Soybean defense responses to the soybean aphid

Yan Li[1], Jijun Zou[2], Min Li[2], Damla D. Bilgin[3], Lila O. Vodkin[2], Glen L. Hartman[2,4] and Steven J. Clough[2,3,4]

[1]Physiological and Molecular Plant Biology Program, University of Illinois at Urbana-Champaign, IL, USA; [2]Department of Crop Sciences, University of Illinois at Urbana-Champaign, IL, USA; [3]Institute for Genomic Biology, University of Illinois at Urbana-Champaign, IL, USA; [4]USDA-ARS and National Soybean Research Center, Urbana, IL, USA

Author for correspondence:
G. L. Hartman
Tel: +1 217 244 3258
Fax: +1 217 244 7703
Email: ghartman@uiuc.edu

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Summary

• Transcript profiles in aphid (*Aphis glycines*)-resistant (cv. Dowling) and -susceptible (cv. Williams 82) soybean (*Glycine max*) cultivars using soybean cDNA microarrays were investigated.

• Large-scale soybean cDNA microarrays representing approx. 18 000 genes or *c*. 30% of the soybean genome were compared at 6 and 12 h post-application of aphids. In a separate experiment utilizing clip cages, expression of three defense-related genes were examined at 6, 12, 24, 48, and 72 h in both cultivars by quantitative real-time PCR.

• One hundred and forty genes showed specific responses for resistance; these included genes related to cell wall, defense, DNA/RNA, secondary metabolism, signaling and other processes. When an extended time period of sampling was investigated, earlier and greater induction of three defense-related genes was observed in the resistant cultivar; however, the induction declined after 24 or 48 h in the resistant cultivar but continued to increase in the susceptible cultivar after 24 h.

• Aphid-challenged resistant plants showed rapid differential gene expression patterns similar to the incompatible response induced by avirulent *Pseudomonas syringae*. Five genes were identified as differentially expressed between the two genotypes in the absence of aphids.

Key words: cDNA microarray, incompatible interaction, plant–aphid interaction, plant defense, plant resistance, soybean aphid.

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Introduction

The soybean aphid, *Aphis glycines* Matsumura, was first identified in North America in 2000 (Hartman *et al*., 2001) and has rapidly spread throughout the northern Midwest (Venette & Ragsdale, 2004). Damage to soybean caused by *A. glycines* includes plant stunting, leaf distortion, and reduced pod set (Sun *et al*., 1990; Hill *et al*., 2004). Resistance to *A. glycines* has been found in soybean germplasm accessions (Hill *et al*., 2004) and the resistance in soybean cv. Dowling was characterized as antibiosis (Li *et al*., 2004), but resistance in other genotypes was characterized as antixenosis (Diaz-Montano *et al*., 2006). Up until 2008, no soybean aphid biotypes were reported (Kim *et al*., 2008). Other aphid biotypes have been observed in greenbug (*Schizaphis graminum*) and the Russian wheat aphid (*Diuraphis noxia*) (Harvey *et al*., 1997; Porter *et al*., 1997; Basky, 2003; Smith *et al*., 2004). A basic understanding of the mechanism of soybean resistance to the aphid may provide an insight into how new biotypes develop and how to develop more durable resistance.

As one of the largest groups of phloem-feeding insects, aphids are a serious problem to many crops. Previous studies of plant response to aphids suggested that jasmonic acid (JA)-, ethylene-, and salicylic acid (SA)-regulated signaling

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pathways were at least partially activated by aphid feeding. A lipoxygenase, believed to be involved in JA synthesis in plants and induced by wounding (Bell *et al*., 1995; Leon & Sanchez-Serrano, 1999), was up-regulated in several plant–aphid interactions, including incompatible and compatible interactions in tomato to the potato aphid (*Macrosiphum euphorbiae*) and green peach aphid (*Myzus persicae*) (Martinez de Ilarduya *et al*., 2003), sorghum to the greenbug aphid (Zhu-Salzman *et al*., 2004), and *Arabidopsis* to the green peach aphid (Moran & Thompson, 2001). A gene encoding the PR1 protein was reported to be a good marker in the SA-dependent pathway (Uknes *et al*., 1992; Rogers & Ausubel, 1997) and was induced by aphid feeding in tomato and *Arabidopsis* (Moran & Thompson, 2001; Martinez de Ilarduya *et al*., 2003; De Vos *et al*., 2005). PR1 protein was antifungal (Niderman *et al*., 1995), and induced by a glucan elicitor, wounding, viruses, SA, and aphids (Hajimorad & Hill, 2001; Graham *et al*., 2003; Martinez de Ilarduya *et al*., 2003). Other PR proteins were also differently expressed between resistant (R) and susceptible (S) cultivars in response to aphids. In wheat, the activities of β-1,3-glucanase, peroxidase, and chitinase were induced to higher levels in R than in S cultivars (van der Westhuizen *et al*., 1998a,b). Russian wheat aphid infestation also induced SA accumulation and peroxidase activity in R compared with S wheat (Mohase & van der Westhuizen, 2002). Greenbug induced *PR10* gene expression in sorghum (Zhu-Salzman *et al*., 2004).

There is limited information about the molecular mechanism of plant resistance to aphids (Kaloshian, 2004; Smith & Boyko, 2007). The first cloned insect resistance gene, *Mi-1.2*, is a member of the leucine zipper, nucleotide binding, leucine-rich repeat (LZ-NBS-LRR) family of plant resistance genes (Milligan *et al*., 1998), which confers resistance to root-knot nematode (*Meloidogyne incognita*) and also to a biotype of the potato aphid (*Macrosiphum euphorbiae*) in tomato (Rossi *et al*., 1998). *Mi-1.2*-mediated aphid resistance behaved similarly to the gene-for-gene resistance model in the field (Goggin *et al*., 2001). *Mi-1.2* did not directly confer aphid resistance because *Mi-1.2*-mediated aphid resistance was not correlated with the transcript abundances of *Mi-1.2* (Goggin *et al*., 2004). Plants with a single recessive mutation in another locus, *Rme1* (confers resistance to *Meloidogyne* spp.), abolished aphid resistance, which indicated that the *Mi-1.2*-mediated aphid resistance required *Rme1* (Martinez de Ilarduya *et al*., 2001). The *Mi-1.2* RNA concentrations in tomato did not change in response to potato aphids or nematodes (Martinez de Ilarduya & Kaloshian, 2001). The only reported differentially induced aphid response between resistant (*Mi-1.2/Mi-1.2*) and susceptible (*mi-1.2/mi-1.2*) plants was the earlier systemic induction and accumulation of pathogenesis-related 1 (*PR-1*) in the incompatible tomato–aphid interaction. More extensive aphid-induced gene expression profiling would be useful to understand the defense responses in resistant plants to aphid infestation.

