2015

Transcriptional responses of tolerant and susceptible soybeans to soybean aphid (*Aphis glycines* Matsumura) herbivory

Travis J. Prochaska
University of Nebraska-Lincoln, Travis.Prochaska@gmail.com

Teresa Donze-Reiner
University of Nebraska-Lincoln, tdonze@wcupa.edu

L. Marchi-Werle
University of Nebraska-Lincoln

N. A. Palmer
University of Nebraska-Lincoln, nathan.palmer@ars.usda.gov

Thomas E. Hunt
University of Nebraska-Lincoln, thunt2@unl.edu

See next page for additional authors

Authors
Travis J. Prochaska, Teresa Donze-Reiner, L. Marchi-Werle, N. A. Palmer, Thomas E. Hunt, Gautam Sarath, and Tiffany Heng-Moss

Arthropod-Plant Interactions (2015) 9:347–359
DOI 10.1007/s11829-015-9371-2

ORIGINAL PAPER

Transcriptional responses of tolerant and susceptible soybeans to soybean aphid (*Aphis glycines* Matsumura) herbivory

T. J. Prochaska[1] · T. Donze-Reiner[1] · L. Marchi-Werle[1] · N. A. Palmer[2] · T. E. Hunt[3] · G. Sarath[2] · T. Heng-Moss[1]

Received: 22 May 2014 / Accepted: 16 March 2015 / Published online: 29 March 2015

Abstract The soybean aphid, *Aphis glycines* Matsumura, was introduced in 2000 to North America and has become one of the most significant pests to soybean, *Glycine max* (L.) Merrill, production. Possible solutions to this problem are the use of resistant plants and the understanding of the genes involved in plant resistance. In this study, we sought to better understand the genes involved in the tolerance response of soybean plants to the soybean aphid, utilizing tolerant (KS4202) and susceptible (K-03-4686) plants. Studies were conducted under greenhouse conditions. Leaf samples of both tolerant and susceptible plants were collected at day 5 and day 15 after infestation and analyzed by sequencing-by-synthesis on an Illumina GA II X instrument. In the tolerant genotype, 3 and 36 genes were found to be differentially expressed in the infested plants compared to the control treatments at day 5 and day 15, respectively. A similar comparison in the susceptible genotype revealed 0 and 11 genes to be differentially expressed at day 5 and day 15, respectively. Predominately, genes related to plant defense, such as WRKY transcription factors, peroxidases, and cytochrome p450s, were up-regulated in the tolerant genotype 15 days post-infestation by aphids. In contrast, none of these genes were similarly up-regulated in the susceptible plants, suggesting that consistent elevation of defense responses is important to plant tolerance. However, significant genotypic differences in global gene expression were also found when transcriptomes from control uninfested plants were compared at both day 5 and 15. qPCR validation of select genes confirmed our RNA-seq data. These comparisons indicate that potentially broader regulation of transcriptomes also contributes to the tolerance response and provides data that the tolerant genotype (KS4202) could be useful in soybean breeding programs trying to minimize production losses accruing from soybean aphid feeding.

Keywords *Glycine max* · Soybean · *Aphis glycines* · Soybean aphid · Plant resistance · Tolerance

Handling Editor: Joseph Dickens.

Electronic supplementary material The online version of this article (doi:10.1007/s11829-015-9371-2) contains supplementary material, which is available to authorized users.

✉ T. Heng-Moss
thengmoss2@unl.edu

[1] Department of Entomology, University of Nebraska-Lincoln, 103 Entomology Hall, Lincoln, NE 68583-0816, USA

[2] Grain, Forage and Bioenergy Research Unit, USDA-ARS, 362 F Plant Science Hall, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

[3] NEREC Haskell Agricultural Laboratory, Department of Entomology, University of Nebraska-Lincoln, 57905 866 Rd., Concord, NE 68728, USA

Introduction

Soybeans [*Glycine max* (L.) Merrill] are an important global commodity and are grown across large areas of the USA. Since the first introduction of the soybean aphid, *Aphis glycines* Matsumura, in the early 2000s, it has emerged as a major pest of soybeans. Aphids have now spread to 30 states and several south Canadian provinces (Hartman 2001; Alleman 2002; Venette and Ragsdale 2004; Beckendorf et al. 2008; NAPIS 2011) and have caused considerable economic damage to soybean growers (NAPIS 2011; Ragsdale et al. 2011; McCarville et al. 2011; Ragsdale et al. 2007; Venette and Ragsdale 2004).

Different strategies have been developed to manage this pest including chemical, biological, and cultural control methods (Wang and Ba 1998; Wang et al. 2000; Ostlie 2002; Hill et al. 2004; Wu et al. 2004; Rutledge and O'Neil 2006; Brosius et al. 2007). Recently, plant resistance has gained attention as a viable management option. Soybeans that exhibit antibiosis, antixenosis, and tolerance have been identified (Hill et al. 2004, 2006a, b; Mensah et al. 2005; Diaz-Montano et al. 2006; Kang et al. 2008; Mian et al. 2008; Pierson et al. 2010; Prochaska et al. 2013). Genes that confer resistance to the soybean aphid through antibiosis have been reported (Wiarda et al. 2012). These include *Rag1*, found from the cultivar Dowling, and *Rag2* that were identified in the variety PI 200538 (Hill et al. 2006a, 2009). Within North America, *Rag1* has been incorporated into soybean cultivars that are sold commercially. However, virulent *A. glycines* biotypes have also been identified.

Microarray analyses using cultivar (cv) Dowling as a resistant source and cv Williams 82 as a source for susceptibility found that cv Dowling showed a differential expression of 140 genes when challenged with *A. glycines* as compared to the susceptible cv William 82 plants. Specifically, three plant defense-related genes were upregulated earlier in the resistant line (Li et al. 2008b). More recently, Studham and Macintosh (2013) investigated the effect of the *Rag1* gene on the transcriptional responses of soybean challenged with soybean aphids using line LD16060 as a source of resistance and SD01-76R as a susceptible source. Using a microarray analysis combined with qPCR on select genes, they showed that the susceptible plants had significant gene expression changes elicited by aphid herbivory, as compared to the resistant soybean line. They suggested that the resistant line constitutively expresses many of the defense-related genes. Verification of the microarray data using qPCR showed over a 10-fold change in data for the same genes in repeat experiments, suggesting that significant variation could occur during this validation experiment. However, the basic findings were that aphid infestation changed the plant transcriptome in a mostly predictable manner and that these differences (despite huge experimental variances) were consistent between the susceptible and resistant lines.

Plant tolerance is a form of resistance that allows a plant to harbor a large number of aphids without a significant loss in yield (Smith 2005). Although tolerance has been identified in soybean (Pierson et al. 2010; Prochaska et al. 2013), limited information is available on how soybean aphid feeding impacts the underlying transcriptional machinery of the plant. Using a susceptible and a tolerant soybean line, Pierson et al. (2011) showed that physiological and biochemical differences exist between aphid-infested and aphid non-infested plants. Total photosynthetic capacity was reduced in aphid-infested plants when compared to control (non-infested) plants of the susceptible genotype Asgrow 2703. Few differences existed between aphid-infested and non-infested plants in the tolerant KS4202 genotype (Pierson et al. 2011; Prochaska et al. 2013). Through peroxidase profiling, Pierson et al. (2011) observed unique banding patterns between aphid-infested and non-infested plants, suggesting peroxidases may play a role in the plant response to aphid herbivory.

To more effectively query global plant responses to aphid feeding, it is possible to utilize microarrays and next-generation sequencing (NGS) technology. Microarrays have been routinely used to study plant responses to insect herbivory (Reymond et al. 2000; Halitschke et al. 2003; Voelckel et al. 2004; Park et al. 2005a, b; Smith and Boyko 2007; Li et al. 2008b; Gutsche et al. 2009). Relatively, few of these studies have used plants with divergent responses to aphids (Zhu-Salzman et al. 2004; Park et al. 2005a, b; Couldridge et al. 2007; Kempema et al. 2007; Li et al. 2008b; De Vos and Jander 2009; Studham and Macintosh 2013). Here we have used NGS to compare and contrast changes in leaf transcriptomes from tolerant and susceptible soybean plants in response to infestation by *A. glycines* that may help uncover more about the tolerant response found in soybean KS4202.

