PLANT DEFENCE RESPONSES

TO ROOT-KNOT NEMATODE INTERACTION

"COST-ARRAY-2007"

Soledad Sanz-Alférez

PLANT-NEMATODE INTERACTION

- Root-knot nematodes

(*Meloidogyne* sp.)

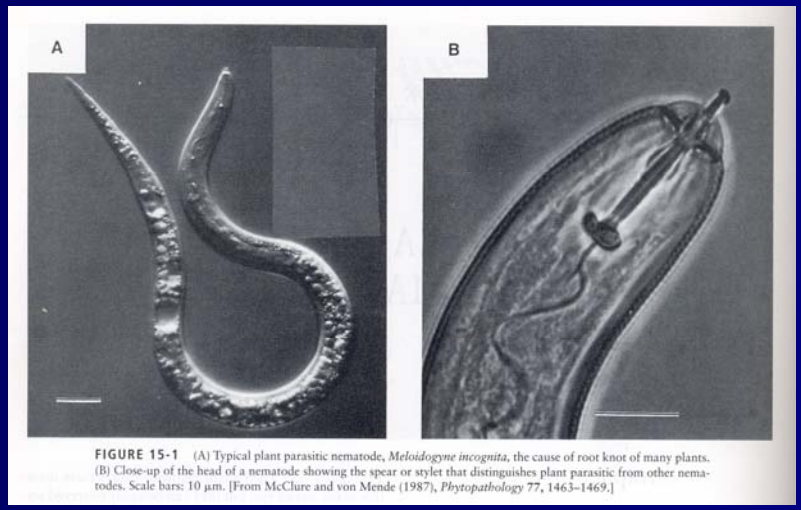
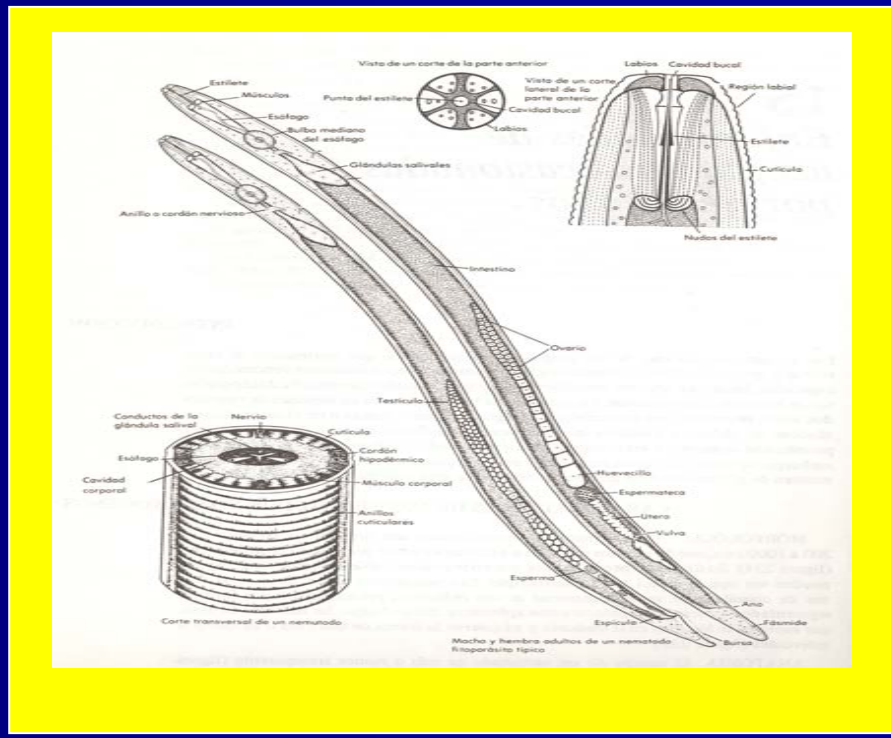


FIGURE 15-1 (A) Typical plant parasitic nematode, *Meloidogyne incognita*, the cause of root knot of many plants. (B) Close-up of the head of a nematode showing the spear or stylet that distinguishes plant parasitic from other nematodes. Scale bars: 10 μm. [From McClure and von Mende (1987), *Phytopathology* 77, 1463–1469.]

