

# Expression analysis of the *Rpp1*-mediated immune reaction to *Phakopsora pachyrhizi* using the Affymetrix Soybean Genome Array

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## Introduction

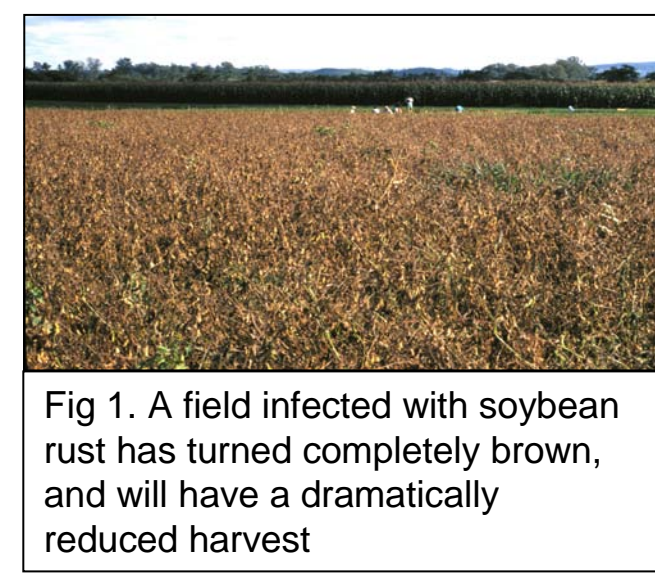
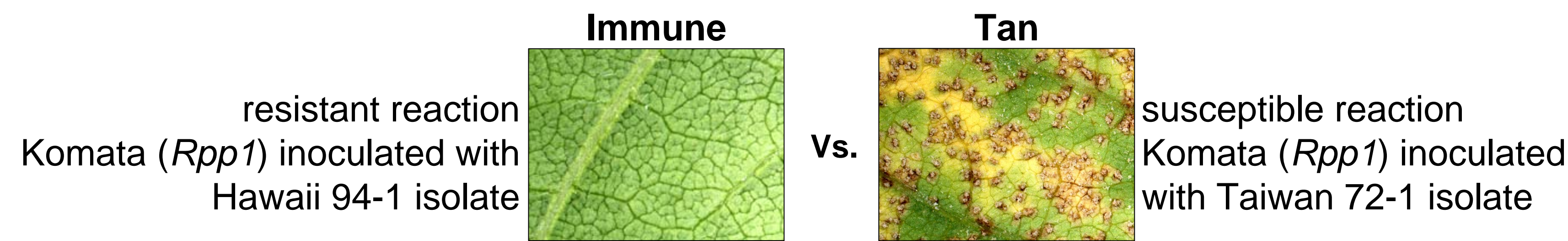


Fig 1. A field infected with soybean rust has turned completely brown, and will have a dramatically reduced harvest

Soybean rust is a devastating foliar disease of legumes, caused by the fungus *Phakopsora pachyrhizi*. Field infections can sometimes lead to 100% yield loss (Fig 1.), and since the U.S. produces over 70 million tons of soybeans per year, soybean rust is an important concern for U.S. agriculture. As of early November 2006, soybean rust had been identified in 15 U.S. states, as far north as Illinois and Indiana. Soybean rust is currently managed through the use of fungicides, and the development of rust tolerant or resistant varieties of soybeans is a top priority. There are currently four independent rust resistance genes (*Rpp1*-*Rpp4*) that have been identified in non-commercial cultivars. Several isolates of soybean rust have been identified that overcome the resistance provided by *Rpp1*-*Rpp4*. Therefore, new sources of rust resistance are needed.

Microarray technology allows the expression of thousands of genes to be simultaneously measured without any prior knowledge of the genes. Using this technology, will be able to identify soybean genes that change expression in a rust resistant reaction. The cultivar Komata (PI200492) carries the *Rpp1* rust resistance gene. When inoculated with specific isolates of *P. pachyrhizi*, Komata displays resistance to rust that has been termed the "Immune reaction" and is characterized by a lack of visible symptoms. In this study we compare the gene expression in the Immune reaction (Fig. 2, below left) to gene expression in the fully susceptible "Tan" reaction (Fig. 2, below right) using the Affymetrix Soybean Genome Array. Genes that show significantly different expression between Immune and Tan reactions are good candidates to be involved in rust resistance.

