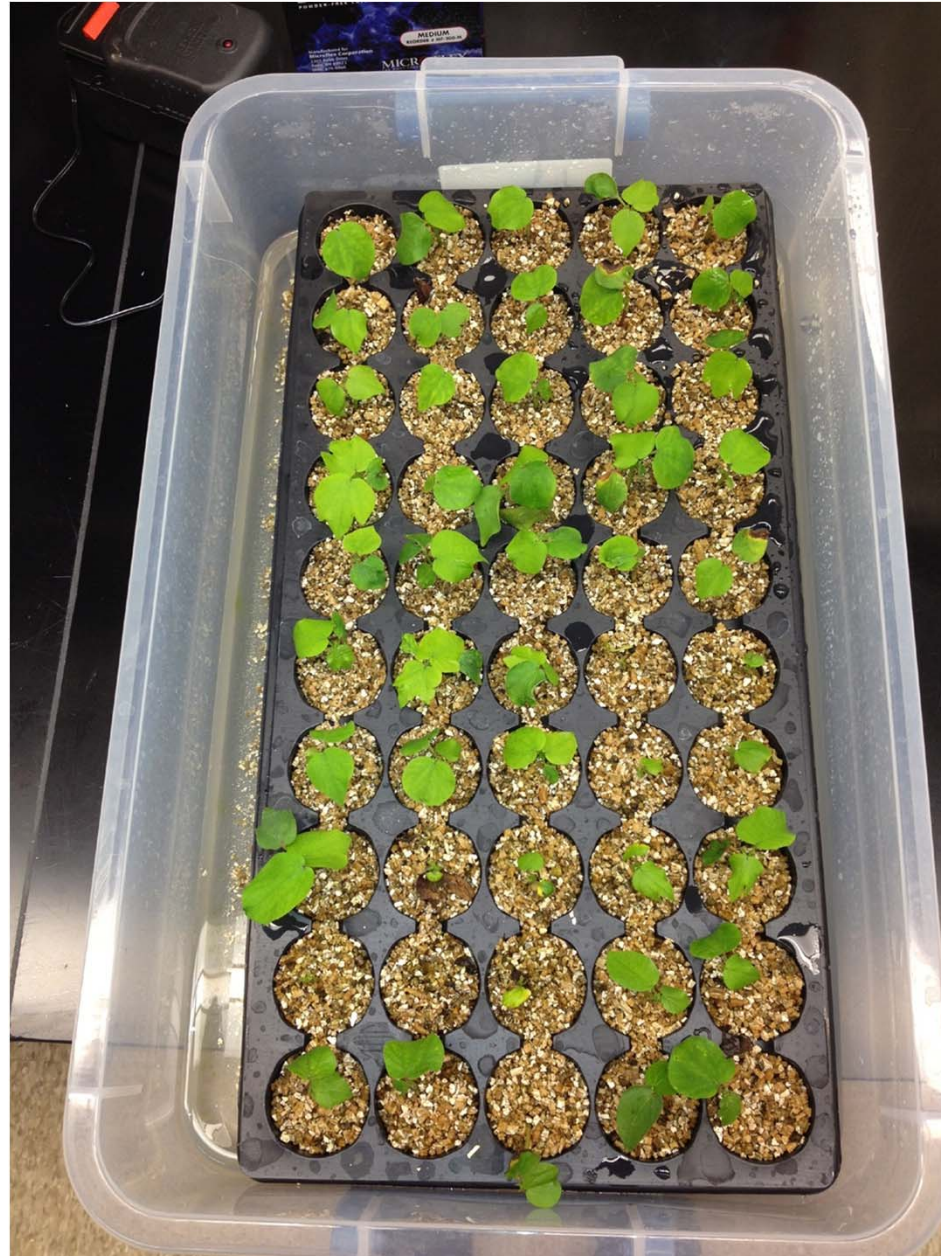
**A. rhizogenes-mediated cotton transformation method:**  
**(6) Incubate cotton at 28 degrees C.**