Materials and methods

Two soybean genotypes were selected for Illumina sequencing to gain a better understanding of the tolerant response to soybean aphid feeding. The genotypes selected for sequencing included the tolerant genotype KS4202 and the susceptible genotype K03-4686 (Pierson et al. 2010, 2011; Chandran 2011; Prochaska et al. 2013). Four seeds of each genotype were planted in potting media (34 % peat, 31 % perlite, 31 % vermiculite, and 4 % soil mix) in 15-cm-diameter round plastic pots (Hummert International, Earth City, MO, USA). Plants were thinned to one plant per pot once seedlings emerged from the soil. Soybeans were grown to the V5 vegetative stage (Fehr and Caviness 1977) in a greenhouse setting under 400-W high-intensity lamps with a 16:8 (L:D) hour photoperiod at a temperature of 23 ± 2 °C.

V5 stage soybean plants were infested with 20 adult aphids on the uppermost fully opened trifoliate. Soybean aphids were obtained from a laboratory maintained colony (Biotype 1, Illinois Biotype). The treatment design was a 2 × 2 × 2 factorial design with two soybean genotypes (tolerant and susceptible), two infestation treatments (control (non-infested) and 20 aphids per plant), and two harvest dates (5 and 15 days). All plants were caged with tubular plastic cages with vents covered with organdy

fabric to confine the aphids. Day 5 was selected expecting that no physical damage would be visible, but that some metabolic changes would occur. Day 15 was selected as we expected physical and metabolic changes to occur based on observations seen by Pierson et al. (2010, 2011) and Prochaska et al. (2013). The experimental design was a completely randomized design with six replications.

Before destructively harvesting plants for Illumina sequencing, damage ratings were performed using a 1–5 scale, where 1 = ≤10 % yellowing discoloration; 2 = 11–30 % yellowing discoloration; 3 = 31–50 % yellowing discoloration; 4 = 51–75 % yellowing discoloration; and 5 = ≥76 % of leaf area with yellowing discoloration or dead tissue (Hill et al. 2004; Pierson et al. 2011). Aphid number and plant stage were also recorded. At the time of harvest, plants were in the V6–V7 vegetative stages. Aphids were removed from the plants with a camel hairbrush. Following aphid removal, the top two trifoliates (youngest plant tissue) were harvested, flash-frozen in liquid nitrogen, and stored at −80 °C until analyzed.

From each of the six biological replicates, three samples were randomly selected and submitted to the University of Nebraska—Lincoln Biotechnology Center for RNA sequencing using Illumina technology. Total RNA was isolated from the soybean leaf samples, and quality was verified by a bioanalyzer (2100 Bioanalyzer, Agilent, Santa Clara, CA, USA) prior to generation of cDNA libraries (Chomczynski and Sacchi 1987). Libraries were analyzed using the Illumina Genome Analyzer IIx (www.illumina.com) and 56 cycles of sequencing-by-synthesis chemistry using manufacturer supplied protocols. Sequence reads were aligned to the soybean genome—*G. max* 109 (Goodstein et al. 2011)—using the Bowtie mapping software (Langmead et al. 2009) and gene expression counts calculated using HTSeq (Anders 2010). Differential expression analysis was done in R (R Core Team 2013) using the Bioconductor (Gentleman et al. 2004) distributed package DESeq2 (Anders and Huber 2010). Only significant genes at the false discovery rate (FDR) of <0.10 are reported. The cutoff for average $\log_2$ fold change between the aphid-infested and control samples was ±2.0.

cDNA synthesis and qPCR validation

Using 2.5 µg of total RNA treated with RNase-free DNase I (Life Technologies, Rockville, MD, USA), first strand cDNA synthesis was completed using ThermoScript RT-PCR system (Life Technologies) according to the manufacturer's protocol. All qPCR was performed on a 7500 Fast Real-Time PCR system (Applied Biosystems) using Bio-Rad SsoAdvanced SYBR Green (Bio-Rad Laboratories, CA, USA) following the manufacturer's protocol which consists of 95 °C for 30 s, then 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Four specific genes used for validation were (1) Glyma06g15030: (fwd: 5′-CCGCCATGATCAAGATGGGA-3′, rev: 5′-AACCCACCACGGAATCCAAA-3′), (2) Glyma16g02960 (fwd: 5′-ATGGCAGCATGATGGATTCC-3′, rev: 5′-TTCTGTGCACGTTGACATGG-3′), (3) Glyma17g34210 (fwd: 5′-TTCAGTGGATGGATGCAACG-3′, rev: 5′-ACTTGGATGAGTGTGGTTGC-3′), and (4) Glyma05g27030 (fwd: 5′-ACGTGGCCATAAGGGTTGAG-3′, rev: 5′-CCAGCAATCTCCCCCAACAT-3′). CYP2 (fwd: 5′-CCCCTCCACTACAAAGGCTCG-3′, rev: 5′-CGGGACCAGTGTGCTTCTTCA-3′) was included in the validation as the endogenous control.

Results and discussion

Damage ratings

Minimal evidence of visible plant damage was observed between infested tolerant and susceptible plants at 5 (tolerant infested: 1.3 ± 0.21, tolerant control: 1.0 ± 0.00, susceptible infested: 1.5 ± 0.22, and susceptible control: 1.0 ± 0.00) and 15 (tolerant infested: 1.2 ± 0.16, tolerant control: 1.0 ± 0.00, susceptible infested: 1.5 ± 0.22, and susceptible control: 1.0 ± 0.00) days after aphid infestation. Furthermore, the number of aphids on the tolerant and susceptible genotypes was similar on day 15 (tolerant: 217.00 ± 79.93 and susceptible: 241.00 ± 38.23). Differences were found on day 5 (tolerant: 25.00 ± 2.44 and susceptible: 53.17 ± 9.73).

Mapping statistics

Soybean cDNA libraries were constructed from leaf tissue of tolerant and susceptible plants infested with soybean aphids throughout a time course, and data are summarized in Table 1. An average of 25.6 million and 37.6 million 56-bp single-end reads were generated from RNA obtained from susceptible plants at day 5 and day 15, respectively. In the tolerant genotype, the average number of 56-bp single-end reads at day 5 and 15 were 23.3 million and 29.2 million. Overall, approximately 98 % of the reads mapped to the reference soybean transcriptome.

Through a $\log_2$ fold change comparison with a FDR < 0.1, relative gene expression levels were compared between infested and control plants for the tolerant genotype. A total of two differentially expressed genes (DEGs) had a higher expression level ($\log_2 > 2.0$), and 0 DEGs had a lower expression level ($\log_2 > -2.0$) in response to aphid feeding at day 5. By day 15, 19 DEGs had a higher expression level in aphid-infested plants when compared to

Table 1 Mapping statistics generated from the Bowtie program alignment for soybean lines KS4202 (tolerant) and K03-4686 (susceptible)

	Total reads	Average total alignment (%)	Unaligned	Unaligned (%)	Reads mapped to genome	Reads mapped to genome (%)
Day 5						
Tolerant control	26,647,947	97.3	712,854	2.7	25,935,093	97.3
	27,280,148	96.8	877,551	3.2	26,402,597	96.8
	24,918,386	98.1	478,816	1.9	24,439,570	98.1
Tolerant infested	25,925,659	97.4	674,525	2.6	25,251,134	97.4
	23,654,349	96.4	860,405	3.6	22,793,944	96.4
	25,332,027	97.1	726,962	2.9	24,605,065	97.1
Susceptible control	22,948,093	98.0	451,232	2.0	22,496,861	98.0
	23,084,055	97.7	536,318	2.3	22,547,737	97.7
	22,085,586	97.7	499,028	2.3	21,586,558	97.7
Susceptible infested	24,997,494	97.8	548,703	2.2	24,448,791	97.8
	22,512,732	98.2	399,812	1.8	22,112,920	98.2
	24,094,267	97.8	520,192	2.2	23,574,075	97.8
Day 15						
Tolerant control	38,059,412	99.2	324,144	0.9	37,735,268	99.2
	39,693,593	99.0	402,263	1.0	39,291,330	99.0
	40,121,007	99.3	274,155	0.7	39,846,852	99.3
Tolerant infested	38,722,186	97.9	800,073	2.1	37,922,113	97.9
	38,758,395	99.1	366,606	1.0	38,391,789	99.1
	30,368,934	98.9	355,028	1.2	30,013,906	98.8
Susceptible control	31,888,099	98.5	494,549	1.6	31,393,550	98.5
	31,765,690	98.7	402,385	1.3	31,363,305	98.7
	27,972,996	98.0	556,222	2.0	27,416,774	98.0
Susceptible infested	27,994,904	97.1	803,146	2.9	27,191,758	97.1
	28,578,842	98.4	465,596	1.6	28,113,246	98.4
	27,064,189	98.7	364,858	1.4	26,699,331	98.7

control plants and five DEGs had a lower expression level between tolerant infested and control plants (Table 2). Comparisons with the susceptible genotype at 5 days after aphid introduction showed no DEGs with higher or lower gene expression levels. By day 15, five DEGs in the infested susceptible plants had a higher expression level and five DEGs had a lower expression level when compared to control (non-infested) plants (Table 2).

