



# *Rpp*-Mediated Gene Expression in Resistant and Susceptible Soybean Lines

**Katherine Schneider**

USDA-ARS, Foreign Disease-Weed Science Research Unit

**Martijn van de Mortel**

Iowa State University, Department of Plant Pathology

IOWA STATE UNIVERSITY



# Outline

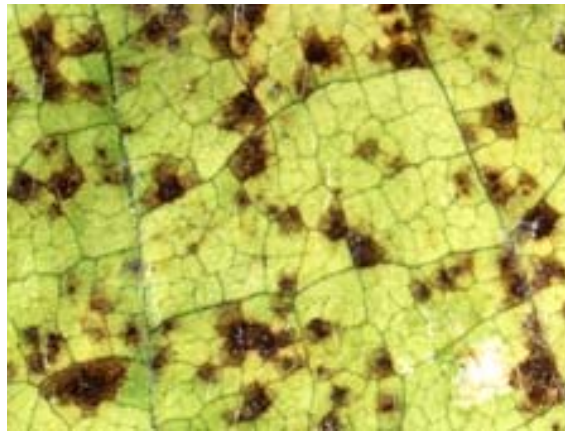
- SBR disease phenotypes
- Our approach to the problem - microarrays
- Experimental design
- Data analysis:
  - Validation of the experimental design
  - Expression patterns
  - Functional categories of differentially expressed genes
  - Example: Secondary metabolism
  - Comparison of different *R*-gene responses
- Conclusions



# Infection phenotypes



immune  
*Rpp1*



red-brown  
*Rpp2 Rpp3 Rpp4*



tan

---

resistant

susceptible

- There are changes in host gene expression that lead to 3 phenotypes
- Need a method that can tell us information about many genes at once

# Overview

---

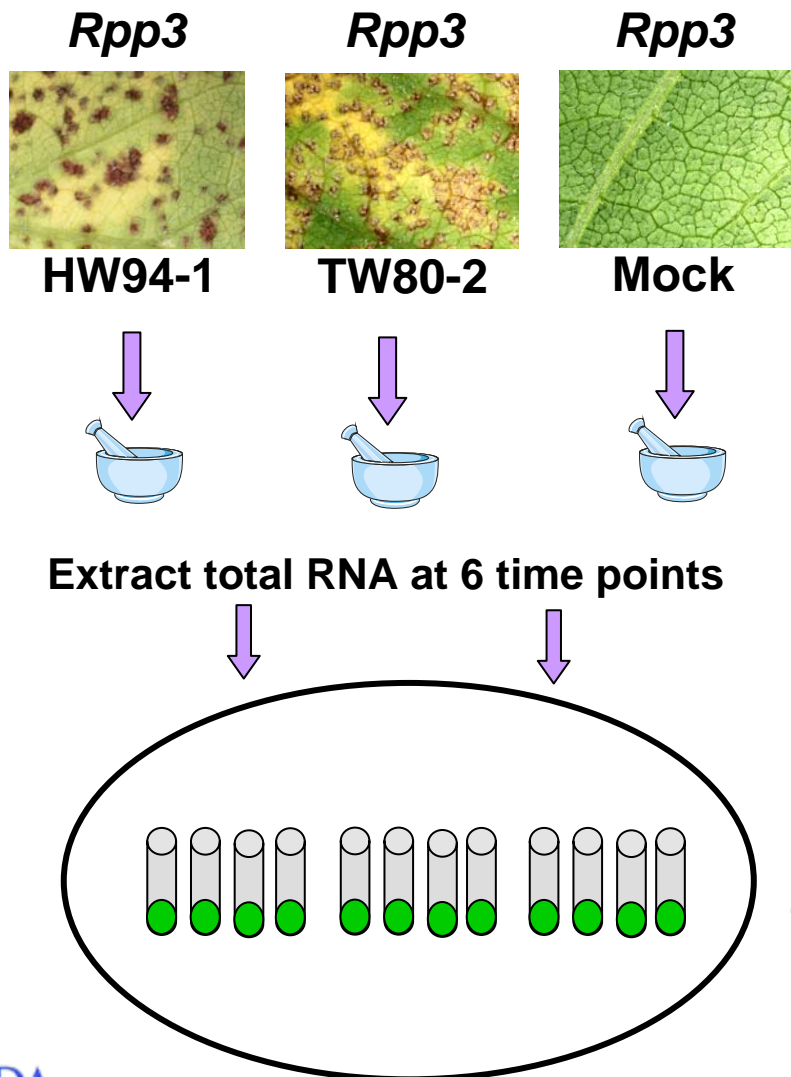
- **Approach:** Study changes in gene expression using microarrays
- **Rationale:** Knowing which genes are 'on' in a resistant plant will help us discover the mechanism of resistance
- **Significance:** Understanding which genes influence resistance will give breeders new targets for development of resistant lines and suggest novel control strategies