A recent review (Thompson & Goggin, 2006) on gene expression profiling of plant responses in several plant–aphid interactions indicated the lack of understanding of the early interactions between hosts and aphids, especially for incompatible interactions that may involve race-specific innate resistance. We address that concern here with an investigation of the early responses in soybean to aphids and identification of genes associated with resistance responses by comparing the differential gene expression between R and S soybean with soybean cDNA microarrays representing approx. 18 000 different soybean genes. *A. glycines* behavior on soybean leaves indicates that resistance in cv. Dowling was actively effective within 8 h after aphid application (Li *et al*., 2004). Electrical penetration graphing showed clear differences in *A. glycines* feeding behavior between resistant cultivars, including cv. Dowling, within 9 h after application (Diaz-Montano *et al*., 2007). Aphids took *c*. 8 h to reach the first sieve element feeding phase on cv. Dowling versus *c*. 3.5 h on susceptible checks. Additionally, only 19% of aphids reached sieve elements on cv. Dowling compared with 94% on the control. Once at the sieve elements of cv. Dowling, *A. glycine* spent *c*. 2.7 min compared with 18.9 min on the susceptible cultivar. Together, these studies suggest that gene expression studies should focus on time points near 8–9 h post-application (hpa) to potentially identify key genes involved in establishing resistance. Therefore, in this current study, the differential gene expressions in response to *A. glycines* were compared between soybean resistant cv. Dowling (R) and susceptible cv. Williams 82 (S) at 6 and 12 hpa. In support of the microarray data, quantitative real-time reverse-transcribed PCR (qRT-PCR) was performed on seven genes. A separate experiment followed the expression pattern of three defense genes to further validate the microarray results and to investigate their expression patterns for a longer time. Cross-comparing our list of genes that were differentially expressed in response to aphid in R and S plants to our previous microarray study of soybean response to compatible and incompatible strains of *Pseudomonas syringae* (Zou *et al*., 2005) would reveal if soybean activated different or overlapped defense pathways or mechanisms against pathogens and insects.

Additionally, we examined gene expression from R and S plants without aphids to identify genes with constitutive expression differences. Any genes showing significantly higher expression in R than S through three time points were considered candidates for the aphid-resistance gene *Rag1* (resistance to *A. glycines*), which was determined to be a single dominant gene using the $F_{2:3}$ population of cvs Dowling × Williams 82 (Hill *et al*., 2006).

Materials and Methods

Soybean cDNA libraries

Two sets of soybean cDNA microarrays, Gm-r1070 and Gm-r1088, were used, representing over 18 000 genes, estimated to

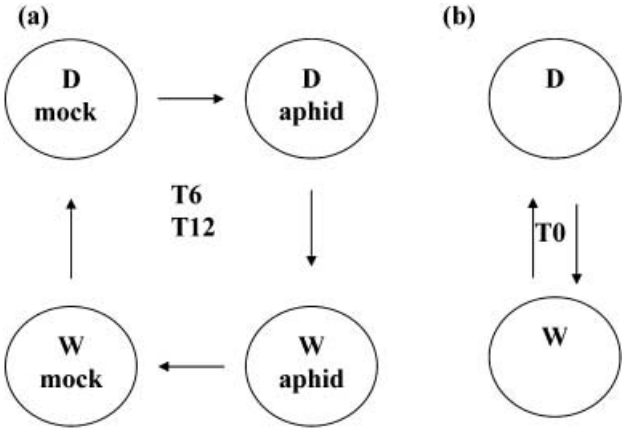

Fig. 1 Design for microarray experiment. Each arrow represents single microarray hybridization. Tails of arrows represent samples labeled with Cy3 dye while heads are Cy5-labeled. The entire experiment was repeated for two biological repeats. (a) Loop design for microarray analysis of resistant (Dowling, D) and susceptible (Williams 82, W) soybean (*Glycine max*) cultivars in response to *Aphis glycines* 6 or 12 h post-application (hpa) after aphid application. (b) Dye-swap design for the comparison between two cultivars at T0 without any treatment.

be approximately one-third of the soybean transcriptome (Vodkin *et al*., 2004). Each slide set consisted of PCR product from 9216 cDNA clones and various control clones. Gm-r1070 consisted of mainly developing seed (green tissue) and floral cDNA (approx. 48 and 43%, respectively); Gm-r188 consists of cDNA from a variety of tissue sources, approx. 60% from germinating cotyledons, seed coat and growing shoots, and approx. 40% from stress, pathogen-challenged, or hormone-treated tissues.

Experimental design

Loop and dye-swap designs were used for the microarrays (Fig. 1). Each loop (Fig. 1a) represented one time point, 6 or 12 hpa, and four samples within one loop could be compared with each other. The cultivar of interest was balanced with respect to dyes since each cultivar was labeled once with both Cy3 and Cy5. The differently expressed genes can be detected with more statistic power in a loop design than a reference design using the same number of arrays (Kerr & Churchill, 2001). Comparisons between aphid and mock treatments on each cultivar were made to identify differentially expressed aphid-response genes. The differences between two cultivars were compared using the mock-treated samples in the loop design, as well as the samples without any treatments at time 0 h in a dye-swap design (Fig. 1b). Independent biological replicates were produced 4 wk apart. For each pair of comparisons, there were at least four replicates (two biological each consisting of two technical).

Soybean plants and aphid clone

Seeds of two soybean (*Glycine max* (L.) Merrill) cvs Dowling (R to soybean aphid) and Williams 82 (S to soybean aphid) were sown in 12 cm diameter plastic pots filled with soil-less potting medium (Sunshine Mix, LC1, Sun Gro Horticulture Inc., Bellevue, WA, USA), and covered with coarse-grade vermiculite (Hummert International, Earth City, MO, USA) contained in plastic trays without holes (Hummert International). Plants were grown in a growth chamber at 22°C (night) and 26°C (day) under a 14 h photoperiod (approx. 300 μmol m^{-2} s^{-1}). The seedlings were bottom-watered.

The soybean aphid clone (virus-free) was established from a single first instar nymph isolated from a collection in Urbana, Illinois, in 2000, and maintained on a continuous supply of seedlings of the soybean cv. Williams 82 grown in a plant growth chamber at 22°C under continuous 100 μmol m^{-2} s^{-1} PAR irradiation.

Plant treatments for microarray

Ten-day-old plants with two fully expanded unifoliolate leaves of R and S were used for all experimental treatments. Leaves at 0 h without any treatment were collected and used in the dye-swap experiment to compare the constitutive differences at 0 h between the two cultivars. For the loop-design experiment, aphid and mock treatments were completed as follows. In order to keep aphids on the target tissue (one unifoliolate leaf of a plant), a piece of weigh paper of 15 × 15 cm was vertically positioned on the petiole of the leaf to serve as a large lightweight, physical barrier to discourage aphid movement off the leaflet. Forty aphids of various ages (20 wingless adults and 20 nymphs) were gently applied evenly on the abaxial surface of the target unifoliolate leaf of each plant by using a fine tip (1 mm in diameter) paint brush. After 6 and 12 h, infested leaves were cut at the center of the abscission joint connecting leaf to petiole using surgical scissors, and aphids were removed quickly using a soft 5-cm-wide paintbrush. The mock treatment consisted of weigh paper without aphids but the leaves were also brushed when collected. Only one unifoliolate leaf from each plant received aphid or mock treatment. Aphid- or mock-treated unifoliolate leaves for each of three plants were pooled together, respectively, and sealed inside plastic bags and immediately frozen in liquid nitrogen. All of the leaves were stored at –80°C before RNA isolation.

RNA isolation

For the microarray experiment, total RNA was isolated from frozen ground leaf tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in combination with Phase Lock Gel-Heavy (Brinkmann Instruments, Inc., Westbury, NY, USA)

followed by a series of phenol-chloroform extraction, RNA precipitation, and suspension in water. The RNeasy Plant Mini Kit (Qiagen, Valencia, CA) was used to extract RNA from the leaf discs from the clip cage experiment. For quality control, all of the RNA samples were checked with a Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA, USA) to verify lack of RNA degradation. The concentration of total RNA was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) based on OD_{260} values.

cDNA microarrays

Reverse transcription of total RNA and fluorescently labeled cDNA probes were prepared from approx. 50 μg total RNA following the indirect labeling protocol described by Zou *et al.* (2005). Briefly, cDNA probes were synthesized from total RNA. After cDNA was purified and labeled with Cy3 or Cy5, light absorption at 260, 550, and 650 nm was measured to determine the concentration of cDNA, incorporated Cy3 and Cy5, respectively, using a NanoDrop ND1000 spectrophotometer. The hybridization, washing and scanning of the microarray slides were conducted as previously described (Zou *et al.*, 2005). The microarray slides were scanned with a two-laser confocal scanner ScanArrayExpress (Perkin Elmer Life Sciences, Boston, MA, USA), using ScanArrayExpress software.