Studham and MacIntosh (2013) showed that plant defense responses occur early in the presence of aphid feeding (24 h), with continued expression changes at later time points (7 days after infestation). Genetic differences

Table 2 Log_2 fold gene expression changes of infested treatment compared to their respective control with a FDR < 0.1

Log_2 fold change:gene expression	D5 Tolerant	D15 Tolerant	D5 Susceptible	D15 Susceptible
3:4	0	4	0	1
2:3	2	15	0	4
1:2	1	96	6	16
1:−1	17	166	18	13
−1:−2	0	8	3	4
−2:−3	0	4	0	3
−3:−4	0	0	0	1
−4:−5	0	1	0	0
−5:−6	0	0	0	1

between the susceptible and resistant plants appeared to contribute to this differential response. Li et al. (2008b) observed that several genes appear to be expressed at higher levels in resistant plants, whereas susceptible plants showed an increase in the expression of these same genes after 24 h. From our dataset, we observed the number of DEGs to be greater at day 15 in the susceptible aphid-infested versus aphid non-infested plants and in the tolerant aphid-infested versus aphid non-infested plants (Table 2). The differences between our experiments may result from the soybean varieties selected, evaluation intervals (days 1 and 7 vs. days 5 and 15), aphid infestations levels, and possibly a combination between local and systemic responses.

qPCR validation

Gene expression studies were performed using qPCR to validate the NGS datasets, using RNA extracted from a repeat experiment performed in a manner identical to those used for Illumina sequencing. Transcript abundances of select genes (Glyma06g15030, Glyma16g02960, Glyma17g34210, and Glyma05g27030) found to be differentially expressed in the susceptible and tolerant soybeans (Tables 3, 4) were analyzed. Overall, qPCR data (Fig. 1) confirmed RNA-seq analyses, although some variation was noted, similar to studies reported by Studham and Macintosh (2013).

Analysis of differentially expressed genes

Five days after infestation, there were three DEGs in tolerant infested plants. Two of these genes were of unknown function and one (*Glyma10g31610*) was a yellow stripe-like (YSL) ortholog (Table 3). YSLs are membrane located transporters that are important to the intercellular transport of iron and other metals and contribute to the overall metal nutrition in plants (Brear et al. 2013; Conte et al. 2013). Although metal ion transporters have not been analyzed in detail in plant–aphid interactions, it is possible that they could be part of the cascade of changes that are elicited upon aphid feeding (Boyd 2006; Poschenrieder et al. 2006). After 15 days of aphid infestation, 36 genes were differentially expressed in the tolerant infested plants. From those 36 genes, six DEGs were down-regulated and 30 genes were up-regulated (Table 3). Several of these genes encode for proteins with known involvement in plant responses to biotic stress and/or insect feeding, including peroxidases, cytochrome P450s, WRKY transcription factors, lecunie-rich receptor kinases (LRR), a Kunitz trypsin inhibitor, CoA ligases, and pectin lyases.

Class III plant peroxidases serve a central role in the cell wall building process, wound healing, auxin catabolism, the removal of hydrogen peroxide, and defense against pathogen or insect attack (Hiraga et al. 2001; Ni et al. 2001; Kawano 2003; Heng-Moss et al. 2004; Gutsche et al. 2009; Gill and Tuteja 2010), and the related ascorbate peroxidases are essential for detoxifying excess cellular hydrogen peroxide (Jespersen et al. 1997; Ishikawa and Shigeoka 2008; Gill and Tuteja 2010). Further evidence has documented increased levels of peroxidases playing a role in defensive responses to aphid herbivory in a number of plant systems (Argandona et al. 2001; Ni et al. 2001; Park et al. 2005a, b; Smith and Boyko 2007; Gutsche et al. 2009). Changes in peroxidases, based on microarray analyses, have also been documented in the rice/wheat-Hessian fly systems (Liu et al. 2010), and these changes appear to be part of the plant defense against this pest.

We found a peroxidase gene (*Glyma06g15030*) with significantly higher expression levels (with a false discovery rate (FDR) of 0.026 [$\log_2$ fold change (FC) = 2.6]) in the infested tolerant plants when compared to the tolerant control plants at day 15 (Table 3). A second peroxidase gene (*Glyma14g35440*) was found in the tolerant soybean to be differentially expressed at day 15 between the control and infested treatments with a FDR of 0.095 and an average expression found to be about 15 % higher in infested plants compared to control plants at this time point. No peroxidase genes were found to be differentially expressed at day 5. Pierson et al. (2011) reported an increase in peroxidase activity in the tolerant soybean in response to aphid feeding at 24 and 28 days after aphid infestation. In contrast, peroxidase genes were not differentially expressed in the susceptible plants at either day 5 or 15 after aphid infestation. These data are consistent with a previously proposed hypothesis (Heng-Moss et al. 2004; Franzen et al. 2007; Gutsche et al. 2009; Ramm et al. 2013) that tolerant plants have the ability to elevate their level of reactive oxygen species (ROS)-scavenging enzymes, such as peroxidases, which enable them to efficiently remove intracellular ROS that accumulate in response to aphid feeding.

Two genes encoding cytochrome P450s were also found to be differentially expressed in the tolerant soybean at day 15. *Glyma11g06390* (cytochrome P450 family 82) was found to have increased expression during infestation with a FDR of 0.041 (Table 3). The second cytochrome P450 encoding gene (*Glyma05g27030*) had a FDR of 0.062 (Table 3) with increased expression. No differences in gene expression were found in the day 5 time point of the tolerant genotype nor in either time points of the susceptible soybean (Tables 3, 4). In plants, cytochrome P450s, some of which are involved in jasmonic acid (JA)-mediated defense responses (Park et al. 2002), have been shown to be induced in aphid-resistant wheat and sorghum in response to *Diuraphis noxia* and *Schizaphis graminum*,

Table 3 Differential expression for genes found in aphid-tolerant soybean line KS4202