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

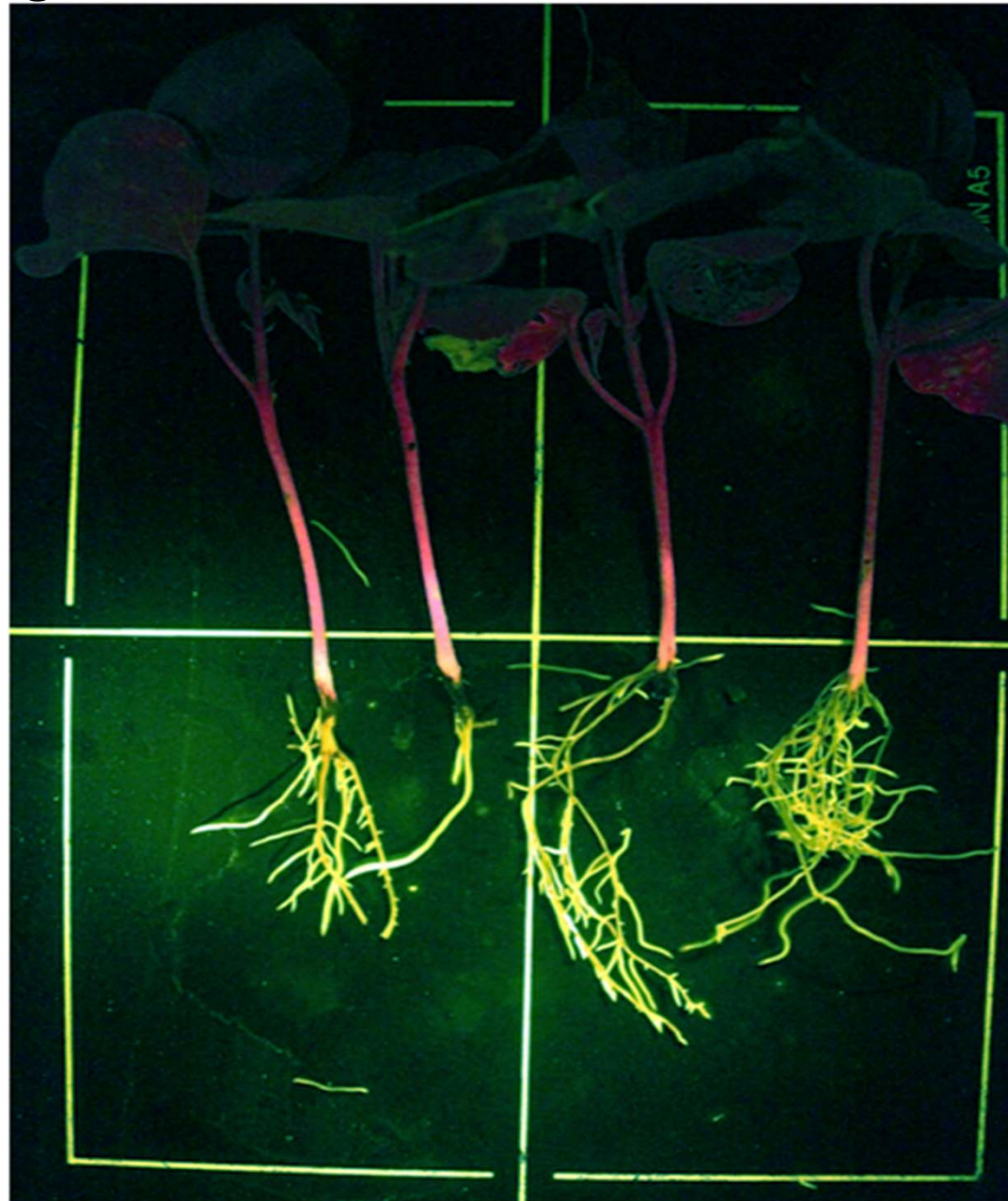


**A. rhizogenes-mediated cotton transformation method:**  
**(8) cotton after two weeks**



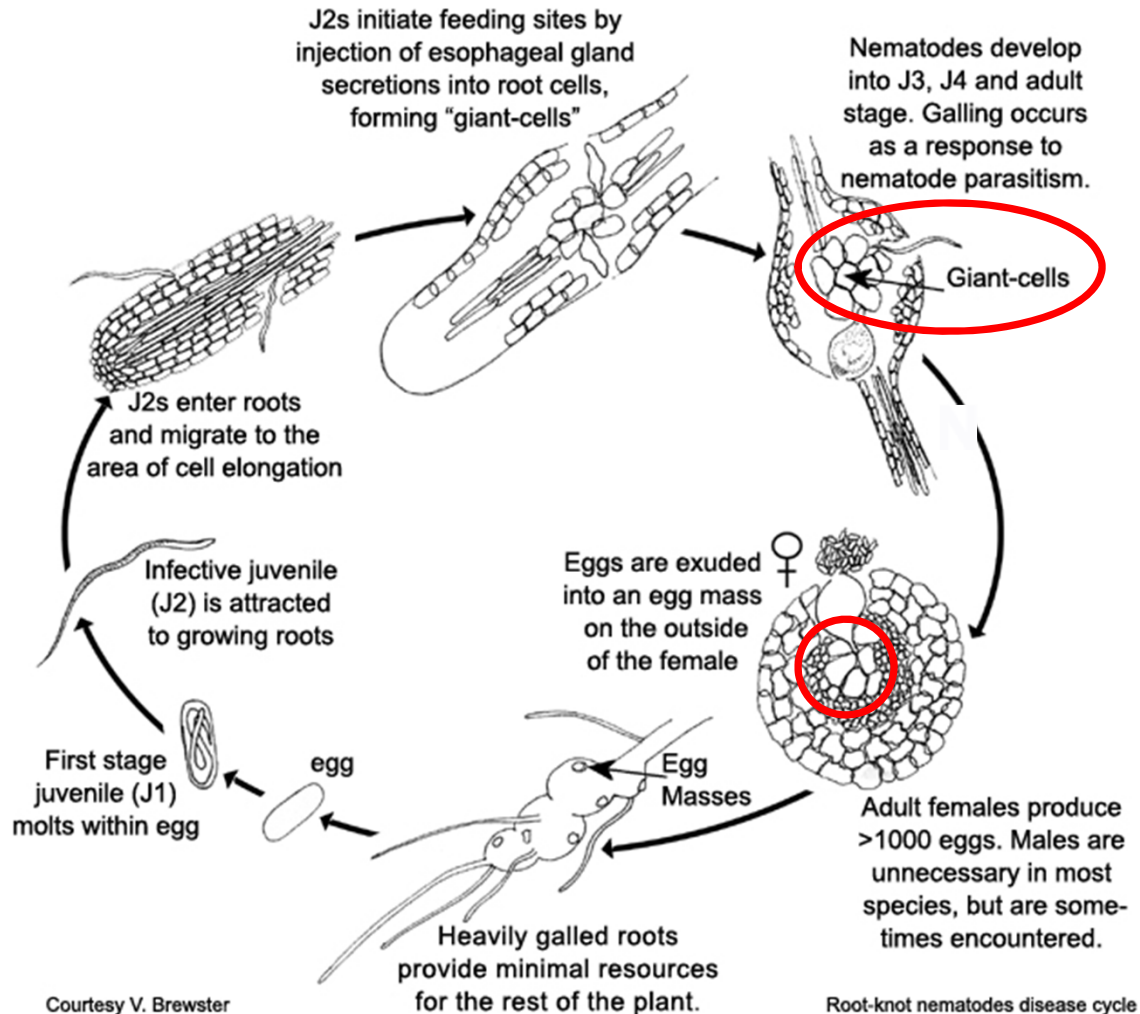