# Microarray analysis

- Method of measuring thousands of mRNAs in a sample easily
- Affymetrix Soybean Genome GeneChips
- Measure the abundance of ~37500 soybean mRNAs simultaneously
- Snapshot of which genes are 'on' and 'off' at a particular time in the cell
- Measure gene expression at multiple times during infection

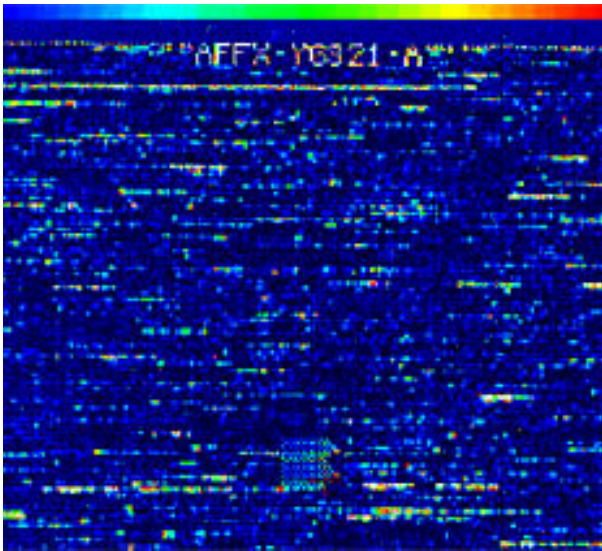
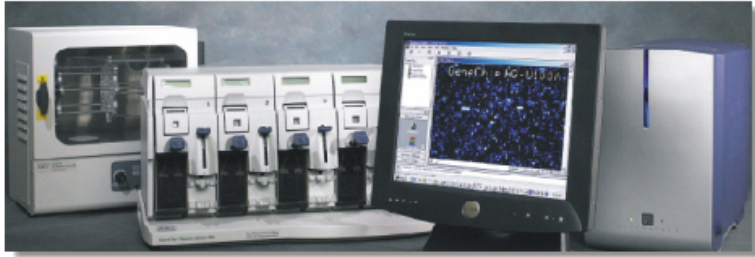


# Experimental design



- 1 genotype:  
*Rpp3*-containing plant introduction
- 3 treatments:  
Virulent SBR isolate (Taiwan 80-2)  
Avirulent SBR isolate (Hawaii 94-1)  
Mock inoculation
- 3 replications
- 6 time points:  
12, 24, 72, 144, 216, 288h
- 54 samples

# Data capture and processing



Raw data



Quality control  
Statistical processing

Barcode: @52011900578836071706401148590090  
 Probe Array Lot: 4011485  
 Expiration Date: Jul 17 2006 12:00AM  
 Experiment User: labuser  
 Algorithm: Statistical  
 HZ=4 VZ=4 BG=2 SmoothFactorBG=100 Epsilon=0.5 BF= Alpha1=0.05 Alpha2=0.065 Tau  
 Background=Avg:43.81,Stdev:0.47,Max:45.0,Min:42.5 Noise=Avg:1.60,Stdev:0.05,Max:1.8

Probe Set Name	Stat Pairs	Stat Pairs Used	Signal	Detection	Detectic
AFFX-BioB-5_at	20	20	260.2	P	
AFFX-BioB-M_at	20	20	362.9	P	
AFFX-BioB-3_at	20	20	283.3	P	
AFFX-BioC-5_at	20	20	726.4	P	
AFFX-BioC-3_at	20	20	776.5	P	
AFFX-BioDn-5_at	20	20	1778	P	
AFFX-BioDn-3_at	20	20	2372	P	
AFFX-CreX-5_at	20	20	7912.6	P	
AFFX-CreX-3_at	20	20	9114.9	P	
AFFX-DapX-5_at	20	20	3215.9	P	
AFFX-DapX-M_at	20	20	4460.3	P	
AFFX-DapX-3_at	20	20	5086.3	P	
AFFX-LysX-5_at	20	20	504.6	P	
AFFX-LysX-M_at	20	20	583	P	