Day	Genotype	Gene ID	Log_2 fold change	FDR	*Arabidopsis* gene ID	Best *Arabidopsis* hit	Functional information
Day 5	Tolerant	Glyma10g31610	2.4	0.05	AT4G24120	YELLOW STRIPE-like 1	Oligopeptide transporter (Le Jean et al. 2005)
		Glyma10g12370	2.2	1.20E−07	AT2G41905	NA	hypothetical protein (Krogh et al. 2001)
		Glyma20g32570	2.2	0.05	NA	NA	NA
Day 15	Tolerant	Glyma05g22960	3.5	3.63E−04	AT5G10050	NAD(P)-binding Rossmann-fold superfamily protein	Protein coding (Tabata et al. 2000)
		Glyma05g03750	3.3	9.37E−04	AT1G04110	Subtilase family protein	Mediates cell-to-cell signaling (Von Groll et al. 2002)
		Glyma15g42590	3.1	0.06	AT2G44480	Beta glucosidase 17	Beta glucosidase (Lin et al. 1999)
		Glyma16g30350	3.1	5.50E−04	AT2G34930	Disease resistance family protein/LRR family protein	Disease resistance family protein (Kobe and Kajava 2001)
		Glyma16g31420	3.1	1.70E−03	AT2G34930	Disease resistance family protein/LRR family protein	Disease resistance family protein (Kobe and Kajava 2001)
		Glyma12g31780	3.0	1.14E−06	AT2G32540	Cellulose synthase-like B4	Protein Coding (Lin et al. 1999)
		Glyma16g30360	3.0	0.04	AT2G34930	Disease resistance family protein/LRR family protein	Disease resistance family protein (Kobe and Kajava 2001)
		Glyma16g30600	3.0	0.03	AT2G34930	Disease resistance family protein/LRR family protein	Disease resistance family protein (Kobe and Kajava 2001)
		Glyma15g01230	2.9	7.52E–06	NA	NA	NA
		Glyma17g34210	2.8	0.04	AT5G26170	WRKY DNA-binding protein (WRKY 50, 51)	SA and JA signaling regulators (Pandey and Somssich 2009)
		Glyma01g38530	2.8	3.16E−09	AT4G36850	PQ-loop repeat family protein/transmembrane family protein	Protein coding (Mayer et al. 1999)
		Glyma06g15030	2.6	0.03	AT5G05340	Peroxidase superfamily protein (Peroxidase 52)	Oxidative Stress (Hiraga et al. 2001)
		Glyma04g42180	2.6	6.09E−04	AT5G56790	Protein kinase superfamily protein	Protein coding (Tabata et al. 2000)
		Glyma11g06770	2.6	0.01	AT4G36850	PQ-loop repeat family protein/transmembrane family protein	Protein coding (Mayer et al. 1999)
		Glyma08g46010	2.5	2.58E−03	AT3G25240	Protein of unknown function (DUF506)	Uncharacterized protein (Salanoubat et al. 2000)
		Glyma15g23830	2.4	0.05	NA	NA	NA
		Glyma10g04230	2.4	0.05	AT2G38940	Phosphate transporter 1;4	Phosphate transporter (Okumura et al. 1998)
		Glyma13g33100	2.2	0.03	NA	NA	NA
		Glyma01g11870	2.2	8.59E–06	AT1G73260	Kunitz trypsin inhibitor 1	Trypsin inhibitor involved with modulating programmed cell death (Li et al. 2008a)
		Glyma01g35620	2.2	0.07	AT4G19380	Long-chain fatty alcohol dehydrogenase family protein	Protein coding (Mayer et al. 1999)
		Glyma02g46440	2.2	0.06	AT4G26770	Phosphatidate cytidylyltransferase family protein	Protein coding (Mayer et al. 1999)
		Glyma08g18700	2.1	0.06	AT1G30260	NA	Uncharacterized protein/cytokinin response (Brenner et al. 2005)
		Glyma11g06390	2.1	0.04	AT4G31940	Cytochrome P450, family 82, subfamily C, polypeptide 4	Early Fe deficiency response (Murgia et al. 2011)
		Glyma18g10330	2.1	0.06	AT4G04450	WRKY family transcription factor (WRKY 6, 31, 36, 42, 47)	Responses to low-Pi stress (Chen et al. 2009)
		Glyma10g34160	2.0	6.56E−04	AT1G20510	OPC-8:0 CoA ligase1	Acyl-coenzyme A synthetase family (Kienow et al. 2008)

Table 3 continued

Day	Genotype	Gene ID	Log_2 fold change	FDR	*Arabidopsis* gene ID	Best *Arabidopsis* hit	Functional information
		Glyma05g32740	2.0	0.02	AT5G63950	Chromatin remodeling 24	Chromatin remodeling (Sarry et al. 2006)
		Glyma05g27030	1.8	0.06	AT3G18270	Cytochrome P450, family 77, subfamily A, polypeptide 5 pseudogene	Chloroplast localization (Hu et al. 2013)
		Glyma14g35440	1.8	0.10	AT4G09010	Ascorbate peroxidase 4	ascorbate peroxidase APX4 (Lundberg et al. 2011)
		Glyma16g02960	1.8	4.40E−03	AT4G11070	WRKY family transcription factor (WRKY 41, 53)	Negative regulators of defense signaling (Pandey and Somssich 2009)
		Glyma03g41750	1.6	0.06	AT5G24110	WRKY DNA-binding protein (WRKY 30)	General stress response (Scarpeci et al. 2013)
		Glyma01g31300	−2.0	0.02	AT5G01600	Ferritin 1	Protein coding (Touraine et al. 2012)
		Glyma03g06420	−2.1	0.02	AT5G01600	Ferritin 1	Protein coding (Touraine et al. 2012)
		Glyma13g02510	−2.3	3.38E−03	AT1G77760	Nitrate reductase 1	Encodes nitrate reductase (Konishi and Yanagisawa 2011)
		Glyma03g37310	−2.8	0.07	AT1G02820	Late embryogenesis abundant 3 (LEA3) family protein	Protein coding (Theologis et al. 2000)
		Glyma20g01930	−2.8	0.02	AT5G12020	17.6 kDa class II heat shock protein	Heat shock protein (Sun et al. 2001)
		Glyma19g27780	−4.3	0.03	AT3G01590	Galactose mutarotase-like superfamily protein	Protein coding (Salanoubat et al. 2000)

respectively (Park et al. 2005a, b; Boyko et al. 2006). Our data generally support these earlier findings. In *Arabidopsis*, the *Glyma11g06390* ortholog (*AT4G31940*) is shown to be tied to the early iron deficiency response, possibly through an iron-deficiency-responsive element (IDE1)-like mediated pathway (Murgia et al. 2011). It is plausible that changes in the YSL transcripts observed at day 5 after aphid infestation in tolerant plants, coupled to the down-regulation of two ferritin genes at day 15, could be indicative of an underlying change in tissue iron levels.

Glyma11g06390 encodes a cytochrome P450 enzyme. P450s can catalyze a number of different reactions, and the role of this soybean P450 in the defense response of the plant is unknown at this time. As an example, the *CYP82E4* gene, a member of the cytochrome P450 family 82 in tobacco, encodes a nicotine *N*-demethylase that can convert nicotine to nornicotine (Siminszky et al. 2005; Xu et al. 2007; Murgia et al. 2011). Nicotine and related metabolites are part of the tobacco defense against insects, and it is possible that the soybean P450 enzyme catalyzes reactions needed to generate defense compounds specific to soybeans.

Four WRKY genes were shown to be differentially expressed in the tolerant soybean. These included *Glyma16g02960* (orthologous to *AtWRKY41* and *AtWRKY53*), *Glyma17g34210*, (*AtWRKY50* and *AtWRKY51*), *Glyma18g10330* (*AtWRKY42, AtWKRY6, AtWKRY31, AtWRKY36*, and *AtWRKY47*), and *Glyma03g41750* (*AtWRKY30*; Table 3). WRKY genes have been reported to be involved in plant defense in other systems, such as wheat (Lapitan et al. 2008; Eck et al. 2010; Botha et al. 2010). In *Arabidopsis*, 74 genes have been found to encode WKRY transcription factors (Pandey and Somssich 2009). WRKYs are involved in a large array of plant responses and frequently can serve redundant functions (Pandey and Somssich 2009). For example, *AtWRKY70* serves as a convergence point that determines the balance between salicylic acid (SA) and jasmonic acid (JA) defensive pathways (Pandey and Somssich 2009). Many WRKYs, including *AtWKRY41* and *AtWRKY53*, serve as negative regulators of defense signaling (Pandey and Somssich 2009). *AtWRKY50* and *AtWRKY51* appear to serve as positive regulators of SA-mediated signaling and as negative regulators of JA-mediated signaling (Gao et al. 2011). *AtWRKY42* and *WRKY6* are part of the WRKY group II-b family. Several of the WRKY genes that are members of the group II-b are involved in *Arabidopsis* response to low-Pi (phosphate) stress by regulating *PHOSPHATE1* (*PHO1*) expression (Chen et al. 2009). Coincidently, *Glyma10g04230* coding for a phosphate transporter is significantly enriched in tolerant plants 15 days after aphid introduction. *AtWRKY30* is a general stress response gene that plays a vital role in the plant's defense against various stresses, especially during early growth stages (Scarpeci et al. 2013). Our data would suggest that the differentially expressed soybean WRKY orthologs participate in similar cascades as has been described in *Arabidopsis*.