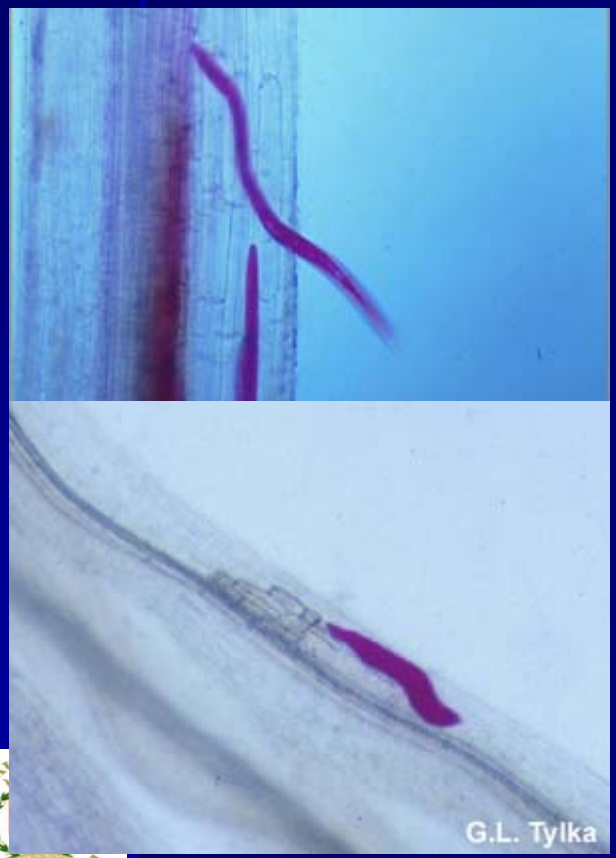


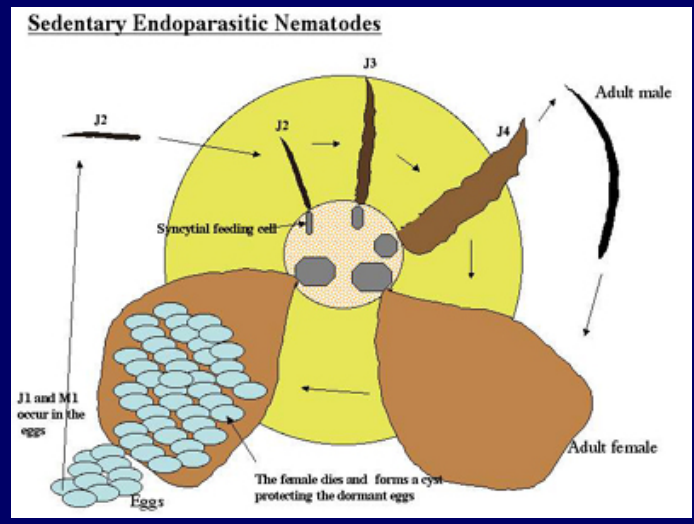
PLANT-NEMATODE INTERACTION

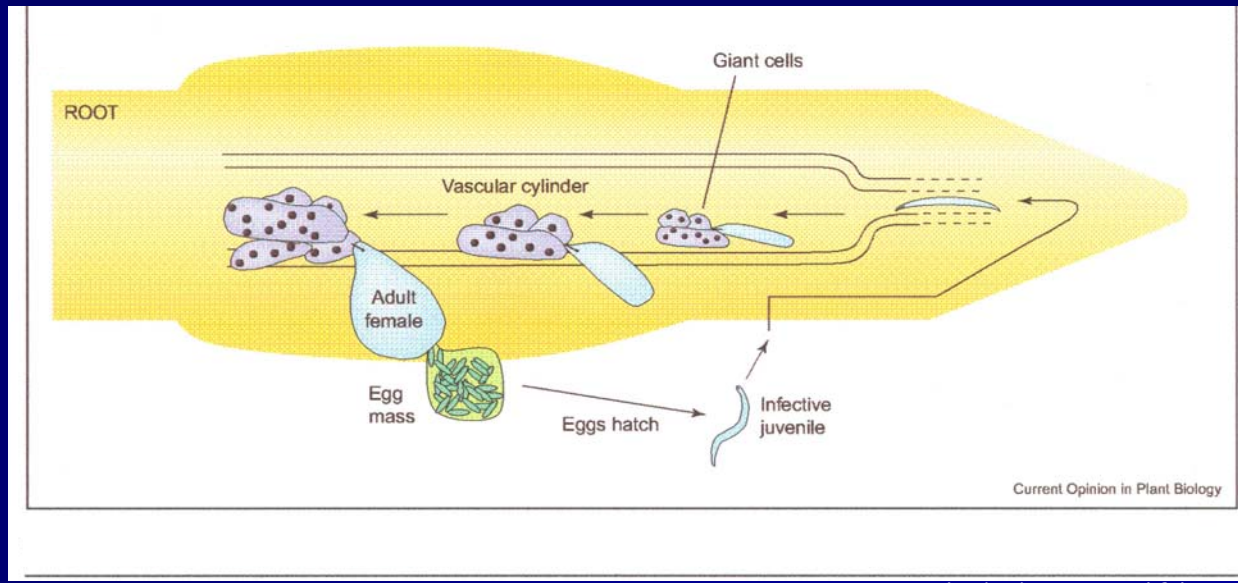
- Host plants
  - Tomato
  - Potato
  - Pepper
  - Squash
  - Eggplant
  - Cucumber
  - ....