Gene lists

# Collaborative effort between USDA-ARS & ISU

- *Rpp1*- USDA-ARS, submitted
- *Rpp2*- ISU, published
- *Rpp3*- ISU & USDA-ARS, study complete, manuscript in process
- *Rpp4*- ISU & USDA-ARS, data collection done, analysis to come
  - Focus on *Rpp3* data and compare to *Rpp1* and *Rpp2*

MPMI Vol. 20, No. 8, 2007, pp. 887–899. doi:10.1094/MPMI-20-8-0887. © 2007 The American Phytopathological Society

MPMI  
SPOTLIGHT

e-Xtra\*

## Distinct Biphasic mRNA Changes in Response to Asian Soybean Rust Infection

Martijn van de Mortel,<sup>1</sup> Justin C. Recknor,<sup>2</sup> Michelle A. Graham,<sup>3</sup> Dan Nettleton,<sup>2</sup> Jaime D. Dittman,<sup>1</sup> Rex T. Nelson,<sup>2</sup> Cláudia V. Godoy,<sup>4</sup> Ricardo V. Abdelnoor,<sup>4</sup> Alvaro M. R. Almeida,<sup>4</sup> Thomas J. Baum,<sup>1</sup> and Steven A. Whitham<sup>1</sup>

<sup>1</sup>Department of Plant Pathology, <sup>2</sup>Department of Statistics, and <sup>3</sup>United States Department of Agriculture–Agricultural Research Service CICGR Unit and Department of Agronomy, Iowa State University, Ames 50011, U.S.A.; <sup>4</sup>Embrapa Soybean Research Center, Cx P 231, Londrina, 86001-970 PR, Brazil

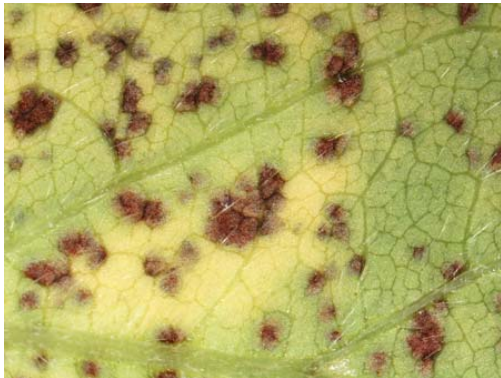
Submitted 30 March 2007. Accepted 30 April 2007.