Table 4 Differential expression for genes found in aphid-susceptible soybean line K03-4686

Day	Genotype	Gene ID	Log_2 fold change	FDR	*Arabidopsis* gene ID	Best *Arabidopsis* hit	Functional information
Day 5	Susceptible	NA	NA	NA	NA	NA	NA
Day 15	Susceptible	Glyma13g02510	3.23	0.01	AT1G77760	Nitrate reductase 1	Nitrate assimilation (Konishi and Yanagisawa 2011)
		Glyma19g00730	2.64	0.03	AT1G75250	RAD-like 6	Transcription factor (Theologis et al. 2000)
		Glyma02g42990	2.50	0.01	AT2G40330	PYR1-like 6	Abscisic acid sensors (Santiago et al. 2009)
		Glyma13g21350	2.38	0.00	AT1G76870	NA	Uncharacterized protein (Theologis et al. 2000)
		Glyma11g10130	2.11	0.00	AT1G50460	Hexokinase-like 1	Protein coding (Karve et al. 2008)
		Glyma13g27590	2.00	0.04	AT4G21870	HSP20-like chaperones superfamily protein	Chaperone (Garcia-Ranea et al. 2002)
		Glyma07g01660	−2.53	0.00	AT1G14520	Myo-inositol oxygenase 1	Protein coding (Kanter et al. 2005)
		Glyma08g42840	−2.59	0.01	AT3G20395	RING/U-box superfamily protein	Protein coding (Salanoubat et al. 2000)
		Glyma01g00930	−2.86	0.00	AT5G56550	Oxidative stress 3	Oxidative stress (Blanvillain et al. 2009)
		Glyma14g07990	−3.86	0.07	AT1G19530	NA	Uncharacterized Protein (Theologis et al. 2000)
		Glyma05g26390	−5.35	0.01	AT1G48100	Pectin lyase-like superfamily protein	Protein coding (Theologis et al. 2000)

Four genes encoding for disease family resistance proteins/leucine-rich repeat (LRR) proteins were found to be differentially expressed in the tolerant soybean at day 15 (*Glyma16g31420*, *Glyma16g30360*, *Glyma16g30350*, and *Glyma16g30600*; Table 3). No significant differences were found at day 5 in the tolerant genotype. In plants, all of the aphid resistance genes, reported so far, encode nucleotide binding site-LRR proteins (Crute and Dunn 1980; Chen et al. 1997; Rossi et al. 1998; Milligan et al. 1998; Nombela et al. 2003; Wroblewski et al. 2007). These large, and often abundant, proteins aid in the detection of diverse pathogens including bacteria, viruses, fungi, insects, and nematodes.

One gene encoding for a Kunitz trypsin inhibitor (*Glyma01g11870*) was found to be up-regulated in the tolerant soybean at day 15 (Table 3). Protease inhibitors have been widely studied in animals, plants, and microorganisms with their roles in plants often associated with defense against pests (Lee et al. 1999). Lee et al. (1999) showed that transgenic rice plants appear to be more resistant to the brown planthopper (*Nilaparvata lugens* Stål) over control plants after the use of a recombinant plasmid to introduce a Kunitz trypsin inhibitor into the protoplasts. Their studies indicated that the introduction of Kunitz trypsin inhibitors could be used to control the brown planthopper in R1 and R2 generation rice plants and potentially be used to control other insect pests in rice.

One gene encoding for acyl-coenzyme A (CoA) ligase (*Glyma10g34160*) was found with higher gene expression in the tolerant soybean at day 15 (Table 3). Kienow et al. (2008) showed that four carboxylic acid activating enzymes, including that of CoA ligase, displayed activity toward different biosynthetic precursors of jasmonic acid in response to stress. In previous studies, jasmonic acid has been shown to play an important role in plant defense against insect pests (McConn et al. 1997; Paré and Tumlinson 1999; Howe and Jander 2008; Gaquerel et al. 2013; Ballaré 2014).

Interestingly, *Glyma13g02540*, encoding a nitrate reductase, was differentially regulated in the tolerant and susceptible plants. Transcripts for this gene were significantly (−2.3-fold) down-regulated in the tolerant plants and significantly up-regulated (3.2-fold) in the susceptible plants. Aphid feeding can lead to a 2-fold increase in nitrate reductase activity in cabbage seedlings infested with the green peach aphid (*Myzus persicae*) (Wilson et al. 2011). These authors suggest that possible signals present in the salivary secretions of the aphid trigger the increase in nitrate reductase activity in cabbage leaf. Data presented here are consistent with the Wilson et al. (2011) hypothesis. Susceptible soybean plants appear to mirror (at least for nitrate reductase) what has been shown in aphid-infested, apparently susceptible, cabbage plants. For the

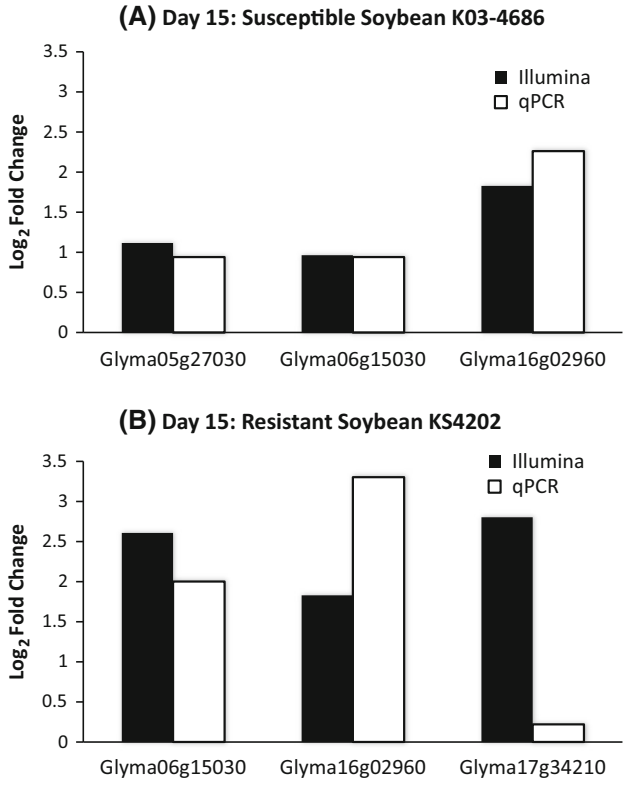

Fig. 1 Validation of transcript abundance detected by RNA-seq using qPCR. **a** Log_2 fold change for select genes comparing Illumina results with qPCR expression data in the susceptible soybeans at day 15. **b** Log_2 fold change for select genes comparing Illumina results with qPCR expression data in the tolerant soybeans at day 15

tolerant soybean genotype, a differential response (as compared to the susceptible plants) can be expected (Prochaska et al. 2013; Pierson et al. 2011). However, more work is needed to tease apart the molecular networks that lead to these differences.

No genes were found to be differentially expressed at day 5 after aphid infestation in the susceptible soybean (Table 4). Fifteen days following aphid infestation, five genes were found with lower gene expression and six genes were found with higher gene expression in the susceptible genotype (Table 4).

Fifteen days after infestation, one gene encoding for heat shock protein (HSP) was found to be differentially expressed in the susceptible soybean. *Glyma13g27590*, encoding for heat shock protein 70 (HSP70), had a FDR of 0.035 (Table 4). Heat shock proteins perform a variety of functions in plants from protein folding to assembly, translocation, and degradation in cellular processes and can assist in the refolding of proteins under stress conditions. It has been demonstrated in expression profile studies that HSP70 genes are expressed in response to stresses such as heat, cold, drought, chemical, and other environmental stresses in *Arabidopsis* and spinach (Guy and Li 1998; Lin et al. 2001; Sung et al. 2001; Wang et al. 2004).

Glyma05g26390, a gene encoding for pectin lyase, was found to be down-regulated at day 15 in the susceptible soybean (Table 4). Pectin lyases contribute to several biological processes, including the degradation of pectin found in the plant cell wall (Cao 2012). This suggests that the plant is down-regulating expression of pectin lyase, which would lead to a reduced rate of pectin degradation as it attempts to protect itself from the stress of aphid herbivory (Cao 2012). We did not find any pectin lyases to be differentially expressed in the tolerant soybean at day 5 or day 15.