PLANT-NEMATODE INTERACTION

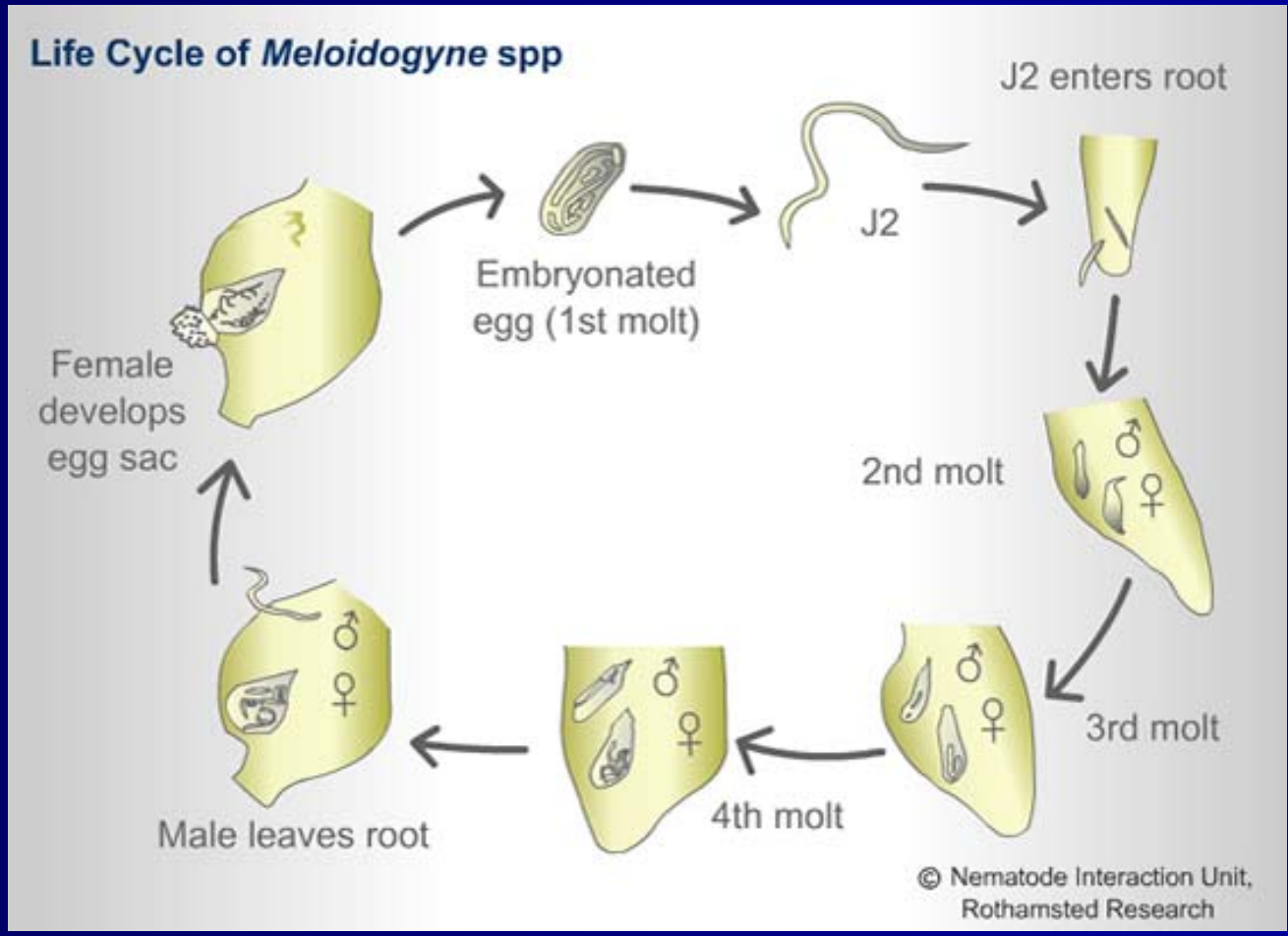
- Interaction



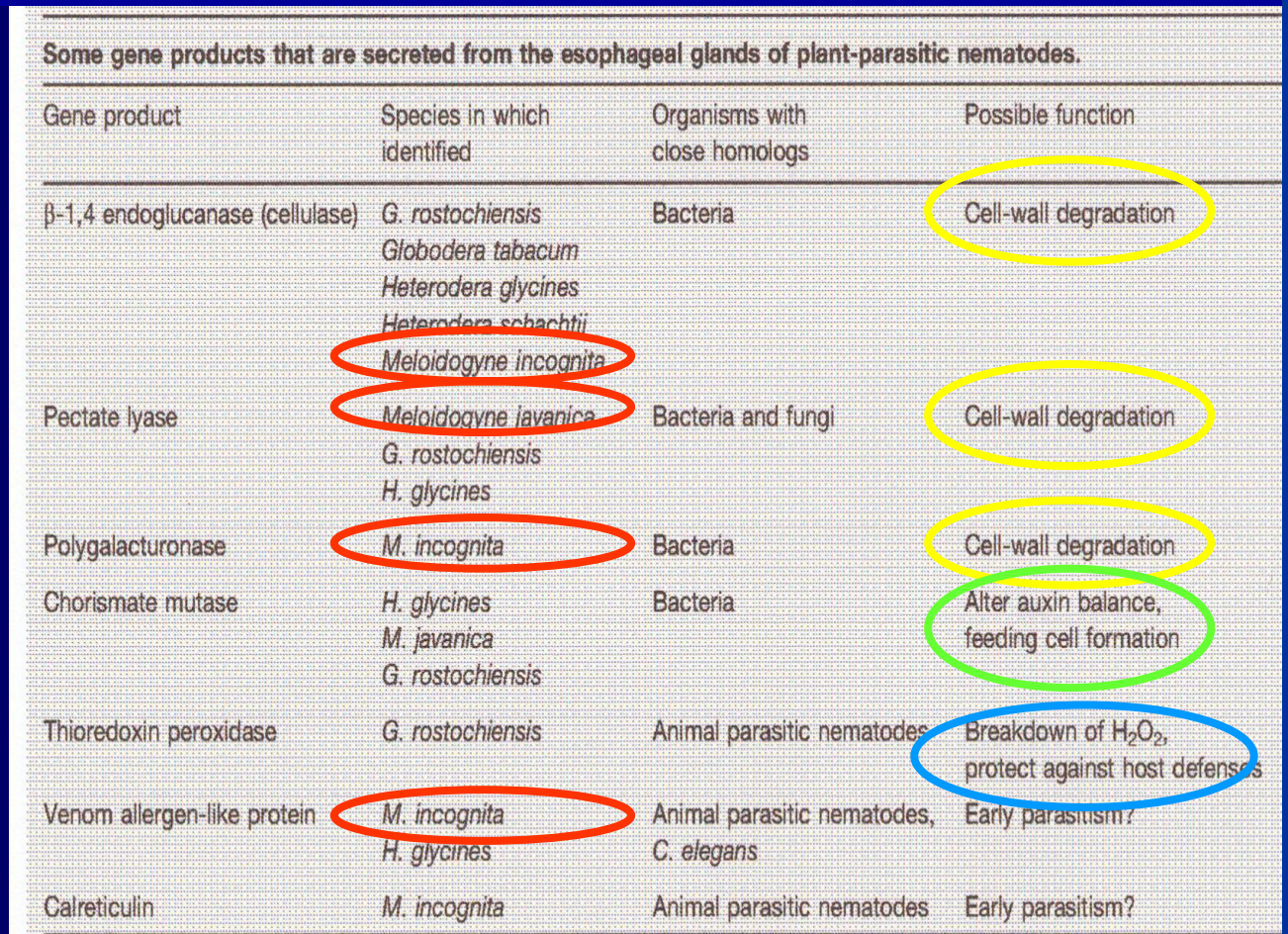




PLANT-NEMATODE INTERACTION



PLANT-NEMATODE INTERACTION

- 

Some gene products that are secreted from the esophageal glands of plant-parasitic nematodes.

Gene product	Species in which identified	Organisms with close homologs	Possible function
β-1,4 endoglucanase (cellulase)	*G. rostochiensis* *Globodera tabacum* *Heterodera glycines* *Heterodera schachtii* *Meloidogyne incognita*	Bacteria	Cell-wall degradation
Pectate lyase	*Meloidogyne javanica* *G. rostochiensis* *H. glycines*	Bacteria and fungi	Cell-wall degradation
Polygalacturonase	*M. incognita*	Bacteria	Cell-wall degradation
Chorismate mutase	*H. glycines* *M. javanica* *G. rostochiensis*	Bacteria	Alter auxin balance, feeding cell formation
Thioredoxin peroxidase	*G. rostochiensis*	Animal parasitic nematodes	Breakdown of H_2O_2, protect against host defenses
Venom allergen-like protein	*M. incognita* *H. glycines*	Animal parasitic nematodes, *C. elegans*	Early parasitism?
Calreticulin	*M. incognita*	Animal parasitic nematodes	Early parasitism?