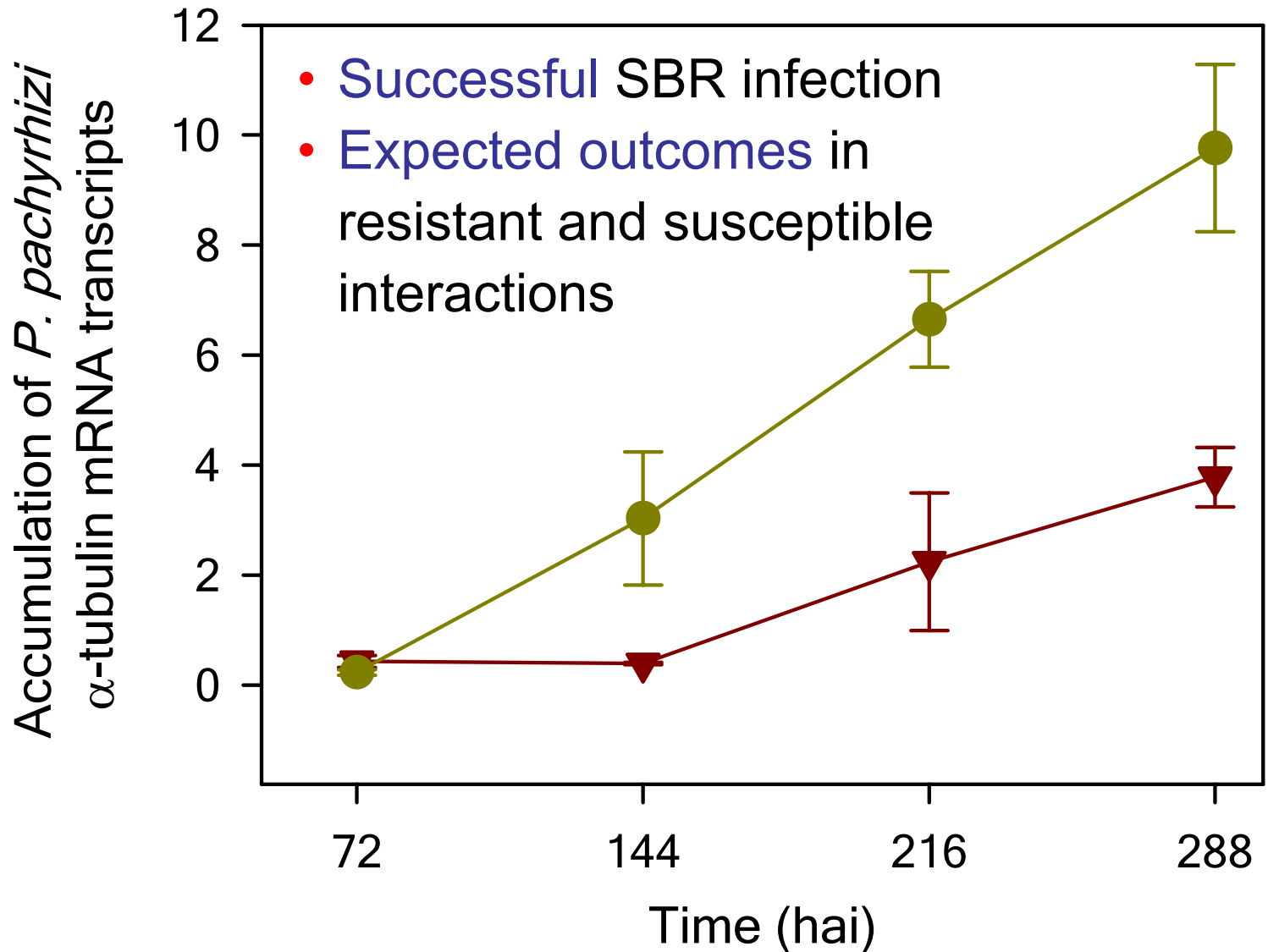
# RESULTS



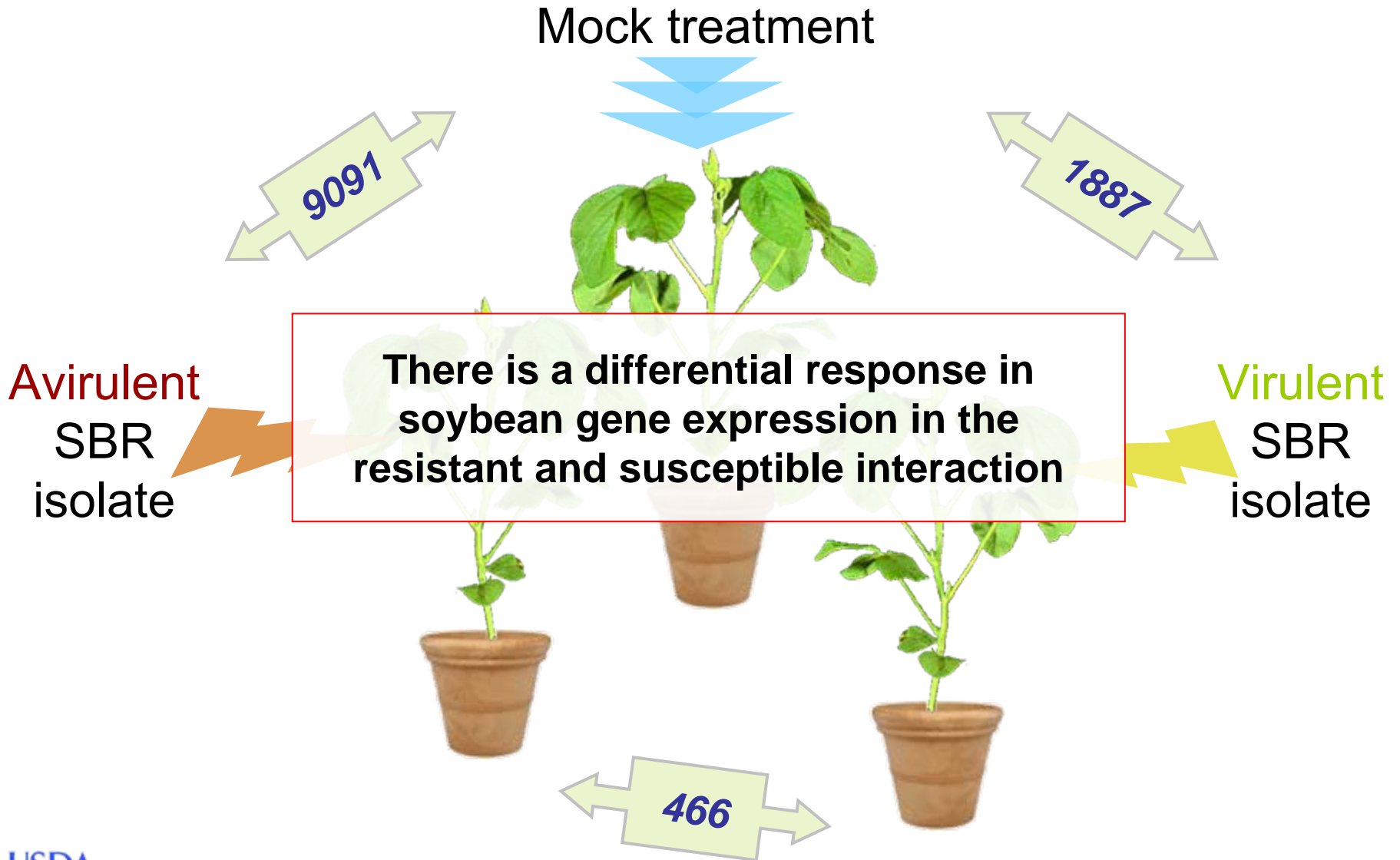
# Validation of experimental design



# Validation: SBR fungus accumulation



# Results microarray analysis



# Function of differentially expressed genes

Affy spot ID	q-value	UniProt Top Hit (BLX, $E < 10^{-4}$ )	Functional class ID	Functional class term
GmaAffx.54524	9.784E-16	Caffeoyl-CoA O-methyltransferase (Fragment)	GO:0009809	lignin_biosynthesis
Gma.15664.1.S	1.15834E-15	2'-hydroxydihydrodaidzein reductase	GO:0009813	flavonoid_biosynthesis
GmaAffx.76384	1.34328E-15	Disease resistance-responsive family protein	GO:0006952	defense_response
GmaAffx.55767	1.37842E-15	Putative calmodulin-like protein	GO:0000004	biological_process_unknown
Gma.5947.1.S1	1.37877E-15	Pathogenesis-related class 10 protein SPE-16 (Fragment)	GO:0006952	defense_response
Gma.18084.1.S	1.49147E-15	Calcium-binding EF-hand; Ferric reductase-like trans		

Functional class ID	Total on GeneChip	# diff. expr.	Adj p-value	Description
GO:0009809	80	45	5.2E-07	lignin biosynthetic process
GO:0009813	79	45	3.0E-07	flavonoid biosynthetic process
GO:0006952	358	126	0.001	defense response
GO:0009807	20	11	1.89	lignan biosynthetic process
GO:0006499	522	156	1.01	N-terminal protein myristoylation
GO:0009621	39	17	3.84	No GO term
GO:0000004	7970	1928	317	biological_process_unknown
GO:0006952	358	126	0.001	defense response