Fig 2. Photographs illustrating the Immune reaction and the Tan reaction on Komata plants



## Methods

•Soybean plants carrying the *Rpp1* resistance gene (PI200492, Komata) were inoculated with *P. pachyrhizi* isolates **Hawaii 94-1** and **Taiwan 72-1** that result in an **Immune** or **Tan** reaction, respectively.

•After inoculation the plants were incubated at 20° C with constant dew for approximately 24 hours.

•Whole leaves were harvested and flash-frozen in liquid nitrogen at 6, 12, 24, and 48 hours post inoculation.

•Leaflets were pooled from 4 to 6 plants at each time point, from three independent experiments, and RNA was extracted using Trizol/GITC<sup>4</sup> (Fig. 3).

•RNA samples from various time points were labeled with biotin using the Affymetrix One-Cycle kit<sup>5</sup> and hybridized to arrays for 16 hours.

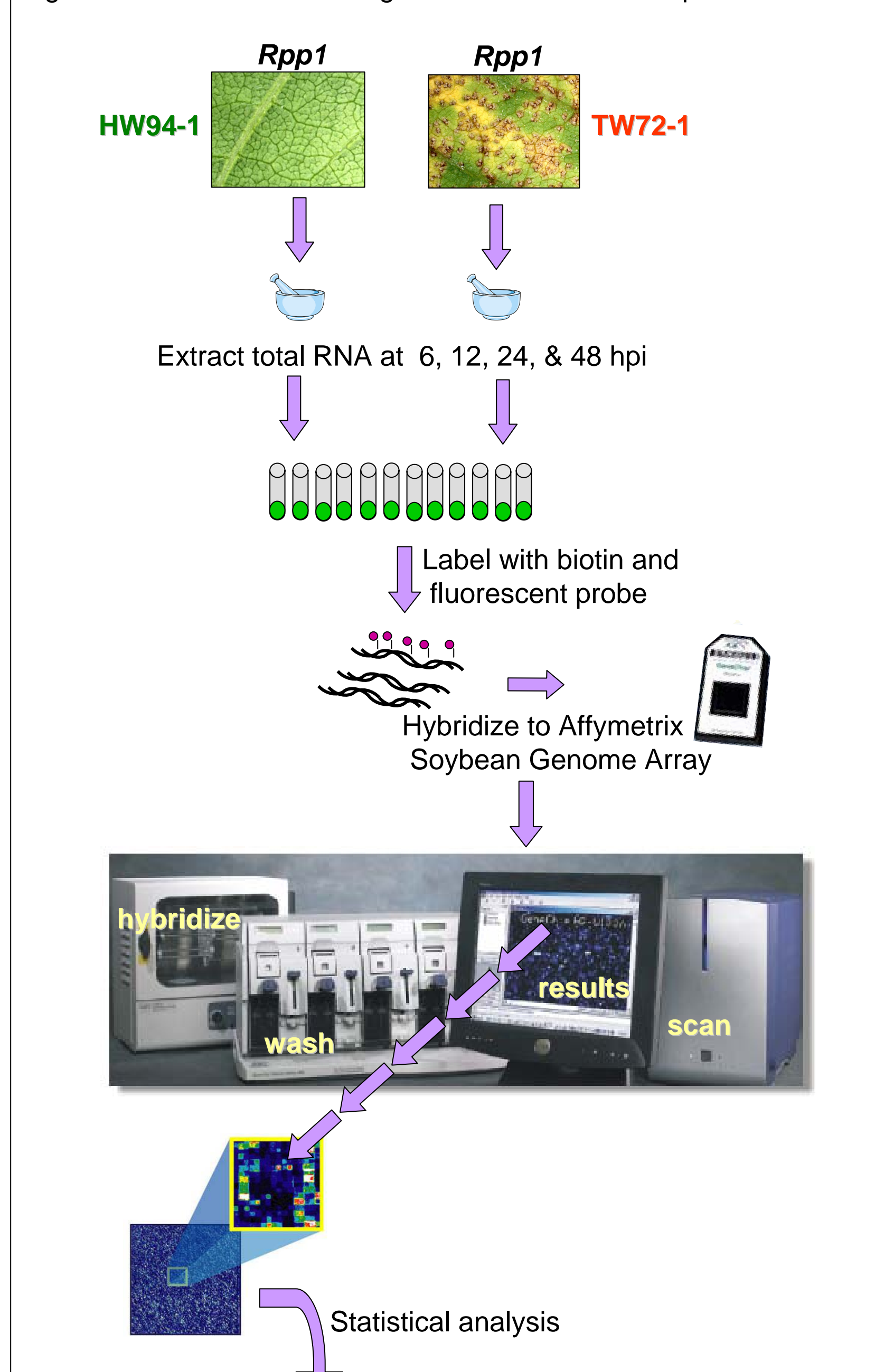
•RNA samples were washed and stained with R-phycoerythrin Streptavidin<sup>6</sup> using the Affymetrix Fluidics Station 450, and scanned using the Affymetrix Gene Chip Scanner 3000.