*A. rhizogenes*-mediated cotton transformation method:  
(9) transgenic cotton

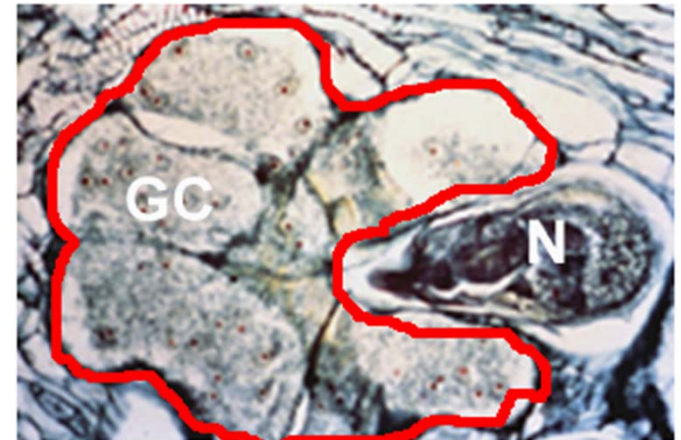


# cotton pipeline: cell collection

## Root knot nematode life cycle



## giant cells made by RKN

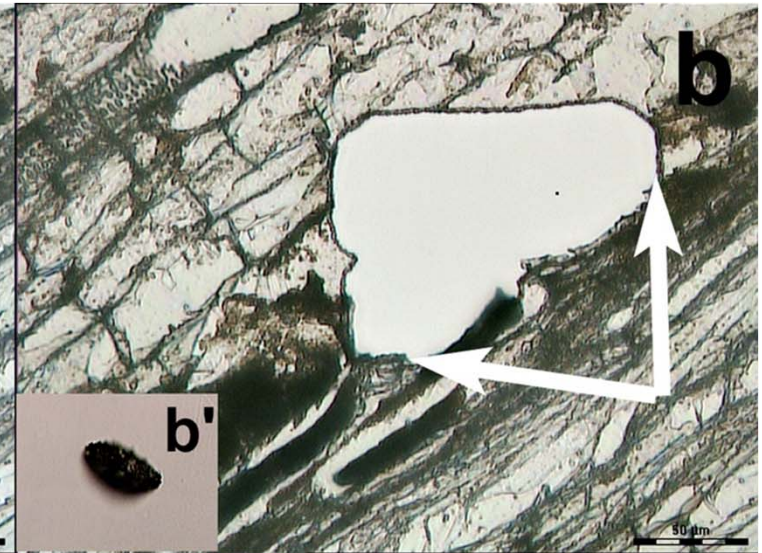
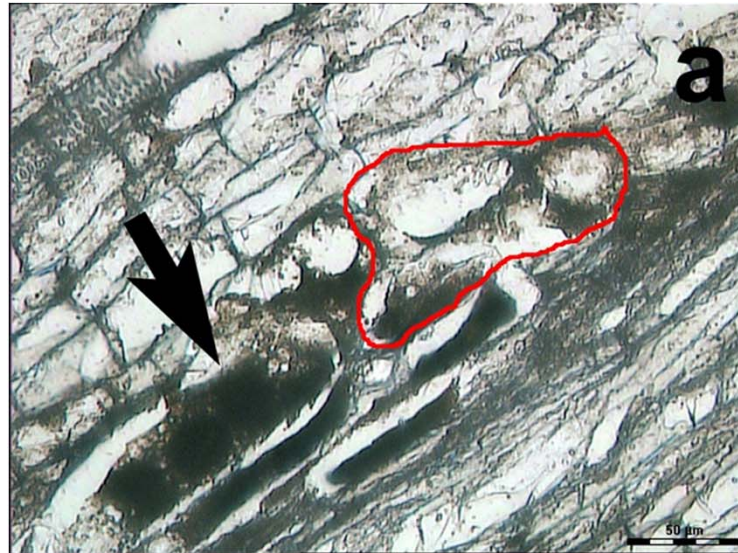




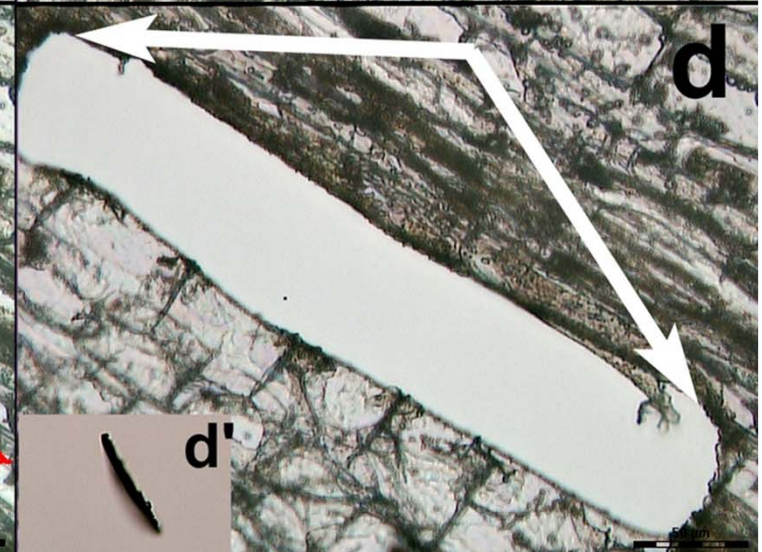
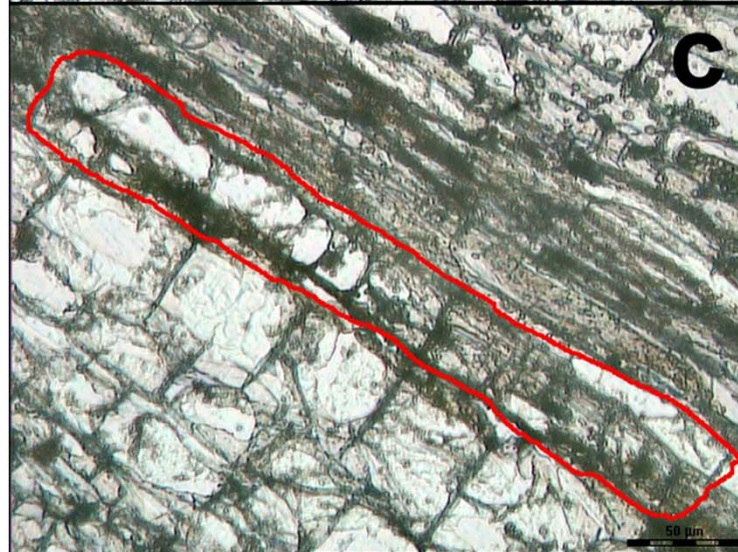
**before LM**