Gene expression trends between non-infested tolerant and susceptible control plants

Analysis of differences in the transcriptomes of tolerant and susceptible plants was undertaken to provide data on the baseline differences in gene expression in these two genotypes of soybeans. Various DEGs were found in the tolerant control versus susceptible control plants. The day 5 analysis showed 709 DEGs to be down-regulated and 341 DEGs to be up-regulated in the tolerant control soybean (Supplementary Tables 1A and 1B). These genes included 22 cytochrome P450s, 34 LRR proteins, two HSPs, 13 peroxidases, and 13 WRKYs (Supplementary Tables 1A and 1B). By day 15, we identified 105 DEGs to be down-regulated (Supplementary Table 1C) and 151 DEGs to be up-regulated in the non-infested tolerant when compared to non-infested susceptible plants (Supplementary Tables 1D). Genes found to be up-regulated at this time point in the tolerant soybean included 12 cytochrome P450s, 17 LRR proteins, five HSPs, two peroxidases, and one WRKY. These data point to the differences in the transcriptomes of the susceptible and tolerant genotypes. The large differences in the DEGs suggest that tolerance could have some basis in elevated expression of stress-ameliorating proteins, such as peroxidases and cytochrome P450s and plausibly in stress-sensing proteins such as the WRKYs and LRR. Similar results have been reported in other studies (Ramm et al. 2013; Studham and Macintosh 2013). GO analysis did not result in data enrichment.

Conclusions

This study has allowed us to utilize next-generation sequencing technology in order to more effectively query soybean plant responses to aphid feeding. Gutsche et al. (2009) reported DEGs assigned to several metabolic categories, including plant defense and scavenging of ROS in

barley. This research finds several similarities in soybean, including genes whose roles are connected to plant defenses and the scavenging of ROS. Overall, this project provides a comprehensive dataset that allows us to characterize transcriptional changes in response to soybean aphid herbivory and provides a better understanding of the genes contributing to the tolerance response and the underlying tolerance mechanism.

Acknowledgments We acknowledge Rachael Sitz, Kyle Koch, Mitchell Stamm, Ruth Miller, and David Orr for technical assistance; Dr. Yuannan Xia for RNA extraction and sequencing; Dr. Jean-Jack Reithoven for data interpretation; and William T. Schapaugh, Jr., for providing the soybean germplasm for these studies. This research was supported in part by the University of Nebraska Agricultural Experiment Station Projects NEB-28-097 and NEB-41-034, the Nebraska Soybeans Board, and the North Central Soybean Research Program. The U.S. Department of Agriculture, Agricultural Research Service, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of commercial products and organizations in this manuscript is solely to provide specific information. It does not constitute endorsement by USDA-ARS over other products and organizations not mentioned.

Ethical standard This article does not contain any studies with human subjects, vertebrates, or regulated invertebrates performed by any of the authors.

Conflict of interest The authors declare that they have no competing interests, and all research has been carried out with an appropriate ethical framework.

References

Alleman RJ, Grau CR, Hogg DB (2002) Soybean aphid host range and virus transmission efficiency. In: Proceedings of Wisconsin Fertilizer, Aglime and Pest Management Conference. http://www.soils.wisc.edu/extension/FAPM/2002proceedings/Alleman-Conf-2002.pdf

Anders S (2010) HTSeq: Analysing high-throughput sequencing data with Python. http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html

Anders S, Huber W (2010) Differential expression analysis for sequence count data. Genome Biol 11:R106

Argandona VH, Chaman M, Cardemil L, Munoz O, Zuniga GE, Corcuera LJ (2001) Ethylene production and peroxidase activity in aphid-infested barely. J Chem Ecol 27:53–68

Ballaré CL (2014) Light regulation of plant defense. Annu Rev Plant Biol. doi:10.1146/annurev-arplant-050213-040145

Beckendorf EA, Catangui MA, Riedell WE (2008) Soybean aphid feeding injury and soybean yield, yield components, and seed composition. Agron J 100:237–246

Blanvillain R, Kim JH, Wu S, Lima A, Ow DW (2009) Oxidative stress 3 is a chromatin-associated factor involved in tolerance to heavy metals and oxidative stress. Plant J 57:654–665

Botha AM, Swanevelder ZH, Lapitan NLV (2010) Transcript profiling of wheat genes expressed during feeding by two different biotypes of *Diuraphis noxia*. Environ Entomol 39:1206–1231

Boyd RS (2006) The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. Plant Soil 293:153–176

Boyko EV, Smith CM, Vankatappa T, Bruno J, Deng Y, Starkey SR, Klaahsen D (2006) The molecular basis of plant gene expression during aphid invasion: wheat Pto- and Pti-like sequences modulate aphid–wheat interaction. J Econ Entomol 99:1430–1445

Brear EM, Day DA, Smith PMC (2013) Iron: an essential micronutrient for the legume-rhizobium symbiosis. Front Plant Sci. 4:1–15

Brenner WG, Romanov GA, Köllmer I, Bürkle L, Schmülling T (2005) Immediate-early and delayed cytokinin response genes of *Arabidopsis thaliana* identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades. Plant J. 44:314–333

Brosius TR, Higley LG, Hunt TE (2007) Population dynamics of soybean aphid and biotic mortality at the edge of its range. J Econ Entomol 100:1268–1275

Cao J (2012) The pectin lyases in *Arabidopsis thaliana*: evolution, selection and expression profiles. PLoS One 7:e46944. doi:10.1371/journal.pone.0046944

Chandran P (2011) Different sources of resistance in soybean against soybean aphid biotypes. M.S. Thesis. Kansas State University, Manhattan, KS

Chen JQ, Rahbé Y, Delobel B, Sauvion N, Guillaud J, Febvay G (1997) Melon resistance to the aphid *Aphis gossypii*: behavioural analysis and chemical correlations with nitrogenous compounds. Entomol Exp Appl 85(1):33–44

Chen YF, Li LQ, Xu Q, Kong YH, Wang H, Wu WH (2009) The WRKY6 transcription factor modulates PHOSPHATE1 expression in response to low Pi stress in *Arabidopsis*. Plant cell. 21:3554–3566

Chomczynski P, Sacchi N (1987) Single step RNA isolation from cultured cells or tissue. Anal Biochem 162:156–159

Conte SS, Chu HH, Rodriguez DC, Punshon T, Vasques KA, Salt DE, Walker EL (2013) *Arabidopsis thaliana* Yellow Stipe1-Like4 and Yellow Stripe1-Like6 localize cellular membranes and are involved in metal homeostasis. Front Plant Sci 4:1–15

Couldridge C, Newbury HJ, Ford-Lloyd B, Bale J, Pritchard J (2007) Exploring plant responses to aphid feeding using a full *Arabidopsis* microarray reveals a small number of genes with significantly altered expression. Bull Entomol Res 97:523–532

Crute IR, Dunn JA (1980) An association between resistance to root aphid (*Pemphigus bursarius* L.) and downy mildew (*Bremia lactucae* Regel) in lettuce. Euphytica 29(2):483–488

De Vos M, Jander G (2009) *Myzus persicae* (green peach aphid) salivary components induce defense responses in *Arabidopsis thaliana*. Plant, Cell Environ 32:1548–1560

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. J Econ Entomol 99:1884–1889

Eck LV, Schultz T, Leach JE, Scofield SR, Peairs FB, Botha AM, Lapitan NLV (2010) Transcript profiling of wheat genes expressed during feeding by two different biotypes of *Diuraphis noxia*. Plant Biotechnol 8:1023–1032

Fehr WR, Caviness CE (1977) Stages of soybean. Iowa State University Cooperative Extension Service Special Report 80. Iowa State University, Ames, IA

Franzen LD, Gutsche AR, Heng-Moss TM, Higley LG, Sarath G, Burd JD (2007) Physiology and biochemical responses of resistant and susceptible wheat to injury by the Russian wheat aphid. J Econ Entomol 100:1692–1703

Gao QM, Venugopal S, Navarre D, Kachroo A (2011) Low oleic acid-derived repression of jasmonic acid-inducible defense responses requires the WRKY50 and WRKY51 proteins. Plant Physiol 155:464–476