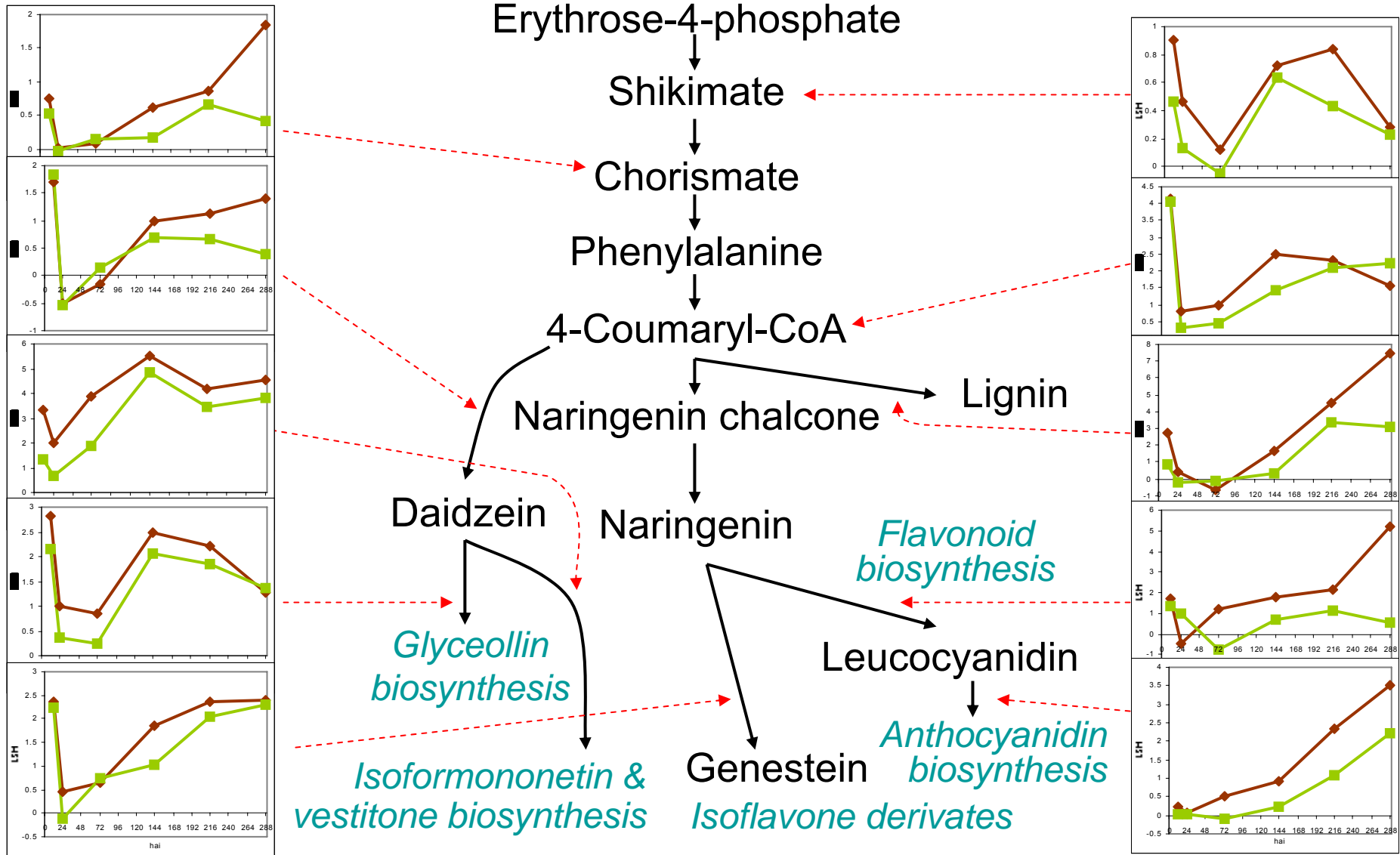
# Functional classes of SBR genes

---

Statistical analysis was used to determine overrepresented functional classes:

- I. Defense/stress responses
- II. Secondary metabolism
- III. Photosynthesis
- IV. Miscellaneous

# Shikimate/phenylpropanoid pathway



# Common and different features observed

- **Biphasic response** in differential expression profiles
- *Rpp1* - Early oxidative burst, compressed time frame, abundance of defense-response genes, secondary metabolism
- *Rpp2* – Defense/stress-responsive genes, secondary metabolism, transport functions, transcription/regulation
- *Rpp3* - Defense/stress-responsive genes, secondary metabolism, photosynthesis, transcription factors

# Conclusions



# Conclusions

## Rpp3 resistance gene

- Different outcomes SBR infection in resistant and susceptible interactions:
  - Fungal biomass
  - Differentially expressed genes
- Biphasic SBR gene regulation
- Main functional categories: Defense/stress functions, secondary metabolism and photosynthesis, gene regulators
- Importance of secondary metabolism in SBR resistance

## Comparison of Rpp1, -2- and -3-mediated resistance

- Similar overall biphasic expression profile
- Unique responses / common functional classes



# Conclusions, continued

---

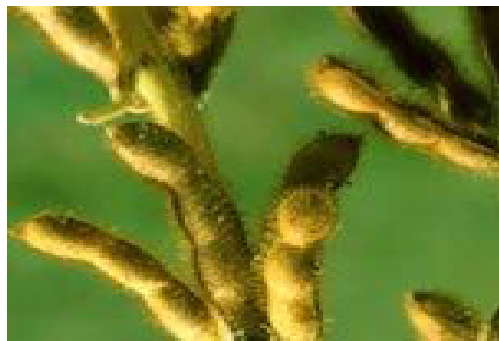
## Comparison of *Rpp1*, -2- and -3-mediated resistance

- **Unique** responses / **common** functional classes
  - Protection against oxidative stress
  - Secondary metabolism for defense compounds and physical reinforcement
  - Regulation of transcription



# Future directions

- Test functional role of candidate genes
- *Rpp4* experiment data analysis
- Meta-analysis of all four *R*-gene backgrounds







# Expression patterns of common SBR genes