•Raw data were subjected to quality control procedures and background correction, normalization, and summarization were applied.

•Summarized data was analyzed using Bioconductor<sup>7</sup> software and the statistical language "R" to produce a set of differentially expressed genes.

•Differentially expressed genes were identified by BLAST X similarity search to proteins in GenBank<sup>9</sup> and assigned to GO functional categories using the UniProt and PFAM<sup>10</sup> databases.

Fig 3. A schematic illustrating the work flow of the experiment



Probe Set Name	Star Pairs	Star Pairs Used	Signal	Detection	Detection p-value	Stat Common Pairs	Signal Log Ratio Low	Signal Log Ratio High	Change	Change p-value
AFFX-B0B-5_at	20	20	62.5	P	0.003212	20	0.2	0	0.5	0.990372
AFFX-B0B-M_at	20	20	93.6	P	0.000081	20	0.3	0	0.6	0.690521
AFFX-B0B-3_at	20	20	56.5	P	0.000044	20	0.3	-0.2	0.7	0.537481
AFFX-B0C-5_at	20	20	211.3	P	0.000052	20	0.1	0	0.2	0.99967
AFFX-B0C-3_at	20	20	192.4	P	0.000044	20	0.2	0.1	0.3	0.99507
AFFX-B0Dn-5_at	20	20	573.2	P	0.000044	20	0.3	0.2	0.5	0.5
AFFX-B0Dn-3_at	20	20	902.6	P	0.000044	20	-0.1	-0.2	0	0.999999
AFFX-CreX-5_at	20	20	2851.5	P	0.000044	20	0.2	0.1	0.2	0.1
AFFX-CreX-3_at	20	20	3406.3	P	0.000044	20	0.2	0.1	0.2	0.999999
AFFX-DapX-5_at	20	20	754.4	P	0.000044	20	-0.1	-0.1	0	0.1
AFFX-DapX-M_at	20	20	1290.2	P	0.000052	20	-0.2	-0.2	-0.1	0.1
AFFX-DapX-3_at	20	20	1616.4	P	0.000044	20	-0.1	-0.2	0	0.1
AFFX-Lyx-5_at	20	20	86	P	0.000044	20	-0.3	-0.4	-0.1	0.1
AFFX-Lyx-M_at	20	20	116.2	P	0.000052	20	-0.4	-0.6	-0.1	0.1
AFFX-Lyx-3_at	20	20	303.8	P	0.000044	20	-0.2	-0.4	-0.1	0.1
AFFX-PheX-5_at	20	20	155.2	P	0.000052	20	-0.1	-0.3	0	0.1

Fig 4. An example of the raw data that is produced by the Affymetrix GCOS software