PLANT-NEMATODE INTERACTION

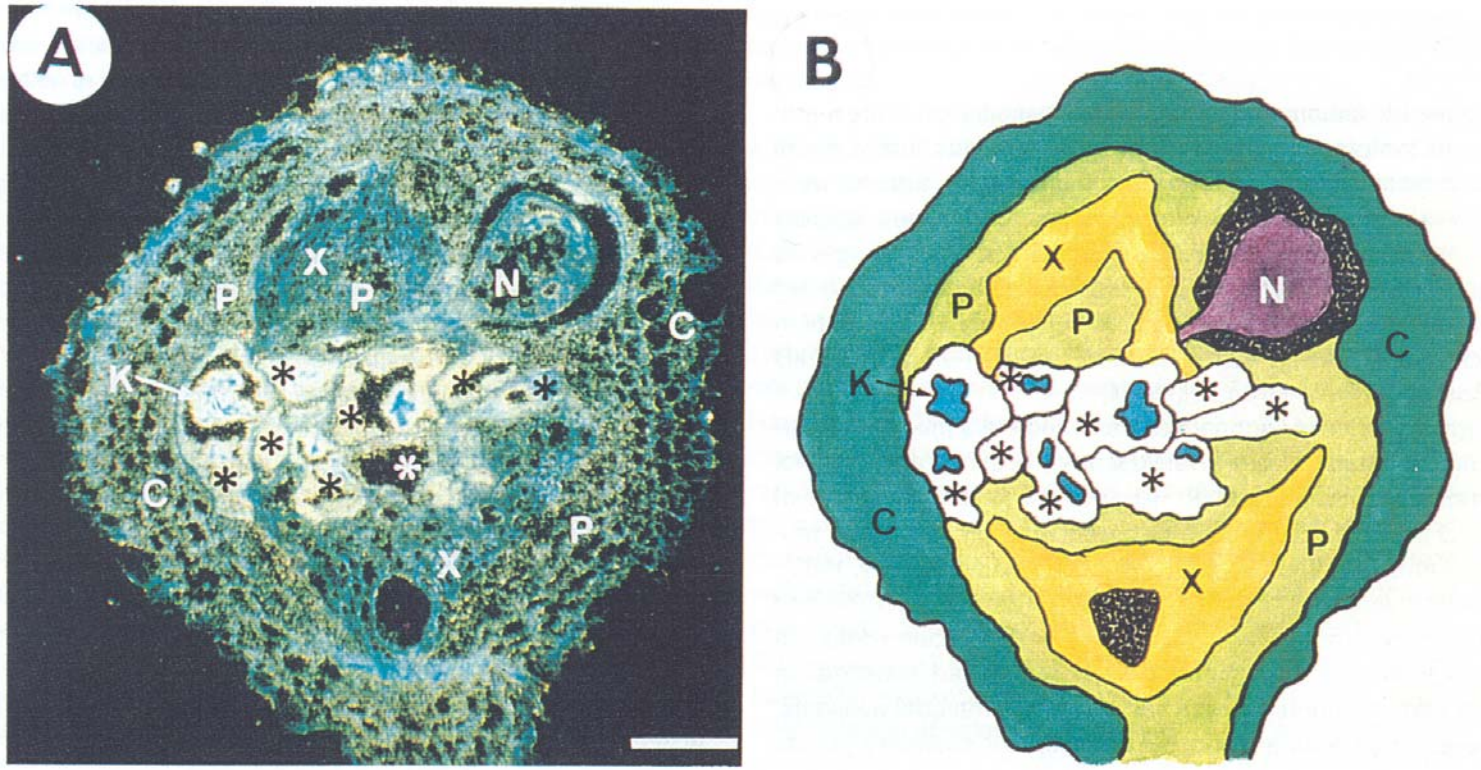


Figure 2. Cross-Section of a 4-Week-Old Gall Induced by *M. incognita* on Tomato Roots.

(A) In situ hybridization, using probe *Lemmi9*, a gene expressed in giant cells. This is a dark-field micrograph, and light-colored dots represent the *Lemmi9* hybridization signal. Bar = 200 μm.
(B) Schematic drawing of the gall in **(A)**.
This figure was reproduced from Van der Eycken et al. (1996) with permission of Blackwell Science Ltd. C, cortex; K, clustered nuclei; N, nematode; P, parenchymatic gall cells; X, xylem; *, giant cell.

PLANT-NEMATODE INTERACTION

- **Infected root cells – gene expression changes**
  - **Cell cycle activation**
    - CYC1At
    - CDC2
  - **Osmotic stress**
    - TobRB7
    - LEA like
  - **Phytohormones**
    - auxin
  - **Cytoskeleton arquitecture**
    - **actin**
    - **tubulin**
    - **formin-like**