Microarray data analysis

Blocks and spots on the array images were defined and the intensities of spots and background were quantified using GenePix Pro (Axon Instruments, Union City, CA, USA). The quantified raw data files (including light fluorescence intensities of Cy3, Cy5, and background) as well as the images were uploaded into GeneTraffic (Iobion, La Jolla, CA, USA) for data storage, visualization, and analysis. The background-subtracted Cy3 and Cy5 signal intensity values were normalized using a linear-logarithmic transformation and analyzed by analysis of variance (ANOVA) in a linear mixed model with array as random effect and dye and sample as fixed effects, using R/MAANOVA statistical package (Wu *et al.*, 2003). Genes that showed significant ($P < 0.05$ from contrast *t*-tests) differences in expression in the desired comparisons were saved as a gene list and uploaded to GeneTraffic. Fold-change calculations of significant genes were performed in GeneTraffic after local background subtraction and locally weighted scatter plot smoother (lowess)-subgrid normalization. Our gene selection cutoff required that a gene meet the statistical *P*-value cutoff of 0.05 in R/MAANOVA and, as an added stringency measure, that the average of the two biological replicates have a 1.5-fold increase or decrease before being considered 'significantly' differentially expressed.

Quantitative real-time PCR (qRT-PCR)

The qRT-PCR was performed according to the procedure described by Zou *et al.* (2005). The expression of a soybean β-actin (AW350943) was used as an internal standard to normalize the possible differences in template amounts (Zou *et al.*, 2005). The primers (Supplementary material, Table S1) were designed based on the tentative consensus sequences with emphasis on the unique oligomer region or singleton EST sequence from TIGR, using Primer Express 2.0 software (Applied Biosystems, Foster City, CA, USA). The specificity of the primers was validated by the presence of a single peak in the dissociation curve analysis run after the qRT-PCR. The qRT-PCR data were analyzed using the relative quantification $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001).

Leaf clip cage experiment

In order to add further verification to the microarray results and to study the expression patterns of the selected genes using qRT-PCR over a longer time period, a new set of samples from a separate experiment was collected at additional time points from leaf clip cages (12 mm outside diameter, 1-mm-thick walls and 12 mm high) as described in our previous study (Li *et al.*, 2004). Ten-day-old plants with two fully expanded unifoliolate leaves were used. Three plants were used for each sample. Five wingless adults and five nymphs of various ages were put inside the leaf cage and clipped on the abaxial surface of one unifoliolate leaf of each plant. Empty cages were used as mock treatment. At 6, 12, 24, 48, and 72 hpa, clip cages were removed and aphids were brushed off. Leaf areas under the cages were cut using a 12-mm-diameter core borer and used for RNA isolation. Three leaf discs from three plants per treatment were pooled together and stored at −80°C before RNA isolation.

Results

Responses in resistant vs susceptible soybean to aphids

Gene expression was compared between two cvs, resistant Dowling and susceptible Williams 82, under mock and aphid treatments at 6 and 12 hpa. Comparison combinations focused on aphid vs mock in R, aphid vs mock in S, R vs S under mock treatment, and R vs S under aphid treatment, following a loop design (Fig. 1a). Differential gene expression of R and S plants in response to aphid vs mock was used to select aphid-response genes within each time point (Figs 1a, 2). The differentially expressed genes were selected based on significance ($P < 0.05$) and a fold-change ratio (> 1.5 or < -1.5). Only four genes overlapped between R and S in response to aphids (Fig. 2).

Most of the 18 000 genes represented on the microarrays did not show differential expression between the two cultivars

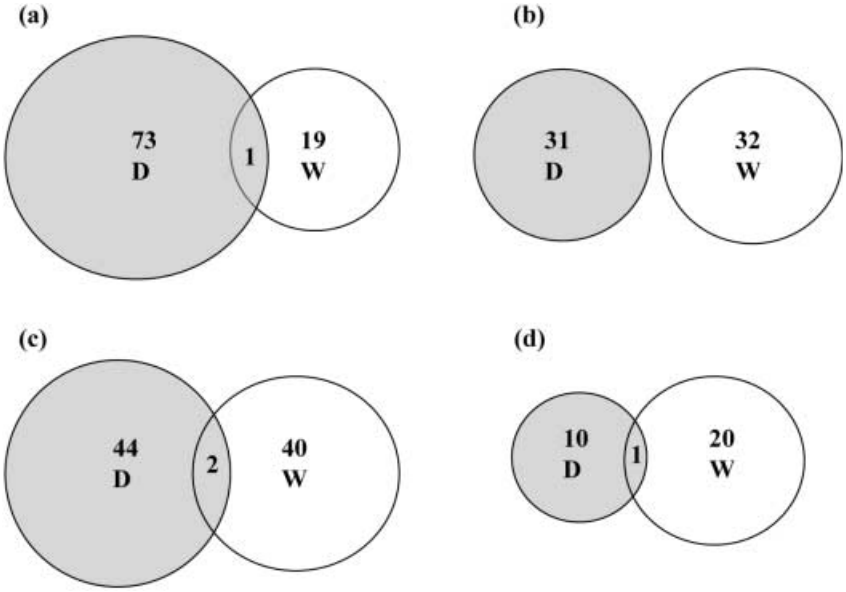

Fig. 2 The number of genes significantly ($P < 0.05$) above 1.5-fold change up-regulated at 6 h post application (hpa) (a); down-regulated at 6 hpa (b); up-regulated at 12 hpa (c); and down-regulated at 12 hpa (d) in resistant (Dowling, D) and susceptible (Williams 82, W) soybean (*Glycine max*) cultivars.

under mock treatment. However, we identified 140 aphid-response genes that showed specific response in R but not S (Table S2) at 6 and 12 h (the shaded area in Fig. 2 without the overlapped four genes; the sum of the numbers of genes in the shaded area is > 140 because of overlapped genes between T6 and T12). If a gene was up-regulated in R but down-regulated in S, it was considered as an R-specific response since its differential expression in R was different from that in S. As selected, these genes showed significantly differential expression after aphid feeding in R but not the same trend in S. On the other hand, 95 genes (nonshaded area in Fig. 2) showed responses to aphids that were specific to S (Table S3). In subsequent text, the gene annotations given are the summarized call based on analysis of both 5′ and 3′ EST comparisons to NCBI nr protein (BLASTx), TIGR TC, and Aridopsis (MIPS) databases (detailed information provided in Table S2).

Resistance-associated responses to aphids in soybean

The 140 R-specific aphid-response genes were classified into 16 functional groups based on the annotation from multiple databases (Table S2). These functional categories included groups of genes related to cell development, cell wall, cytoskeleton, defense, DNA/RNA, membrane, oxidation, primary metabolism, protein, secondary metabolism, senescence, signaling, stress, miscellaneous, no hits, and unknown. We focused on five groups, including cell wall, defense, DNA/RNA, secondary metabolism, and signaling.

Six of seven significant cell wall-related genes were down-regulated in R plants to aphids. Three homologs of pectate lyases (Gm-r1070-3571, Gm-r1070-4002, and Gm-r1070-7069) were down-regulated.