## Results

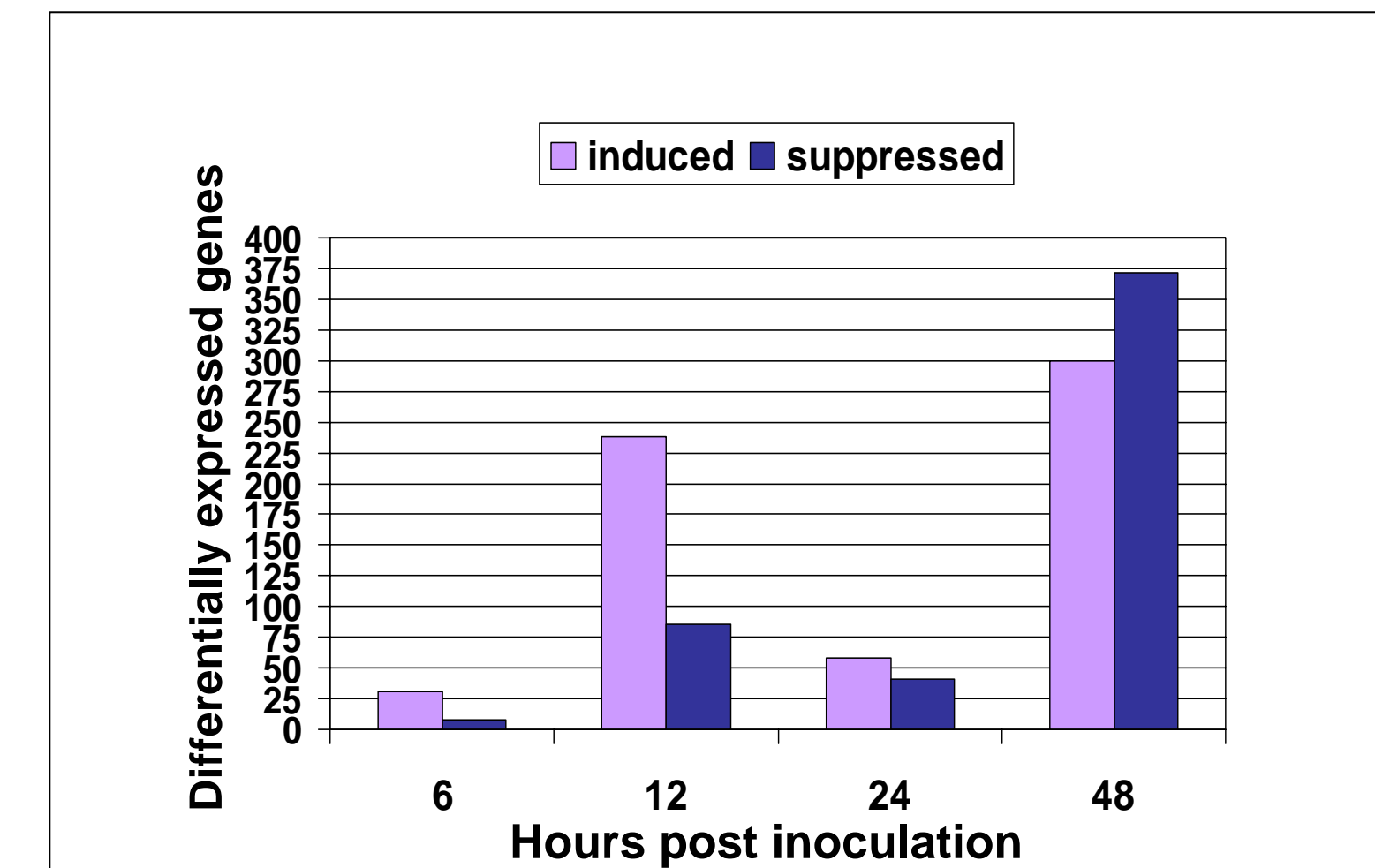


Fig 5. The distribution of transcripts identified as statistically significantly different ( $p < 0.05$ , with Benjamini False Discovery Rate control<sup>11</sup>) between the Immune and susceptible reactions. A total of 1133 genes were differentially expressed in the Immune reaction.