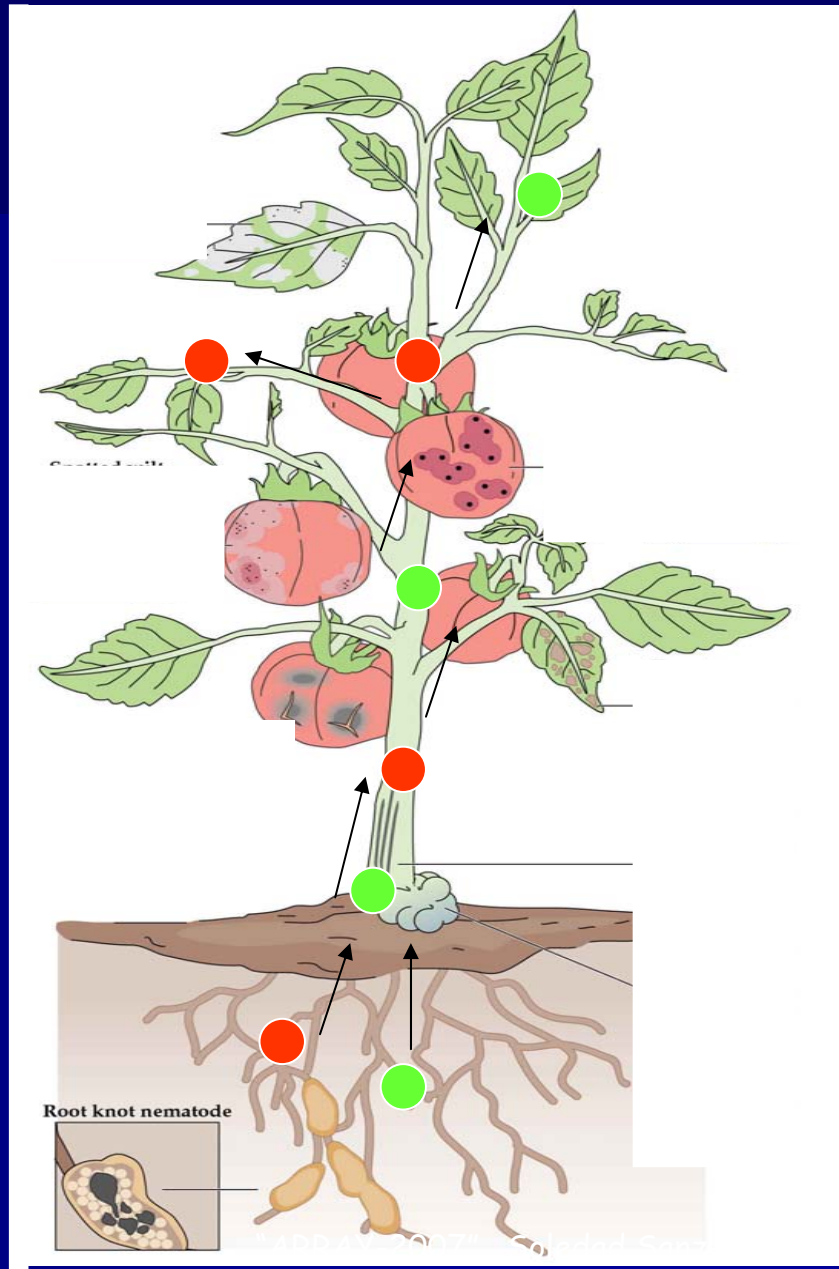
IMMEDIATE RESPONSES OF INVADED CELLS

… /…

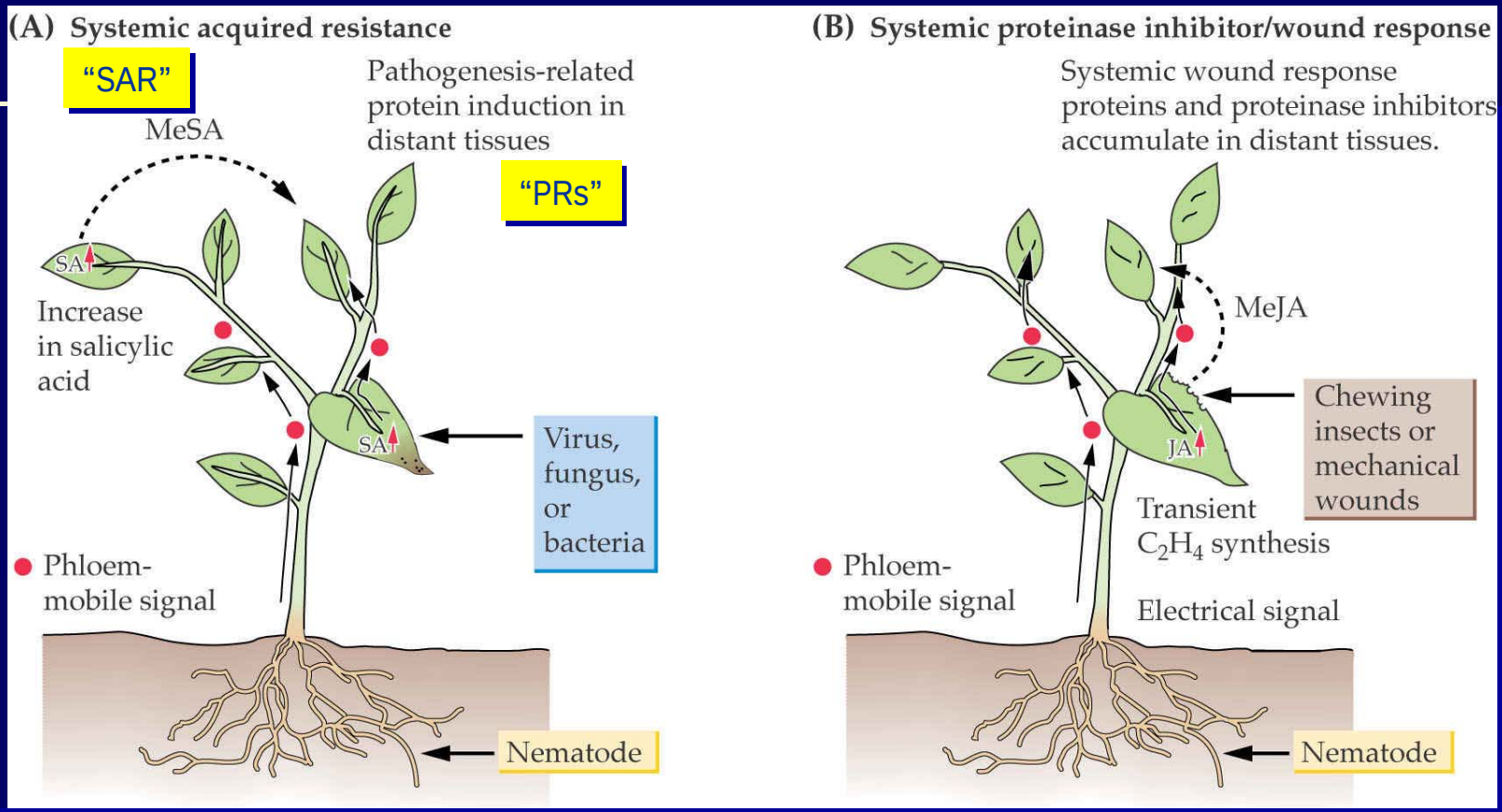
PLANT DEFENCE RESPONSES

SYSTEMIC RESPONSES
AND
GENE ACTIVATION

LOCAL RESPONSES
AND
GENE ACTIVATION