Significant defense-related genes included gene homologs to *R* genes, PR proteins, HR-associated proteins, and antimicrobial proteins. All genes were significantly up-regulated in R in response to aphids at 6 and/or 12 hpa, except for the snakin homolog (Gm-r1070-7107) which was down-regulated. Two *hsr23J* (HR-induced protein) homologs (Gm-r1070-628 and Gm-r1070-4998) were induced at 6 and 12 hpa. Three *R* gene homologs (Gm-r1088-3067, Gm-r1070-3058, and Gm-r1070-2852) and two *R* gene-related proteins (X21-binding protein Gm-r1088-3841 and NPR1 like-protein Gm-r1088-1044) were all up-regulated in R at 6 and/or 12 hpa. In addition to *PR1a* precursor (Gm-1088-8829), another two PR protein genes, soybean *P21* (Gm-r1070-5189) and thaumatin (Gm-r1088-8018), which belongs to PR-5 family, also were induced.

The DNA/RNA functional group included 14 differentially expressed homologs of transcription factor (TF) and genes related to DNA or RNA processing. Eleven genes in this group were induced by aphids in R, and three genes were homologs of *WRKY* TFs (Gm-r1070-4784, Gm-r1070-4040, and Gm-r1070-2914).

All significant genes in the secondary metabolism (most genes related to phenylpropanoid pathway) were up-regulated in R, including homologs of chalcone synthase, isoflavone synthase, flavanone 3-hydroxylase-like protein, and cytochrome P450s. Secondary metabolites play an essential role in many plant defense responses, and several genes associated with synthesis of these chemicals that may serve as both antimicrobial and antioxidants were differentially expressed in response to aphids.

For the significant genes of the signaling group, two homologs of the gibberellin-interacting protein GIP1 (Gm-r1070-1066 and Gm-r1070-4900) and a homolog

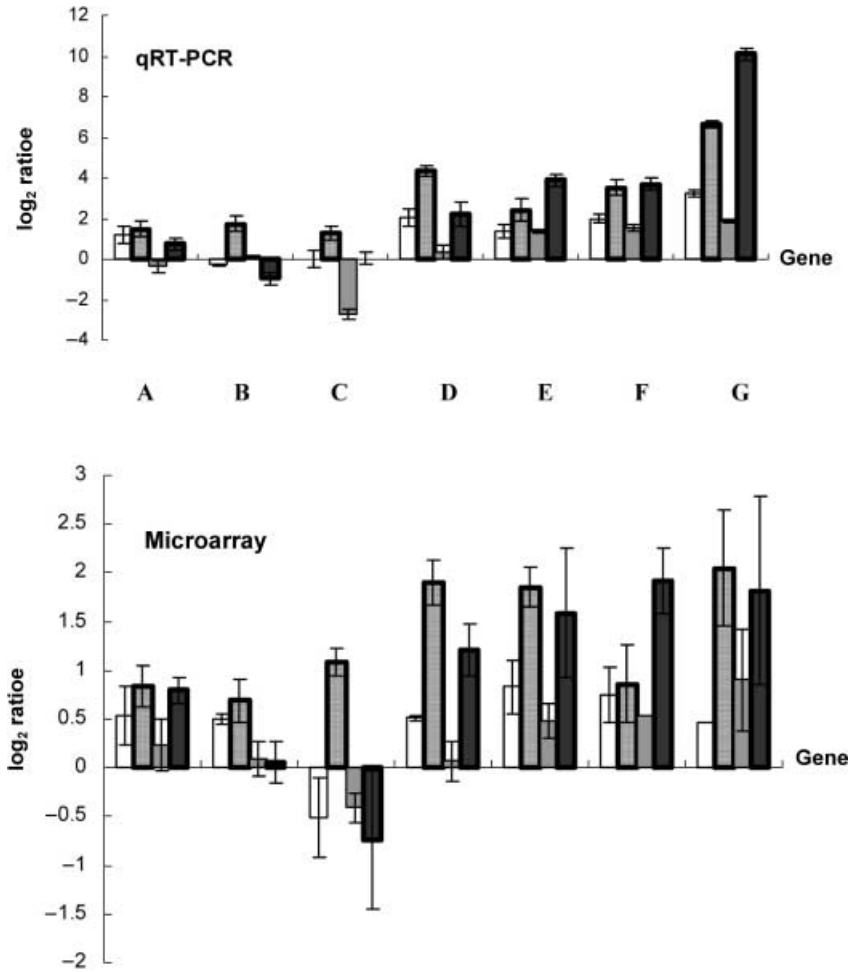

Fig. 3 Confirmation of microarray results using quantitative real-time reverse-transcribed PCR (qRT-PCR). Transcriptional responses of resistant (Dowling, D) and susceptible (Williams 82, W) soybean (*Glycine max*) cultivars in response to soybean aphids (*Aphis glycines*) at 6 and 12 h post application (hpa) were evaluated by both microarray and qRT-PCR. The changes in transcript abundance after aphid feeding were expressed as $\log_2$-transformed fold change compared with the mock treatment. qRT-PCR data showed the mean ratios from three replicates and the error bars represent the standard error (SE) of the mean. The mean ratios and SE of the mean for microarray data were obtained from GeneTraffic. The annotations for the genes are listed as the following: A, calreticulin; B, HR-associated Ca^{2+}-binding protein; C, ferritin; D, DNA-binding protein; E, soybean P21 protein; F, matrix metalloproteinase GmMMP2; G, PR1a precursor.

of calmodulin1 (Gm-b10BB-40) were down-regulated. Four calcium-associated genes (Gm-r1070-2651, Gm-r1088-1400, Gm-r1088-8402, and Gm-r1088-6724), two kinase homologs (Gm-r1070-6315 and Gm-r1070-8303), and a homolog to the jasmonate biosynthetic gene allene oxidase (Gm-r1070-2203) were induced in R in response to aphid at 6 hpa.

Array confirmation and expression patterns of defense-related genes by qRT-PCR

To validate the microarray results, seven genes were analyzed by qRT-PCR using the same RNA samples used for the microarrays. The results were consistent with the microarray results, as the genes assayed showed similar differential gene expression in response to aphids (Fig. 3), except that the fold change from qRT-PCR was generally higher than that observed for microarrays. Three genes related to defense, including soybean *P21* (Gm-r1070–5189), *MMP2* homolog (Gm-r1070-749), and *PR1a* precursor homolog (Gm-r1088-8829), increased in abundance from 6 to 12 hpa based on qRT-PCR (Fig. 3).

Three defense-related genes that were up-regulated in the microarray analysis (*P21*, *MMP2*, and *PR1a*) were further verified at 6 and 12 hpa and were analyzed for their expression patterns over a longer time course (6, 12, 24, 48, and 72 hpa) using a leaf clip cage. All three genes had a higher induction (aphid vs mock) before 48 hpa in R than in S (Fig. 4). The induction of soybean *P21* and the *MMP2* homolog reached a peak at 24 h in R, and the *PR1a* precursor homolog peaked in expression at 48 h in R. Defense gene expression in the S genotype responded to the aphid infestation more slowly and less robustly as transcription abundances of these genes were induced only after 24 hpa.