Gaquerel E, Stitz M, Kallenbach M, Baldwin IT (2013) Jasmonate signaling in the field, part II: insect-guided characterization of genetic variations in jasmonate-dependent defenses of transgenic and natural *Nicotina attenuate* populations. Method Mol Biol. 1011:97–109

Garcia-Ranea JA, Mirey G, Camonis J, Valencia A (2002) P23 and HSP20/α-crystallin proteins define a conserved sequence domain present in other eukaryotic protein families. FEBS Lett 529:162–167

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5:R80

Gill SG, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2011) Phytozome: a comparative platform for green plant genomics. Nucl Acids Res 40:D1178–D1186

Gutsche A, Heng-Moss T, Sarath G, Twigg P, Xia Y, Lu G, Mornhinweg D (2009) Gene expression profiling of tolerant barley in response to *Diuraphis noxia* (Hemiptera: Aphididae) feeding. Bull Entomol Res 99:163–173

Guy CL, Li QB (1998) The organization and evolution of the spinach stress 70 molecular chaperone family. Plant Mol Biol 10:539–556

Halitschke R, Gase K, Hui D, Schmidt DD, Baldwin IT (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuate*. VI. Microarray analysis reveals that most herbivore-specific transcriptional changes are mediated by fatty acid-amino acid conjugates. Plant Physiol 131:1894–1902

Hartman GL, Domier LL, Wax LM, Helm CG, Onstad DW, Shaw JT, Solter LF, Voegtlin DJ, D'Arcy CJ, Gray ME, Steffey KL, Isard SA, Orwick PL (2001) Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. http://planthealthprogress.org/current/briefs/aphisglycines/article.htm

Heng-Moss TM, Sarath G, Baxendale FP, Novak D, Bose S, Xinhi N, Quisenberry S (2004) Characterization of oxidative enzyme changes in buffalograsss challenged by *Blissus occiduus*. J Econ Entomol 97:1086–1095

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

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

Hill CB, Li Y, Hartman GL (2006b) Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Sci 46:1606–1608

Hill CB, Kim K, Crull L, Diers BW, Hartman GL (2009) Inheritance of resistance to the soybean aphid in soybean PI 200583. Crop Sci 49:1193–1200

Hiraga S, Sasaki K, Ito H, Ohashi Y, Hatsui H (2001) A large family of class III plant peroxidases. Plant Cell Physiol 42:462–468

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59:41–66

Hu Z, Xu F, Guan L, Qian P, Liu Y, Zhang H, Huang Y, Hou S (2013) The tetratricopeptide repeat-containing protein slow green1 is required for chloroplast development in *Arabidopsis*. J Exp Bot. http://jxb.oxfordjournals.org/content/early/2014/01/12/jxb.ert463.abstract

Ishikawa T, Shigeoka S (2008) Recent advances in ascorbate biosynthesis and the physiological significance of ascorbate peroxidase in photosynthesizing organisms. Biosci Biotechnol Biochem 72:1143–1154

Jespersen HM, Kjaersgard IVH, Ostergaard L, Welinder KG (1997) From sequence analysis of three novel ascorbate peroxidases from *Arabidopsis thaliana* to structure, function and evolution of seven types of ascorbate peroxidase. Biochem J 326:305–310

Kang ST, Mian MAR, Hammond RB (2008) Soybean aphid resistance in PI 243540 is controlled by a single dominant gene. Crop Sci 48:1744–1748

Kanter U, Usadel B, Guerinearu F, Li Y, Pauly M, Tenhaken R (2005) The inositol oxygenase gene family of *Arabidopsis* is involved in the biosynthesis of nucleotide sugar precursors for cell-wall matrix polysaccharides. Planta 221:243–254

Karve A, Rauh BL, Xia X, Kandasamy M, Meagher RB, Sheen J, Moore BD (2008) Expression and evolutionary features of the hexokinase gene family in *Arabidopsis*. Planta 228:411–425

Kawano T (2003) Roles of the reactive oxygen species generating peroxidase reactions in plant defense and growth induction. Plant Cell Rep 21:829–837

Kempema LA, Cui X, Holzer FM, Walling LL (2007) *Arabidopsis* transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in response to aphids. Plant Physiol 143:849–865

Kienow L, Schneider K, Bartsch M, Stuible HP, Weng H, Miersch O, Wasternack C, Kombrink E (2008) Jasmonates meet fatty acids: functional analysis of a new acyl-coenzyme A family from *Arabidopsis thaliana*. J Exp Bot 59:403–419

Kobe B, Kajava AV (2001) The luecine-rich repeat as a protein recognition motif. Curr Opin Struct Biol 11:725–732

Konishi M, Yanagisawa S (2011) The regulatory region controlling the nitrate-responsive expression of a nitrate reductase gene, NIA1, in *Arabidopsis*. Plant Cell Physiol 52:824–836

Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580

Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10:R25

Lapitan NLV, Hess A, Wang H, van Eck L, Scofield S, Botha AM (2008) Different sets of wheat genes are used in Dn7-mediated resistance to feeding by two biotypes of Russian wheat aphid. In: Appels R, Eastwood R, Lagudah E, Langridge P, Mackay M, McIntyre L, Shart P (eds) 11th international wheat genetics symposium. Sydney University Press, Brisbane, Australia, p P128

Le Jean M, Schikora A, Mari S, Briat JF, Curie C (2005) A loss of function mutation in AtYSL1 reveals its role in iron and nicotianamine seed loading. Plant J. 44:769–782

Lee SI, Lee SH, Koo JC, Chun HJ, Lim CO, Mun JH, Song YH, Cho MJ (1999) Soybean Kunitz trypsin inhibitor (SKTI) confers resistance to the brown planthopper (*Nilaparvata lugens* Stål) in transgenic rice. Mol Breed 5:1–9

Li J, Brader G, Palva ET (2008a) Kunitz trypsin inhibitor: an antagonist of cell death triggered by phytopathogens and fumonisin b1 in *Arabidopsis*. Mol Plant 1:482–495

Li Y, Zou J, Li M, Bilgin DD, Vodkin LO, Hartman GL, Clough SJ (2008b) Soybean defense responses to the soybean aphid. New Phytol 179:185–195

Lin X, Kaul S, Rounsley S, Shea TP et al (1999) Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana*. Nature 402:761–768

Lin BL, Wang JS, Liu HC, Chen RW, Meyer Y, Barakat A, Delseny M (2001) Genomic analysis of the Hsp70 superfamily in *Arabidopsis thaliana*. Cell Stress Chaperon 6:201–208

Liu X, Williams CE, Nemacheck JA, Wang H, Subramanyam S, Zheng C, Chen MS (2010) Reactive oxygen species are involved in plant defense against a gall midge. Plant Physiol 152:985–999

Lundberg E, Storm P, Schröder WP, Funk C (2011) Crystal structure of the TL29 protein from *Arabidopsis thaliana*: an APX homolog without peroxidase activity. J Struct Biol 176:24–31

Mayer K, Schüller C, Wambutt R, Murphy G et al (1999) Sequence and analysis of chromosome 4 of the plant *Arabidopsis thaliana*. Nature 402:769–777

McCarville MT, Kanobe C, MacIntosh GC, O'Neal M (2011) What is the economic threshold of soybean aphid (Hemiptera: Aphididae) in enemy-free space? J Econ Entomol 104(3):845–852

McConn M, Creelman RA, Bell E, Mullet JE, Browse J (1997) Jasmonate is essential for insect defense in *Arabidopsis*. PNAS 94:5473–5477

Mensah C, DiFonzo C, Nelson RL, Wang D (2005) Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci 45:2228–2233

Mian MAR, Hammond RB, St Martin SK (2008) New plant introductions with resistance to the soybean aphid. Crop Sci 48:1055–1061

Milligan SB, John B, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM (1998) The root 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

Murgia I, Tarantino D, Soave C, Morandini P (2011) *Arabidopsis CYP82C4* expression is dependent on Fe availability and circadian rhythm, and correlates with genes involved in the early Fe deficiency response. J Plant Physiol 168:894–902

National Agriculture Pest Information System (NAPIS) (2011) Reported status of soybean aphid–*Aphis glycines*. NAPHIS, Purdue University, West Lafayette, Indiana