PLANT DEFENCE RESPONSES

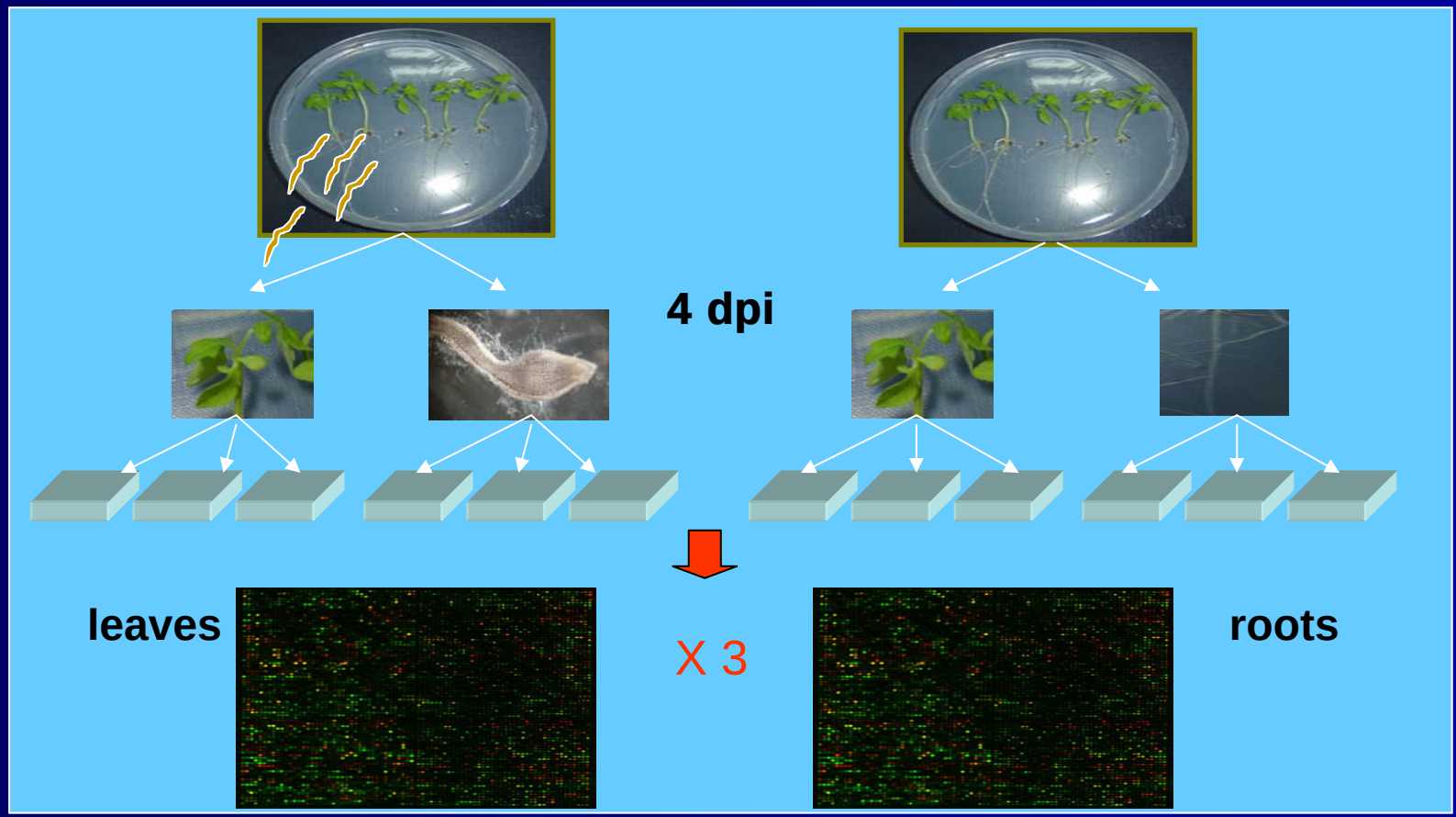


- Local and systemic plant responses induced by nematode

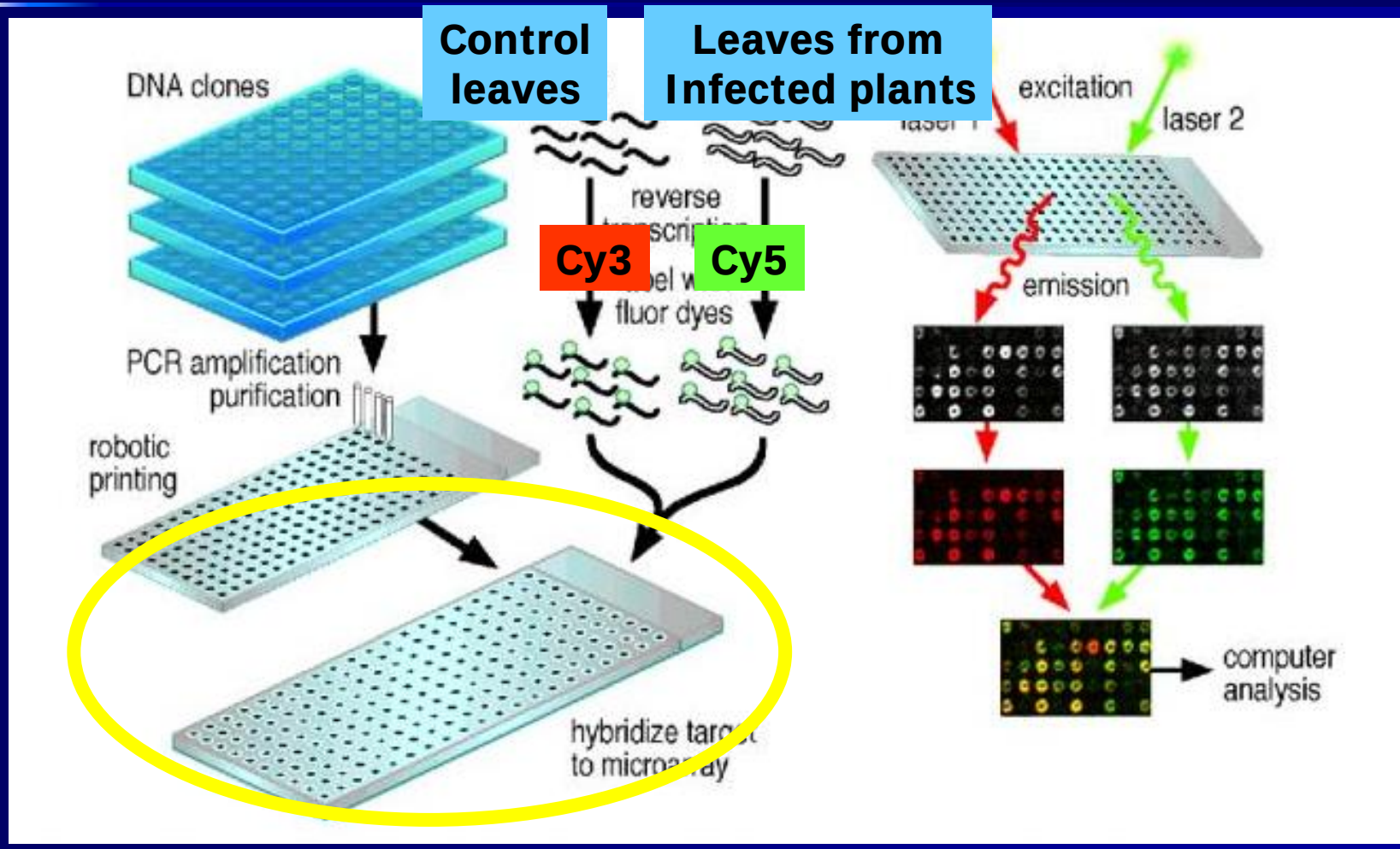
PLANT DEFENCE RESPONSES by GENE EXPRESSION PROFILING