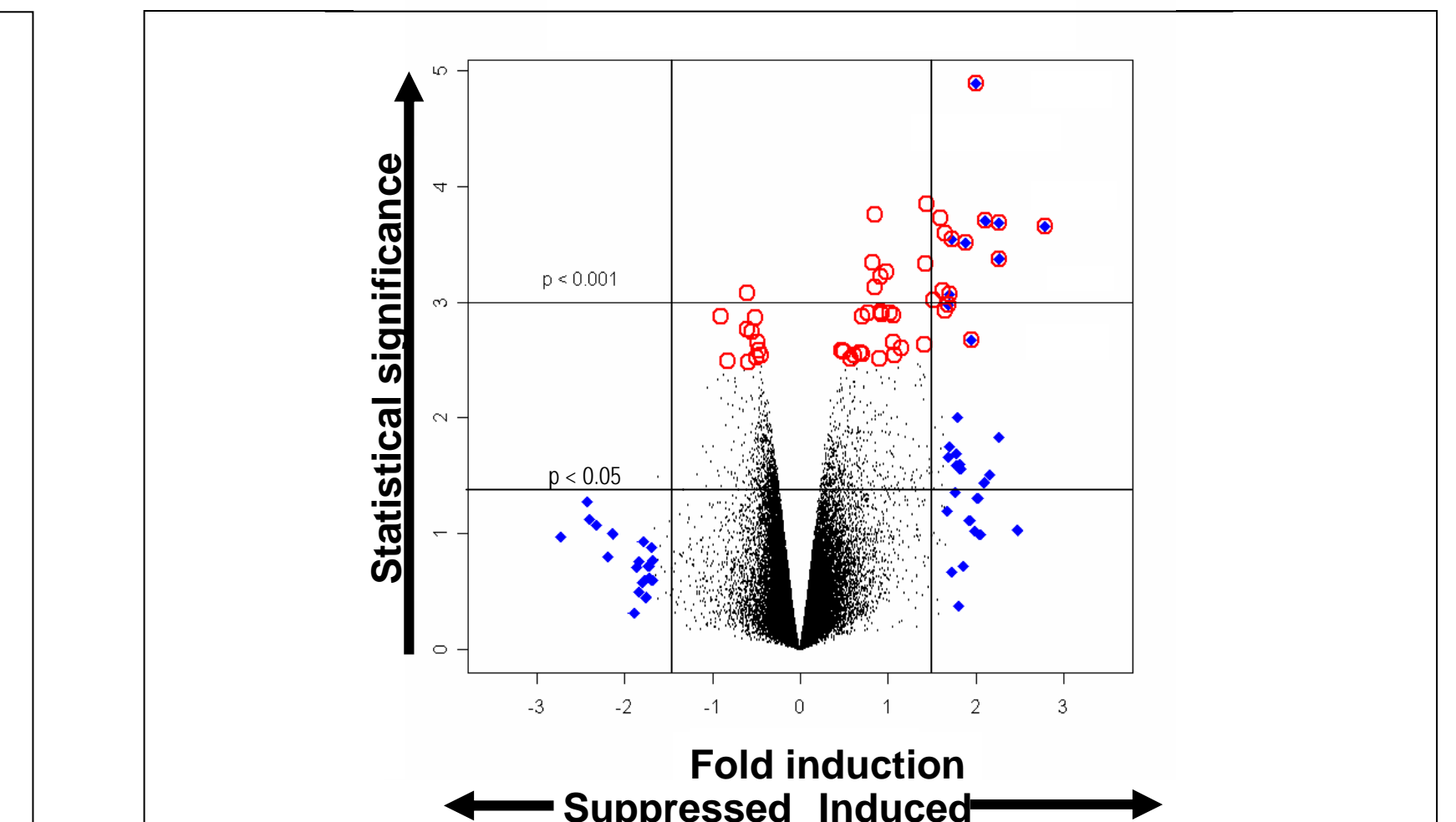


Fig 6. A "volcano" plot of the fold induction of each transcript featured on the Affymetrix Soybean Genome Array versus the  $\log_{10}$  of the  $p$  value. Each spot on the graph represents a single spot on the array. The blue diamonds represent the top 50 genes with the greatest change in expression, and the red circles represent the top 50 transcripts with the smallest  $p$  values (most statistical significance). Transcripts with both blue diamonds and red circles are the best candidates for further investigation since they represent genes that have the greatest change in expression with the most statistical significance.