Ni S, Quisenberry SS, Heng-Moss T, Markwell J, Sarath G, Klucas R, Baxendale F (2001) Oxidative responses of resistant and susceptible cereal leaves to symptomatic and non-symptomatic cereal aphid (Hemiptera: Aphididae) feeding. J Econ Entomol 94:743–751

Nombela G, Williamson VW, Muniz M (2003) The root-knot nematode resistance gene Mi-1.2 of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol Plant Microbe Interact 16:645–649

Okumura S, Mitsukawa N, Shirano Y, Shibata D (1998) Phosphate transporter gene family of *Arabidopsis thaliana*. DNA Res 5:261–269

Ostlie K (2002) Managing soybean aphid. University of Minnesota Extension Service

Pandey SP, Somssich IE (2009) The role of WRKY transcription factors in plant immunity. Plant Physiol 150:1648–1655

Paré PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiol 121:325–332

Park JH, Halitschke R, Kim HB, Baldwin IT, Feldmann KA, Feyereisen R (2002) A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. Plant J 31:1–12

Park JA, Ahn JW, Kim YK, Kim SJ, Kim JK, Kim WT, Pai HS (2005a) Retinoblastoma protein regulates cell proliferation, differentiation, and endoreduplication in plants. Plant J 42:153–163

Park SJ, Huang Y, Ayoubi P (2005b) Identification of expression profiles of sorghum genes in response to greenbug phloem feeding using cDNA subtraction and microarray analysis. Planta 223:932–947

Pierson LM, Heng-Moss TM, Hunt TE, Reese JC (2010) Categorizing the resistance of soybean genotypes to the soybean aphid (Hemiptera: Aphididae). J Econ Entomol 103:1405–1411

Pierson LM, Heng-Moss TM, Hunt TE, Reese JC (2011) Physiological responses of resistant and susceptible reproductive stage soybean to soybean aphid (*Aphis glycines* Matsumura) feeding. Arthropod-Plant Interact 5:49–58

Poschenrieder C, Tolrà R, Barcelò J (2006) Can metals defend plants against biotic stress? Trends Plant Sci 11:288–295

Prochaska TJ, Pierson LM, Baldin ELL, Hunt TE, Heng-Moss TM, Reese JC (2013) Evolution of late vegetative and reproductive stage soybeans for resistance to soybean aphid (Hempitera: Aphididae). J Econ Entomol 106:1036–1044

Ragsdale DW, McCornack BP, Venette RC, Potter BD, MacRae IV, Hodgson EW, O'Neal ME, Johnson KD, O'Neil RJ, DiFonzo CD, Hunt TE, Glogoza PA, Cullen EM (2007) Economic threshold for soybean aphid (Hemiptera: Aphididae). J Econ Entomol 100(4):1258–1267

Ragsdale DW, Landis DA, Brodeur J, Heimpel GE, Desneux N (2011) Ecology and management of soybean aphid in North America. Annu Rev Entomol 56:375–399

Ramm CM, Saathoff A, Donze T, Heng-Moss T, Baxendale F, Twigg P, Baird L, Amundsen K (2013) Expression profiling of four defense-related buffalograss transcripts in response to chinch bug (Hemitpera: Blissidae) feeding. J Econ Entomol 106:2568–2576

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell 12:707–719

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. Proc Natl Acad Sci USA 95:9750–9754

Rutledge CE, O'Neil RJ (2006) Soybean plant stage and population growth of soybean aphid. J Econ Entomol 99:60–66

Salanoubat M, Lemcke K, Rieger M, Ansorge W et al (2000) Sequence and analysis of chromosome 3 of the plant *Arabidopsis thaliana*. Nature 408:820–822

Santiago J, Rodrigues A, Saez A, Rubio S, Antoni R, Dupeux F, Park SY, Márquez JA, Cutler SR, Rodriguez PL (2009) Modulation of drought resistance by the abscisic acid receptor PYL5 through inhibition of clade A PP2Cs. Plant J 60:575–588

Sarry JE, Kuhn L, Ducruix C, Lafaye A, Junot C, Hugouvieux V, Jourdain A, Bastien O, Fievet JB, Vailhen D, Amekraz B, Moulin C, Ezan E, Garin J, Bourguignon J (2006) The early responses of *Arabidopsis thaliana* cells to cadmium exposure explored by protein and metabolite profiling analyses. Proteomics 6:2180–2198

Scarpeci TE, Zanor MI, Mueller-Roeber B, Valle EM (2013) Overexpression of *AtWRKY30* enhances abiotic stress tolerance during early growth stage in *Arabidopsis thaliana*. Plant Mol Biol 83:265–277

Siminszky B, Gavilano L, Bowen SW, Dewey RE (2005) Conversion of nicotine to nornicotine in *Nicotiana tobacum* is mediated by CYP82E4, a cytochrome p450 monooxygenase. Proc Natl Acad Sci 102:14919–14924

Smith CM (2005) Plant resistance to arthropods. Springer, Dordrecht

Smith CM, Boyko EV (2007) The molecular bases of plant resistance and defense to aphid feeding: current status. Entomol Exp Appl 122:1–16

Studham ME, MacIntosh GC (2013) Multiple phytohormone signals control the transcriptional response to soybean aphid infestation in susceptible and resistant soybean plants. Mol Plant Microbe Interact 26:116–129

Sun W, Bernard C, van de Cotte B, Van Montagu M, Verbruggen N (2001) At-HSP17.6A, encoding a small heat-shock protein in

Arabidopsis, can enhance osmotolerance upon overexpression. Plant J. 27:407–415

Sung DY, Vierling E, Guy CL (2001) Comprehensive expression profile of analysis of the *Arabidopsis* Hsp70 gene family. Plant Physiol 126:789–800

Tabata S, Kaneko T, Nakamura Y et al (2000) Sequence and analysis of chromosome 5 of the plant *Arabidopsis thaliana*. Nature 408:823–826

Theologis A, Ecker JR, Palm CJ, Federspiel NA et al (2000) Sequence and analysis of chromosome 1 of the plant *Arabidopsis thaliana*. Nature 408:816–820

Touraine B, Briat JF, Gaymard F (2012) GSH threshold requirement for NO-mediated expression of the *Arabidopsis* AtFer1 ferritin gene in response to iron. FEBS Lett 586:880–883

Venette RC, Ragsdale DW (2004) Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? Ann Entomol Soc Am 97:219–226

Voelckel C, Weisser WW, Baldwin IT (2004) An analysis of plant–aphid interactions by different microarray hybridization strategies. Mol Ecol 10:3187–3195

Von Groll U, Berger D, Altmann T (2002) The subtilisin-like serine protease SDD1 mediates cell-to-cell signaling during *Arabidopsis* stomatal development. Plant Cell 14:1527–1539

Wang YZ, Ba F (1998) Study on optimum control of the soybean aphid. Acta Phys Sin 25:152–155

Wang YZ, Ma L, Wang JZ, Ren XZ, Zhu WL (2000) Systematic optimum control of diseases and inset pests in summer soybean. J Ecol 20:502–509

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9:244–252

Wiarda SL, Fehr WR, O'Neal ME (2012) Soybean aphid (Hemiptera: Aphididae) development on soybean with Rag1 alone, Rag2 alone, and both genes combined. J Econ Entomol 105(1):252–258

Wilson AC, Sternberg L da SL, Hurley KB (2011) Aphids alter host-plant nitrogen isotope fractionation. PNAS 108:10220–10224

Wroblewski T, Piskurewicz U, Tomczak A, Ochoa O, Michelmore RW (2007) Silencing of the major family of NBS–LRR-encoding genes in lettuce results in the loss of multiple resistance specificities. Plant J 51(5):803–818

Wu Z, Schenk-Hamlin D, Zhan W, Ragsdale DW, Heimpel GE (2004) The soybean aphid in China: a historical review. Ann Entomol Soc Am 97:209–218

Xu D, Shen Y, Chappell J, Cui M, Nielsen M (2007) Biochemical and molecular characterizations of nicotine demethylase in tobacco. Physiol Plant 129:307–319

Zhu-Salzman K, Salzman RA, Ahn JE, Koiwa H (2004) Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant Physiol 134:420–443