Comparison between aphid and *P. syringae* induced responses

In a previous microarray study (Zou *et al.*, 2005), soybean responses to *P. syringae* lacking (Virulent, Vir) or carrying (Avirulent, Avr) the avirulence gene *avrB* were compared. Soybean cv. Williams 82 showed susceptibility to Vir (compatible interaction) but resistance to Avr (incompatible

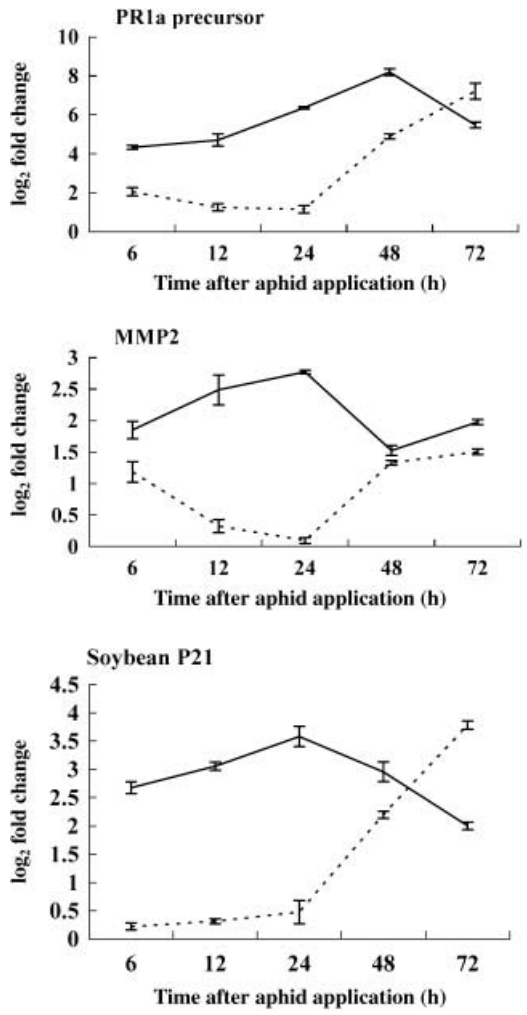

Fig. 4 The expression patterns of three genes in soybean (*Glycine max*) in response to *Aphis glycines* from 6 to 72 h post-application (hpa) using qRT-PCR. Induction ratio (aphid vs mock) was expressed as mean $\log_2$ fold change, and the error bars represent the standard error of the mean. RNA samples were collected from pooled leaf discs in the leaf clip cage experiment, in which five wingless aphid adults and five nymphs were caged in the area where leaf samples were collected. Solid lines, resistant; dotted lines, susceptible.

interaction). Among the 140 R-specific aphid-response genes, 100 showed significant ($P < 0.05$) differential expression between incompatible and compatible induced responses when comparing the incompatible vs compatible at 8 h (Table S4). Hierarchical clustering of these 100 genes across the different experiments revealed that R responses to aphids at 6 hpa (T6-Dowling) were most similar to incompatible induced responses (T8-Avr_vs_$MgCl_2$) (Fig. 5a). On the other hand, only 22 of the 95 S-specific aphid-response genes were identified as differentially expressed ($P < 0.05$) between incompatible and compatible (Table S5). The cluster analysis did not show a close relationship between S responses to aphids and incompatible or compatible (Fig. 5b). Combining R responses to aphids at 6 and 12 hpa shows a clear overlap in expression behavior of the 100 genes that were both significant in this aphid study ($P < 0.05$) and significant in the Avr *P. syringae*-induced incompatible response ($P < 0.05$) at 8 hpi, revealing shared rapid responses for these genes in both R interactions.

Genes with constitutively higher expression in resistant than susceptible soybean

In addition to comparing mock-treated controls, we also directly compared nontreated R with S at the start of the experiment (T0). In this T0 comparison, 68 genes, or less than 0.4% of the spots on the array, showed significantly higher expression ($P < 0.05$, and fold change > 1.5) in R than in S (Table S6). Only five genes showed constitutively higher expression in R than in S throughout T0, T6, and T12. These five genes were as follows: Gm-r1070-2257, no homolog; Gm-r1088-3786, a homolog of an unknown protein; Gm-r1088-7714, a homolog of a soybean NBS-LRR type resistance gene *RPG1-b*; Gm-r1070-4664, similar to a Myb family transcription factor, one of which has been shown to regulate the phenylpropanoid biosynthesis pathway and plant resistance to insect (Johnson & Dowd, 2004); and Gm-r1088–4309, a homolog of LTCOR11 or snakin2, which is potentially antimicrobial.

Discussion

The soybean aphid is an invasive insect pest that is new to North America. The soybean aphid was not reported in North America before July 2000 (Hartman *et al.*, 2001) and has rapidly spread throughout the Midwestern USA and southern Canada causing economic losses (Venette & Ragsdale, 2004). Other than the discovery of finding sources of resistance, and determining the inheritance and map location of the gene for resistance (Hill *et al.*, 2004, 2006; Li *et al.*, 2007), there has been very little research done on the molecular mechanism of soybean resistance to the aphid.

This is the first report to show early regulation of global gene expression in soybean in response to *A. glycines*, and to identify different functional groups of aphid-response genes. It is also the first to show a correlation of gene expression in R plants to aphids and the incompatible response induced by avirulent *P. syringae*. Another study compared the transcriptome changes in *A. thaliana* with those in *P. syringae* and *M. persicae* (De Vos *et al.*, 2005), but was not R-specific. Studies on global transcriptional regulation of plant response to aphids include the sorghum–greenbug interaction (Zhu-Salzman *et al.*, 2004; Park *et al.*, 2006), and the compatible interaction between *A. thaliana* and *M. persicae* (Moran *et al.*, 2002; De Vos *et al.*, 2005; Couldridge *et al.*, 2007). The earliest time point examined by microarray analysis in

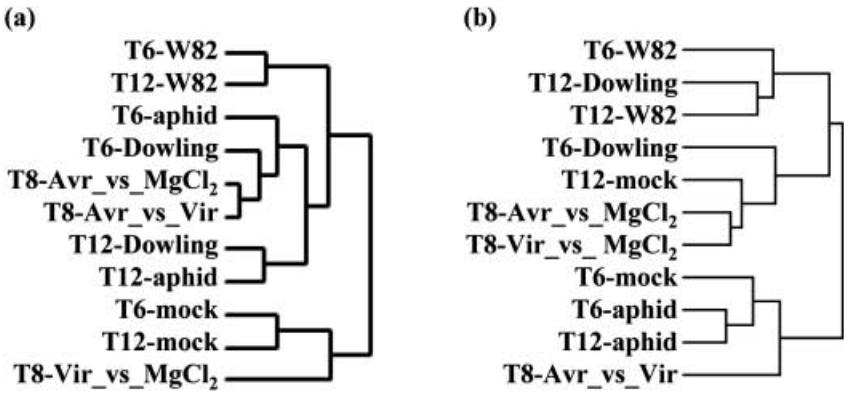

Fig. 5 Hierarchical clustering of soybean (*Glycine max*) responses to *Aphis glycines* and *Pseudomonas syringae* (Zou *et al*., 2005) using Pearson correlation. (a) Using 100 genes showed both R-specific aphid response and differential expression ($P < 0.05$) between Avr and Vir *P. syringae*-induced responses. (b) Using 22 genes showed both S-specific aphid response and differential expression ($P < 0.05$) between Avr and Vir *P. syringae*-induced responses. T6-Dowling, aphid vs mock in resistant Dowling at 6 h post-application (hpa); T6-W82, aphid vs mock in susceptible Williams 82 at 6 hpa; T6-mock, Dowling vs Williams 82 under mock treatment at 6 hpa; T6-aphid, Dowling vs Willams 82 under aphid treatment; T12 is as T6; T8-Avr vs $MgCl_2$, Avr *P. syringae*-induced responses at 8 h; T8-Vir vs $MgCl_2$, Vir *P. syringae*-induced responses at 8 h; T8-Avr vs Vir, differential expression between Avr and Vir *P. syringae*-induced responses at 8 h.

these studies was at 24 hpa, and comparisons between resistant and susceptible plants were made at 72 hpa, when susceptible plants were already badly damaged.