Experimental design

- Tomato plants vs. *Meloidogyne javanica*

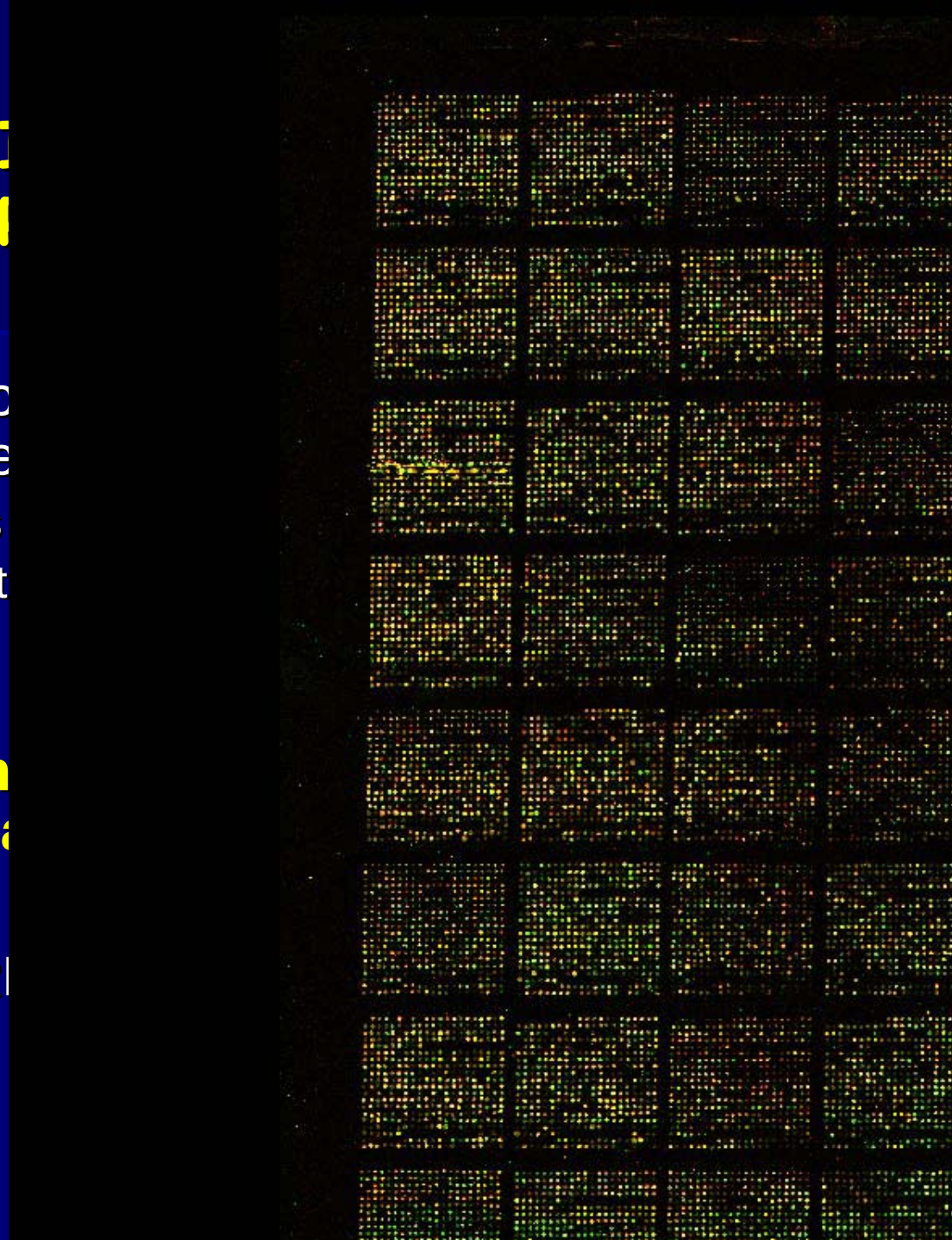


MICROARRAY.
GENE EXPRESSION PROFILING



M
GENE EXPI

- TOM1 cDNA micro
  Thomson Institute
  - 12900 EST clones
  - 8500 independent
  - 384 well plates
- **1. Hybridization**
  infected plants a
- 2. Hybridization RI
  infected roots



cDNA MICROARRAY
Systemic Gene Expression Profiling

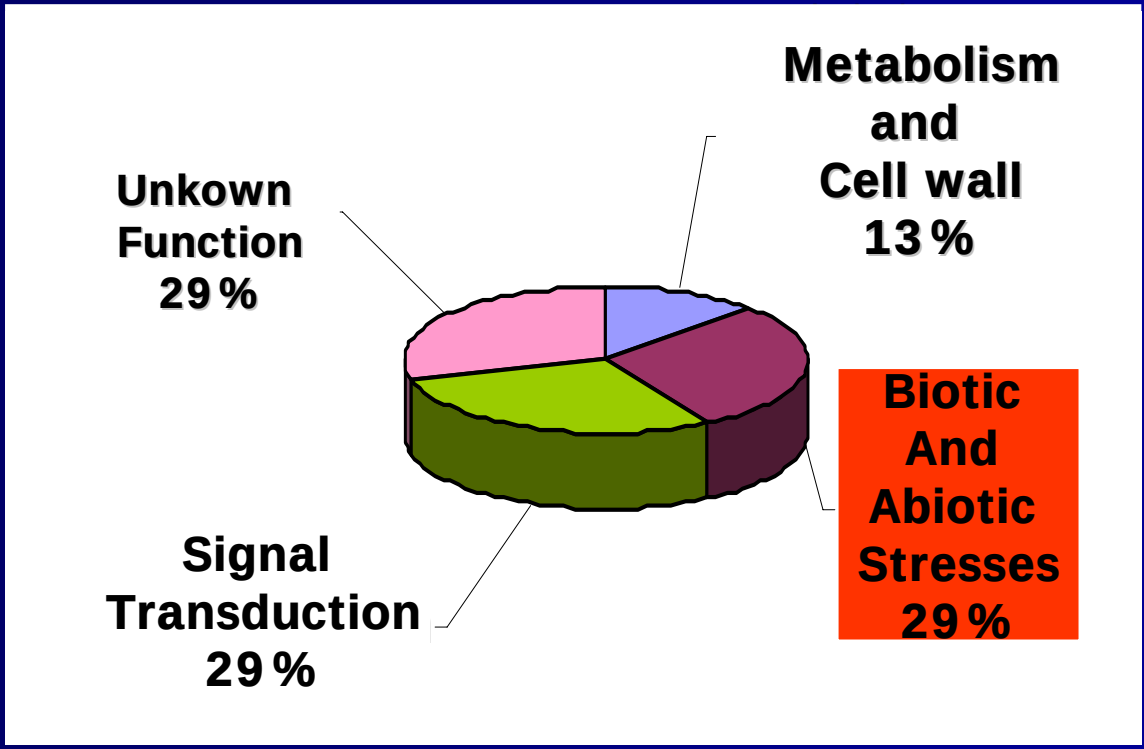
- Results
  - Numerical data
  - Data processing: $\log_2$ transformation, ratio
  - Normalization
  - Statistics: mean, SD, Pvalue (<0.05)
  - Identification of differentially expressed genes (FDR: false discovery rate)

cDNA MICROARRAY
Systemic Gene Expression Profiling

- 236 diferentially expressed genes
  - **181 induced genes**
  - **55 repressed genes**
- Functional categories