**after LM**

**RKN-infected**

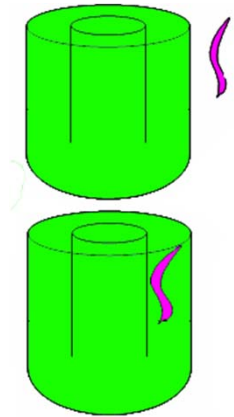


**control cells**



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

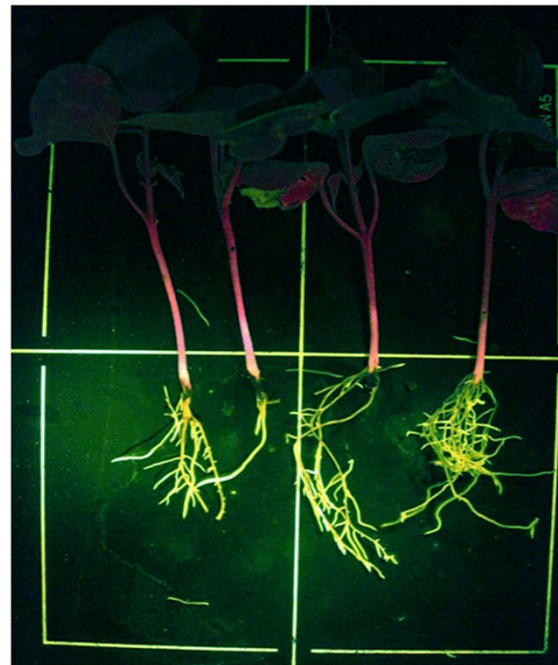
*in planta*  
expression/  
nematode  
inoculation



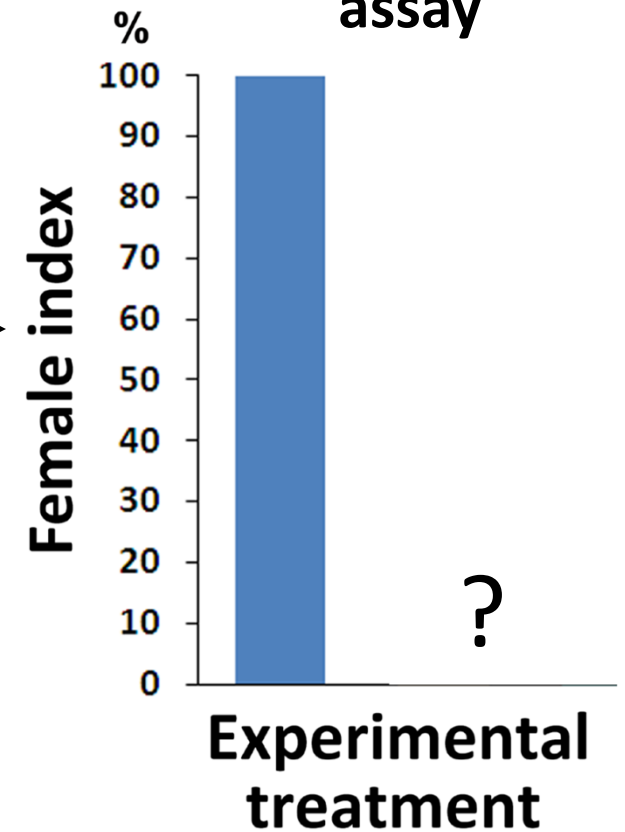
nematode  
feeding



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**



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