Although cDNA microarrays have their limits, many of their initially reported shortfalls (Li *et al*., 2002) have been diminished by the use of improved experimental designs and appropriate statistical analyses (Yauk & Berndt, 2007). Properly used, cDNA microarrays are powerful tools for the identification of candidate genes for further studies and can reveal interesting underlying physiologies when one examines the data across functional categories and/or by cross-comparison to other expression studies. Our microarray data showed that the differential gene expression pattern in aphid resistance had many similarities to Avr *P. syringae*-induced expression patterns (Zou *et al*., 2005), suggesting that the aphid-resistance gene *Rag1* in Dowling might be mediated by an incompatible-like interaction. However, we were unable to detect cell death at 24 or 48 hpa in R or S (data not shown). This lack of detection might be because the aphid-induced cell death in R was only limited to a few cells, making it difficult to detect, or because cell death is not induced in response to aphids in R. Although incompatible reactions are often characterized as a hypersensitive response (HR) that induces cell death, there are examples that an HR can occur without cell death (Yu *et al*., 1998; Cole *et al*., 2001). It is possible that the aphid resistance in Dowling was induced by HR-like responses (including ROS, PR proteins) without causing cell death. Additionally, the probing/feeding behavior of aphids would presumably stimulate fewer cells compared with the leaf spongy mesophyll infiltration used for *P. syringae* inoculation (Zou *et al.* 2005) and would explain our limited ability to detect equally high numbers of differentially expressed genes.

In other plant–aphid interactions, an HR-like reaction was only reported in barley resistance to Russian wheat aphid, where the resistant barley had more collapsed, autofluorescent cells (because of the polymerization of phenolics) which was usually observed in cells undergoing the HR (Belefantmiller *et al*., 1994). No cell death was observed in the resistant tomato to aphid at 24 h using trypan blue stain (Martinez de Ilarduya *et al*., 2003).

Our microarray results showed that many genes were selectively activated in R plants as early as 6 hpa in addition to *PR1a* precursor, which is suggestive that the R gene *Rag1* might be a regulatory gene associated with triggering resistance in the early stage of the soybean–aphid interaction. Although cvs Dowling and Williams 82 used in this study were not isogenic lines, the comparison between these two cultivars without aphids at three time points provided several R gene candidates. Five genes with constitutively higher expression in Dowling throughout all time points were identified, and the NBS-LRR type of R gene (Gm-r1088-7714) would be the top candidate because two other aphid-resistance genes were mapped in a NBS-LRR cluster region (Brotman *et al*., 2002; Klingler *et al*., 2005) in addition to the *Mi-1.2* gene. By BLASTx search, the closest match to Gm-r10880-7714 was *Rpg1-b*, the soybean disease-resistant gene against *P. savastonoi* pv. *glycinea* (avrB) *at* 83% identity. *Rpg1-b* maps to soybean linkage group F (Ashfield *et al*., 2003), whereas *Rag-1* maps to linkage group M (Li *et al*., 2007). It is possible that *Rag-1* is a homolog of *Rpg1-b* if it is a NBS-LRR type of R gene. Furthermore, *Rag-1* could be absent from the array, or there may be no differential expression between two cultivars. The sequence of selected candidate genes in this study will be used to design primers for use in more detailed mapping.

A model was proposed to summarize the resistance-associated defense responses in R in this study (Fig. 6). Before aphids successfully locate phloem cells to feed on phloem sap, they

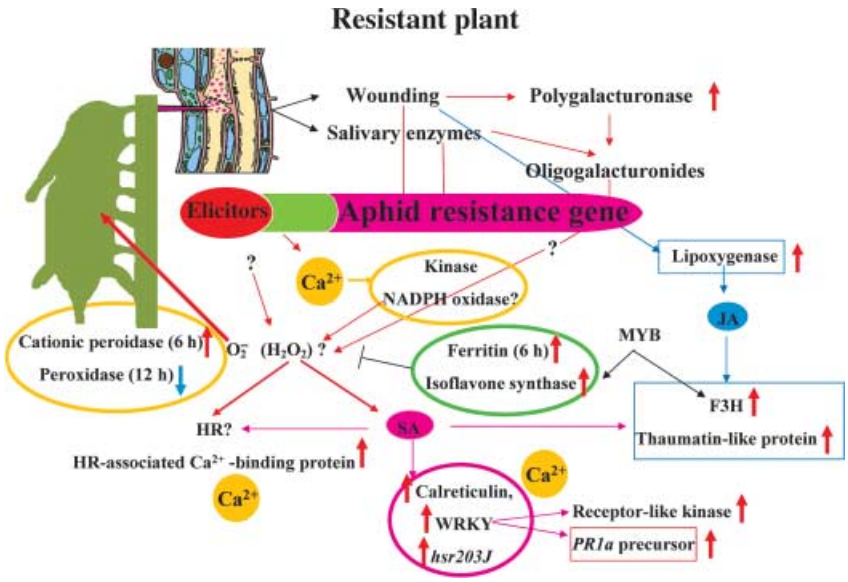

Fig. 6 Proposed model to summarize the defense responses in resistant soybean (*Glycine max*) cv. Dowling at 6 and 12 h post-application (hpa). The genes labeled by upward vertical arrows were up-regulated and those labeled by downward vertical arrows were down-regulated after aphid feeding in this study. HR, hypersensitive reaction; JA, jasmonic acid; SA, salicylic acid; WRKY, WRKY transcription factors.

repeatedly probe by inserting their mouth stylets into plant tissue (Mclean & Kinsey, 1965). This probing activity releases discharged aphid saliva containing peroxidases, β-glucosidases, and other potential signal-generating enzymes, which produces potential chemical signals (such as turonides released from plant cell wall polysaccharides) as a result of digestion by aphid salivary enzymes (Miles, 1999). Additionally, the cells in the stylet path release wound signals as they are punctured (Morgham *et al.*, 1994). When aphids locate phloem cells to feed, additional signals are released (Tjallingii, 2006). Wounding signals can induce synthesis of JA (Bell *et al.*, 1995), which is an essential signal in the defenses against insects, including aphids, in *Arabidopsis* (McConn *et al.*, 1997; Moran & Thompson, 2001; De Vos *et al.*, 2005). The aphid-generated signals could act as elicitors to induce calcium- and reactive oxygen species (ROS)-related signaling. ROS, including H_2O_2, might be directly toxic to insects, leading to decreased herbivory (Bi & Felton, 1995) and contributing to cell wall strengthening (Bradley *et al.*, 1992; Brisson *et al.*, 1994), and may signal the incompatible reaction in challenged cells as well as function as a diffusible signal to induce the genes encoding protectants in adjacent cells (Levine *et al.*, 1994). H_2O_2 can induce SA accumulation (Wu *et al.*, 1997) and SA is a key signaling molecule in local and systemic defenses against pathogens (Dempsey *et al.*, 1999). The SA- and JA-dependent signaling pathways may also overlap.