6 hpi				12 hpi			
Fold Change	p value	Annotation	GO	Fold Change	p value	Annotation	GO
1.59	0.0016	putative ABC transporter	protein binding, signalling, auxin transporter	3.50	3.29934E-05	HSP 91-8	unfolded protein binding
1.57	0.0053	peroxidase	oxidoreductase activity	3.79	0.000183849	isoflavone reductase homolog 1	transcriptional repressor, nitrogen utilization
1.57	0.0072	putative basic blue protein/plantacyanin	copper binding, electron transporter	2.69	0.000241585	70 kDa heat shock cognate protein 1	unfolded protein binding
2.24	0.0076	uroporphyrin methylase	catalytic activity, methyltransferase	5.48	0.000252007	HSP 91-1	ATP binding, unfolded protein binding
1.72	0.0119	nitrile reductase	oxidoreductase, electron transport	4.51	0.000254495	HSP 91-3	DNA binding, RNA polymerase activity, molecular chaperone
1.04	0.0142	Spo12 transcription factor homolog (regulates virulence in yeasts)	transcription factor	3.09	0.000383714	lipase	lipid metabolism
1.60	0.0198	thiazole biosynthetic enzyme, chloroplast precursor	amino acid synthesis, chloroplast located	4.38	0.000391949	HSP 91-5	lipid metabolism
1.52	0.0214	expansin	cell wall organization and biosynthesis	2.79	0.000424096	lipase/carboxylester hydrolase	lipid metabolism
1.08	0.0227	nitrile reductase	catalytic activity, oxidoreductase	3.06	0.000648984	70 kDa heat shock cognate protein 1	unfolded protein binding
1.64	0.0242	SCPL27; catalytic/serine carboxypeptidase	catalytic activity; proteolysis	2.62	0.000779349	trans-cinnoyl-coenzyme A 3-O-methyltransferase	lipid biosynthesis, defense
1.60	0.0267	class III HD-Zip protein CNA1	dna binding	1.78	0.000783461	oxidoreductase	oxidoreductase
1.52	0.0277	cell-associated kinase; serine/threonine kinase	kinase, phosphorylation, plasma membrane	1.95	0.001099915	catechol oxidase	oxidoreductase
1.53	0.0283	catalytic/protein phosphatase type 2C	catalytic activity; phosphatase	2.76	0.001058781	isoflavone reductase homolog 1	transcriptional repressor; nitrogen utilization
1.95	0.0340	inducible nitrile reductase	catalytic activity, oxidoreductase	2.60	0.001371294	membrane attack complex component/perforin	cytolysis, complement activation
1.59	0.0348	chlorophyllid; PAPS reductase homolog	catalytic activity, chloroplast	1.96	0.001642375	transferrin/Gliadin 1,3-beta-glucosidase precursor	carbohydrate metabolism
-1.66	0.0345	similar to pathogenesis related protein PR-1	defense	-5.64	0.0037	acid phosphatase	catalytic activity, lyase
-1.79	0.0329	probable histone H2A.2, subunit of nucleosome	proteolysis	-6.76	0.0043	Fish-like protein PHF precursor	proteolysis
-1.58	0.0263	indole diphosphate glucose epimerase	biogenesis	-4.31	0.0044	putative Na <sup>+</sup> /H <sup>+</sup> antiporter	transporter activity
-2.73	0.0246	cytochrome b6/f complex subunit 4	energy	-6.18	0.0049	putative thiamin biosynthesis protein	cellular metabolism
-1.52	0.0204	ubiquitin-conjugating enzyme	proteolysis	-8.35	0.0056	carotenoid cleavage dioxygenase	oxidoreductase
-1.69	0.0182	plant invertase/pectin methyltransferase inhibitor	defense	-9.11	0.0071	PAP fibrillin	structural molecule activity
-1.63	0.0166	isocupressin	defense, signalling	-5.21	0.0071	putative thiamin biosynthesis protein	cellular metabolism
-1.66	0.0138	actin	structural	-4.95	0.0072	pyruvate decarboxylase	catalytic activity, lyase

Fig 8. A list of genes that are differentially expressed in the Immune vs. Tan reactions. The genes are grouped by time point, with induced genes in the top colored areas, and suppressed genes in the bottom grey areas. Gene names in **Blue** = transcripts related to reduction/oxidation reactions, **Brown** = cell wall synthesis and cell biogenesis, **Orange** = heat shock and abiotic stress, and **Green** = lipid metabolism.

## Discussion

•Soybean genes have been identified that are induced or suppressed in the Immune reaction following infection with soybean rust.

•In the comparison of the Immune reaction to the susceptible reaction, the host genotype (cultivar Komata, *Rpp1*) is identical between samples. Therefore, differential expression of genes should result from the difference between the two treatments, the rust isolate giving an Immune or Tan reaction.

•Many of the genes induced in the Immune reaction are genes that have been shown in other plant-microbe interaction studies to be involved in plant defense and stress responses, such as pathogenesis-related (PR) proteins and isoflavone reductase.

•The induced genes can be grouped into categories by similar functions. Upregulation of genes such as nitrile reductase, glutaredoxin, catechol reductase and isoflavone reductase suggests that redox reactions may play role in the Immune reaction, possibly by regulating cellular metabolism.

•Induction of lipases and lipid/membrane metabolism genes and a group of carbohydrate metabolism and cell wall-related genes such as expansins suggests that remodeling of the cell wall and cellular membranes is a component of the immune reaction, even though no visible lesions are present on the inoculated plants.

•Heat-shock proteins, heat-shock transcription factors, and abiotic stress response genes are upregulated in the Immune reaction. The heat-shock 70 family of genes has been implicated in oxidative stress in *Arabidopsis*, and heat-shock transcription factors have been shown to regulate transcription of defense genes.