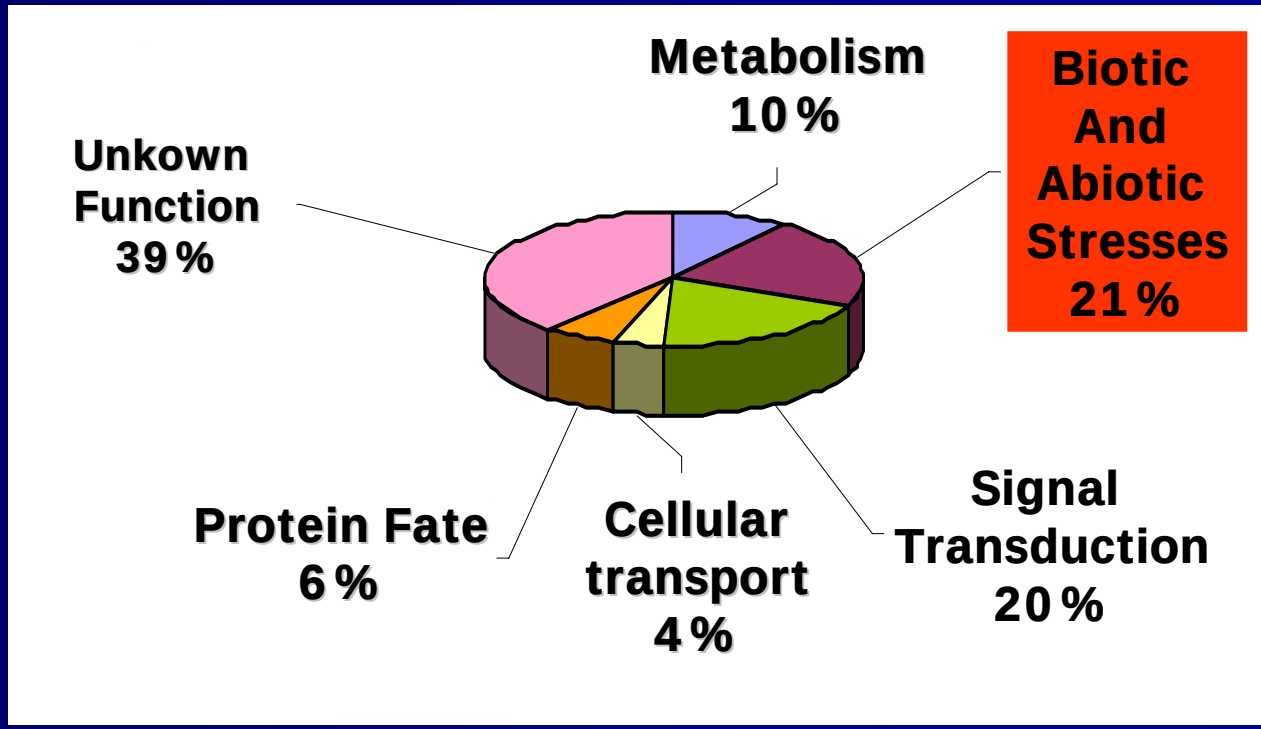
cDNA MICROARRAY
Systemic Gene Expression Profiling

- Main functional categories of Induced genes



cDNA MICROARRAY
Systemic Gene Expression Profiling

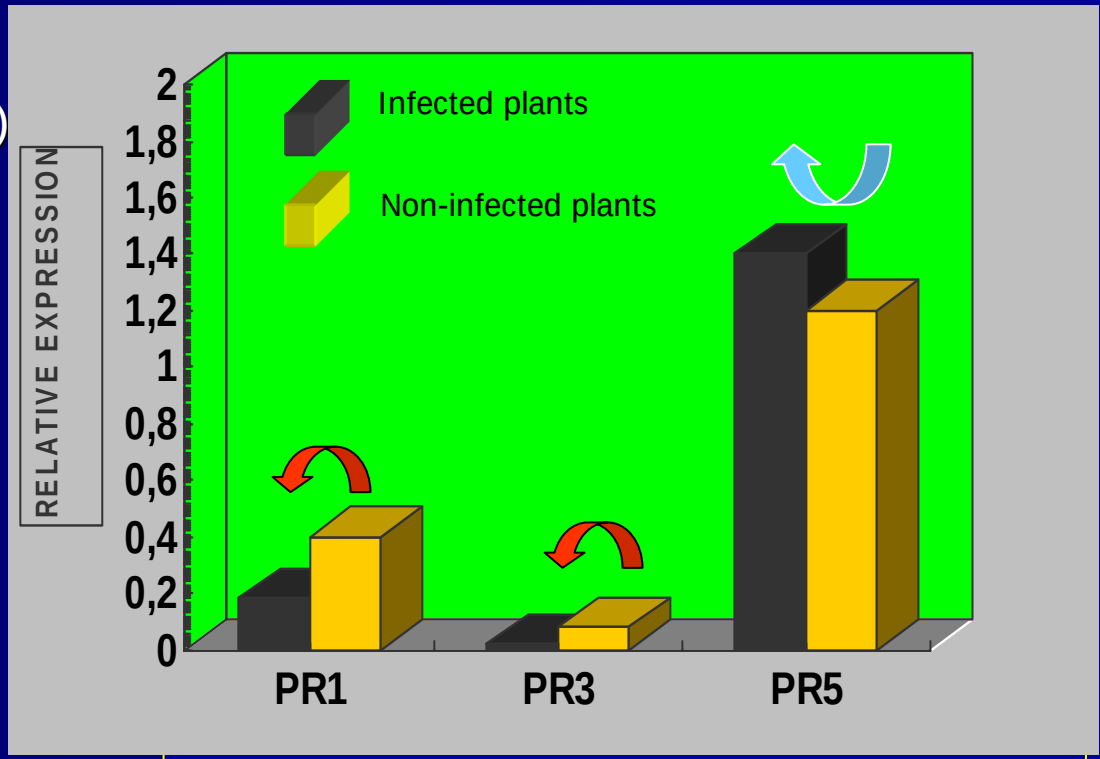
- Main functional categories of repressed genes



cDNA MICROARRAY
Systemic Gene Expression Profiling

- Validation of data by real-time qRT-PCR

- ✓ SYBER Green
- ✓ 18S (housekeeping gene)
- ✓ Relation of Ct values
- ✓ Triplicate samples



cDNA MICROARRAY
Systemic Gene Expression Profiling
Conclusions

- Microarray technology makes it possible to obtain information about patterns of systemic gene expression during plant-nematode interaction
- The interaction of nematode is associated with a suppression of plant defence mechanisms
- Differential expression pattern reflets the complexity of plant responses during nematode interaction

cDNA MICROARRAY
Systemic Gene Expression Profiling

- In the future
  - Microarray data from roots assay (in progress...)
  - Comparison to gene expression patterns by other species of nematodes or pathogens using TED

cDNA MICROARRAY
Systemic Gene Expression Profiling

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  - Dra. Francisca Fernández del Campo
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- Funded by Ministry of Science and Education
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