The defense responses we observed at the RNA concentration in soybean generally coincided with previously observed aphid behavior. In a previous nonchoice test, it was shown that soybean aphids started to avoid feeding on R leaves after 4 hpa and some aphids died as early as 48 hpa, while aphids stayed and colonized leaves of the S genotype to the end of the 72 hpa experiment (Li *et al.*, 2004). Electrical penetration graphing studies likewise showed clear differential feeding behavior between R and S genotypes, with *A. glycines* finding sieve elements within *c.* 4 hpa on a susceptible host, but taking *c.* 8 h on resistant genotypes (Diaz-Montano *et al.*, 2007). In our study, more genes were induced in R than in S at 6 hpa, supporting these observations from behavioral studies that defense-related regulation of gene transcription leading to aphid resistance occurs early. Assuming that the expression of three defense genes we studied by qRT-PCR represents defense in general, one would conclude that the response to *A. glycines* before 48 hpa was induced earlier and more strongly in R than in S. In R, the induction of defense responses declined after 24 or 48 hpa, probably because of the lack of continual feeding or probing by the aphids, caused by avoidance of feeding or cessation of feeding when aphids died after 48 hpa. On the other hand, the induction of defense gene expression in S plants increased after 24 hpa and reached the higher amount of induction after 72 hpa, suggesting timing of the defense induction was important to the aphid resistance in R. Our results are consistent with resistance to *A. glycines* being controlled in a gene-for-gene model involving the early reorganization of aphid-generated signals by a resistance gene triggering rapid downstream defenses to prevent further damage.

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References

Ashfield T, Bocian A, Held D, Henk AD, Marek LF, Danesh D, Penuela S, Meksem K, Lightfoot DA, Young ND *et al.* 2003. Genetic and physical localization of the soybean rpg1-b disease resistance gene reveals a complex locus containing several tightly linked families of nbs-lrr genes. *Molecular Plant–Microbe Interactions* **16**: 817–826.

Basky Z. 2003. Biotypic and pest status differences between Hungarian and South African populations of Russian wheat aphid, diuraphis noxia (kurdjumov) (homoptera: Aphididae). *Pest Management Science* **59**: 1152–1158.

Belefantmiller H, Porter DR, Pierce ML, Mort AJ. 1994. An early indicator of resistance in barley to russian wheat aphid. *Plant Physiology* **105**: 1289–1294.

Bell E, Creelman RA, Mullet JE. 1995. A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **92**: 8675–8679.

Bi JL, Felton GW. 1995. Foliar oxidative stress and insect herbivory – primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. *Journal of Chemical Ecology* **21**: 1511–1530.

Bradley DJ, Kjellbom P, Lamb CJ. 1992. Elicitor-induced and wound-induced oxidative cross-linking of a proline-rich plant-cell wall protein – a novel, rapid defense response. *Cell* **70**: 21–30.

Brisson LF, Tenhaken R, Lamb C. 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* **6**: 1703–1712.

Brotman Y, Silberstein L, Kovalski I, Perin C, Dogimont C, Pitrat M, Klingler J, Thompson GA, Perl-Treves R. 2002. Resistance gene homologues in melon are linked to genetic loci conferring disease and pest resistance. *Theoretical and Applied Genetics* **104**: 1055–1063.

Cole AB, Kiraly L, Ross K, Scheolz JE. 2001. Uncoupling resistance from cell death in the hypersensitive response of *Nicotiana* species to *Cauliflower mosaic virus* infection. *Molecular Plant–Microbe Interactions* **14**: 31–41.

Couldridge, C, Newbury, B, Ford-Lloyd, B, Bale, J, Pritchard J. 2007. Exploring plant responses to aphid feeding using a full *Arabidopsis* microarrray reveals a small number of genes with significantly altered expression. *Bulletin of Entomological Research* **97**: 523–532.

De Vos M, Van Oosten VR, Van Poecke RMP, Van Pelt JA, Pozo MJ, Mueller MJ, Buchala AJ, Metraux JP, Van Loon LC, Dicke M *et al.* 2005. Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Molecular Plant–Microbe Interactions* **18**: 923–937.

Dempsey DA, Shah J, Klessig DF. 1999. Salicylic acid and disease resistance in plants. *Critical Reviews in Plant Sciences* **18**: 547–575.

Diaz-Montano J, Reese JC, Louis J, Campbell LR, Schapaugh WT. 2007. Feeding behavior by the soybean aphid (Hemiptera: Aphididae) on resistant and susceptible soybean genotypes. *Journal of Economical Entomology* **100**: 984–989.

Diaz-Montano J, Reese JC, Schapaugh WT, Campbell LR. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. *Journal of Economical Entomology* **99**: 1884–1889.

Goggin FL, Shah G, Williamson VM, Ullman DE. 2004. Developmental regulation of Mi-mediated aphid resistance is independent of *Mi-1.2* transcript levels. *Molecular Plant–Microbe Interactions* **17**: 532–536.

Goggin FL, Williamson VM, Ullman DE. 2001. Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the tomato resistance gene Mi. *Environmental Entomology* **30**: 101–106.

Graham MY, Weidner J, Wheeler K, Pelow MJ, Graham TL. 2003. Induced expression of pathogenesis-related protein genes in soybean by wounding and the *Phytophthora sojae* cell wall glucan elicitor. *Physiological and Molecular Plant Pathology* **63**: 141–149.

Hajimorad MR, Hill JH. 2001. Rsv1-mediated resistance against soybean mosaic virus-n is hypersensitive response-independent at inoculation site, but has the potential to initiate a hypersensitive response-like mechanism. *Molecular Plant–Microbe Interactions* **14**: 587–598.

Hartman GL, Domier LL, Wax LM, Helm CG, Onstad DW, Shaw JT, Solter LF, Voegtlin DJ, D'Arcy CJ, Gray ME *et al.* 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. *Plant Health Progress*. doi: 10.1094/PHP-2001-0205-01-HN

Harvey TL, Wilde GE, Kofoid KD. 1997. Designation of a new greenbug, biotype k, injurious to resistant sorghum. *Crop Science* **37**: 989–991.

Hill CB, Li Y, Hartman GL. 2004. Resistance to the soybean aphid in soybean germplasm. *Crop Science* **44**: 98–106.

Hill CB, Li Y, Hartman GL. 2006. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. *Crop Science* **46**: 1601–1605.

Johnson ET, Dowd PF. 2004. Differentially enhanced insect resistance, at a cost, in *Arabidopsis thaliana* constitutively expressing a transcription factor of defensive metabolites. *Journal of Agricultural and Food Chemistry* **52**: 5135–5138.

Kaloshian I. 2004. Gene-for-gene disease resistance: bridging insect pest and pathogen defense. *Journal of Chemical Ecology* **30**: 2419–2438.

Kerr MK, Churchill GA. 2001. Experimental design for gene expression microarrays. *Biostatistics* **2**: 183–201.

Kim KS, Hill, CB, Hartman GL, Rouf Mian MA, Diers, BW. (in press). Discovery of soybean aphids biotypes. *Crop Sciences*.

Klingler J, Creasy R, Gao LL, Nair RM, Calix AS, Jacob HS, Edwards OR, Singh KB. 2005. Aphid resistance in *Medicago truncatula* involves antixenosis and phloem-specific, inducible antibiosis, and maps to a single locus flanked by *NBS-LRR* resistance gene analogs. *Plant Physiology* **137**: 1445–1455.

Leon J, Sanchez-Serrano JJ. 1999. Molecular biology of jasmonic acid biosynthesis in plants. *Plant Physiology and Biochemistry* **37**: 373–380.

Levine A, Tenhaken R, Dixon R, Lamb C. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* **79**: 583–593.

Li Y, Hill CB, Carlson SR, Diers BW, Hartman GL. 2007. Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group m. *Molecular Breeding* **19**: 25–34.

Li Y, Hill CB, Hartman GL. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera: Aphididae). *Journal of Economic Entomology* **97**: 1106–1111.

Li J, Pankratz M, Johnson JA. 2002. Differential gene expression patterns revealed by oligonucleotide versus cDNA arrays. *Toxicological Sciences* **69**: 383–390.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative pcr and the 2–δδct method. *Methods* **25**: 402–408.

Martinez de Ilarduya O, Kaloshian I. 2001. *Mi-1.2* transcripts accumulate ubiquitously in root-knot nematode resistant *Lycopersicon esculentum*. *Journal of Nematology* **33**: 116–120.

Martinez de Ilarduya O, Moore AE, Kaloshian I. 2001. The tomato *Rme1* locus is required for *Mi-1*-mediated resistance to root-knot nematodes and the potato aphid. *Plant Journal* **27**: 417–425.

Martinez de Ilarduya O, Xie QG, Kaloshian I. 2003. Aphid-induced defense responses in *Mi-1*-mediated compatible and incompatible tomato interactions. *Molecular Plant–Microbe Interactions* **16**: 699–708.

McConn M, Creelman RA, Bell E, Mullet JE, Browse J. 1997. Jasmonate is essential for insect defense arabidopsis. *Proceedings of the National Academy of Sciences, USA* **94**: 5473–5477.

McLean DL, Kinsey MG. 1965. Identification of electrically recorded curve patterns associated with aphid salivation and ingestion. *Nature* **205**: 1130–1131.

Miles PW. 1999. Aphid saliva. *Biological Reviews* **74**: 41–85.

Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM. 1998. The root knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* **10**: 1307–1319.

Mohase L, van der Westhuizen AJ. 2002. Salicylic acid is involved in resistance responses in the russian wheat aphid-wheat interaction. *Journal of Plant Physiology* **159**: 585–590.

Moran PJ, Cheng YF, Cassell JL, Thompson GA. 2002. Gene expression profiling of *arabidopsis thaliana* in compatible plant-aphid interactions. *Archives of Insect Biochemistry and Physiology* **51**: 182–203.

Moran PJ, Thompson GA. 2001. Molecular responses to aphid feeding in arabidopsis in relation to plant defense pathways. *Plant Physiology* **125**: 1074–1085.

Morgham AT, Richardson PE, Campbell RK, Burd JD, Eikenbary RD, Sumner LC. 1994. Ultrastructural responses of resistant and susceptible wheat to infestation by greenbug biotype-e (Homoptera, Aphididae). *Annals of the Entomological Society of America* **87**: 908–917.

Niderman T, Genetet I, Bruyere T, Gees R, Stintzi A, Legrand M, Fritig B, Mosinger E. 1995. Pathogenesis-related pr-1 proteins are antifungal. Isolation and characterization of three 14-kilodalton proteins of tomato and of a basic PR-1 of tobacco with inhibitory activity against *Phytophthora infestans*. *Plant Physiology* **108**: 17–27.

Park SJ, Huang YH, Ayoubi P. 2006. Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis. *Planta* **223**: 932–947.

Porter DR, Burd JD, Shufran KA, Webster JA, Teetes GL. 1997. Greenbug (Homoptera: Aphididae) biotypes: selected by resistant cultivars or preadapted opportunists? *Journal of Economic Entomology* **90**: 1055–1065.

Rogers EE, Ausubel FM. 1997. *Arabidopsis* enhanced disease susceptibility mutants exhibit enhanced susceptibility to several bacterial pathogens and alterations in *PR-1* gene expression. *Plant Cell* **9**: 305–316.

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM. 1998. The nematode resistance gene Mi of tomato confers resistance against the potato aphid. *Proceedings of the National Academy of Sciences, USA* **95**: 9750–9754.

Smith CM, Belay T, Stauffer C, Stary P, Kubeckova I, Starkey S. 2004. Identification of russian wheat aphid (Homoptera: Aphididae) populations virulent to the *DN4* resistance gene. *Journal of Economic Entomology* **97**: 1112–1117.

Smith CM, Boyko EV. 2007. The molecular bases of plant resistance and defense responses to aphid feeding: current status. *Entomologia Experimentalis et Applicata* **122**: 1–16.

Sun Z, Tian PZ, Wang J. 1990. Study on the uses of aphid-resistant character in wild soybean. I. Aphid-resistance performance of f2 generation from crosses between cultivated and wild soybeans. *Soybean Genetics Newsletter* **17**: 43–48.

Thompson GA, Goggin FL. 2006. Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. *Journal of Experimental Botany* **57**: 755–766.

Tjallingii WF. 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany* **57**: 739–745.

Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, Chandler D, Slusarenko A, Ward E, Ryals J. 1992. Acquired resistance in *Arabidopsis*. *Plant Cell* **4**: 645–656.

Venette RC, Ragsdale DW. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? *Annals of the Entomological Society of America* **97**: 219–226.

Vodkin LO, Khanna A, Shealy R, Clough SJ, Gonzalez DO, Philip R, Zabala G, Thibaud-Nissen F, Sidarous M, Stromvik MV *et al.* 2004. Microarrays for global expression constructed with a low redundancy set of 27 500 sequenced cDNAs representing an array of developmental stages and physiological conditions of the soybean plant. *BMC Genomics* **5**: 73.

van der Westhuizen AJ, Qian XM, Botha AM. 1998a. Beta-1,3-glucanases in wheat and resistance to the Russian wheat aphid. *Physiologia Plantarum* **103**: 125–131.

van der Westhuizen AJ, Qian XM, Botha AM. 1998b. Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation. *Plant Cell Reports* **18**: 132–137.

Wu GS, Shortt BJ, Lawrence EB, Leon J, Fitzsimmons KC, Levine EB, Raskin I, Shah DM. 1997. Activation of host defense mechanisms by elevated production of h2o2 in transgenic plants. *Plant Physiology* **115**: 427–435.

Wu H, Kerr MK, Cui X, Churchill GA 2003. Maanova: a software package for the analysis of spotted cdna microarray experiments. In: Parmigiani G, Garrett ES, Irizarry RA, Zeger SL, eds. *The analysis of gene expression data: methods and softwares*. New York, NY, USA: Springer, 313–341.

Yauk CL, Berndt ML 2007. Review of the literature examining the correlation among DNA microarray technologies. *Environmental and Molecular Mutagenesis* **48**: 380–394.

Yu IC, Parker J, Bent AF. 1998. Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis dnd1* mutant. *Proceedings of the National Academy of Sciences, USA* **95**: 7819–7824.

Zhu-Salzman K, Salzman RA, Ahn JE, Koiwa H. 2004. Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. *Plant Physiology* **134**: 420–431.

Zou JJ, Rodriguez-Zas S, Aldea M, Li M, Zhu J, Gonzalez DO, Vodkin LO, DeLucia E, Clough SJ. 2005. Expression profiling soybean response to pseudomonas syringae reveals new defense-related genes and rapid HR-specific downregulation of photosynthesis. *Molecular Plant–Microbe Interactions* **18**: 1161–1174.

Supplementary Material

The following supplementary material is available for this article online:

Table S1 Primer sequences used for qRT-PCR

Table S2 R-specific responses to aphids at 6 and 12 h post-application (hpa)

Table S3 S-specific responses to aphids at 6 and 12 h post-application (hpa)

Table S4 Comparison between R-specific responses to aphids at 6 and 12 h post-application (hpa) and significant differential expression between Avr- and Vir-induced responses

Table S5 Comparison between S-specific responses to aphids at 6 and 12 h post-application (hpa) and significant differential expression between Avr- and Vir-induced responses

Table S6 Number of genes expressed significantly ($P < 0.05$) higher in Dowling than Williams 82 in absence of the soybean aphid

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