Pak. J. Bot., 55(5): 1795-1804, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-5(4)

GLYCINE MAX TRANSCRIPTOME CHANGES IN RESPONSE TO APHID AND VOLATILES EXPOSURES

HONGYU CHEN[1,2], YING YU[3], XIULING CHEN[4], ZHENZHU ZHANG[5], KUIJUN ZHAO[1*] AND AOXUE WANG[4*]

[1]*College of Agriculture, Northeast Agricultural University, Harbin150030, China*
[2]*School of Pharmaceutical Sciences, Guizhou University of Traditional Chinese Medicine, Guiyang 550025, China*
[3]*School of Basic Medicine, Guizhou University of Traditional Chinese Medicine, Guiyang, China*
[4]*College of Horticulture and Landscape Architecture, Northeast Agricultural University, Harbin150030, China*
[5]*College of Life Sciences, Agriculture and Forestry, Qiqihar University, Qiqihar161006, China*
[*]*Corresponding author's email: kjzhao@neau.edu.cn; axwang@neau.edu.cn*

Abstract

Plant-plant interactions via herbivory-induced plant volatiles (HIPVs) have been shown to trigger plant anti-herbivore defense responses. Such volatiles-regulated genes have been studied comprehensively in lima bean (*Phaseolus lunatus*) and *Arabidopsis thaliana* at the transcriptional level, but not in soybean (*Glycine max)*. The transcriptomes of soybean leaf tissues responding to aphid infestation and volatiles released from neighboring aphid-infested plants were studied by RNA-seq. Soybean aphid infested plants exhibited 2413 up-regulated and 3905 down-regulated genes, while leaf exposure to volatiles resulted in 2153 up-regulated and 3572 down-regulated genes, which were endowed with a wide range of functions including 'hormone responses', 'pathogen-related', and 'oxidative stress' functions. The results ultimately demonstrated that aphid damage and volatiles exposure elicited drastic metabolic changes in soybean leaves. These results also emphasize that RNA-seq analysis of repressed genes should contribute to our understanding of signal transduction in aphid-infested leaves, as well as in leaves exposed to herbivory-induced volatiles.

Key words: *Glycine max*; Transcriptome; Sequencing; Aphis glycines; Volatiles.

Introduction

Some phytophagous insects obtain nutrition from their plant hosts by sucking sap from underground or aerial plant parts. To compensate, plants have developed various distinct inducible defense strategies against insect herbivores, including the activation of proteinase inhibitors, polyphenol oxidases, chitinases, and so on. Induced defense mechanisms also include the production and release of herbivore-induced plant volatiles (HIPVs) that attract carnivorous natural enemies of herbivores (Dicke *et al*., 1990a; Dicke *et al*., 1990b; Turlings *et al*., 1990; Takabayashi *et al*., 1994; Moraes *et al*., 1998; Dicke *et al*., 1999; Aartsma *et al*., 2017). Previous research has shown that volatiles affect herbivore predators while also triggering resistance in neighboring plants exposed to HIPVs (Godard *et al*., 2008; Ton *et al*., 2010) both in the laboratory (Bruin *et al*., 1992; Arimura *et al*., 2000a; Arimura *et al*., 2000b) and under natural conditions (Rhoades 1983; Karban *et al*., 2000; Heil & Bueno, 2007). More specifically, volatiles from insect herbivore-infested plant leaves can activate expression of specific defense genes in neighboring plants (Arimura *et al*., 2000b) to attract herbivore predators to ultimately reduce insect herbivore feeding and oviposition rates (Dicke *et al*., 1990a; Arimura *et al*., 2000a; Arimura *et al*., 2000b).

Several studies have employed cDNA microarrays to identify volatiles-responsive genes within whole-genome transcription systems of lima bean (*Phaseolus lunatus*) (Arimura *et al*., 2000a) and *Arabidopsis thaliana* (Zhang *et al*., 2012). To date, investigations of the expression patterns of some defense genes have demonstrated that both volatiles-based and direct insect damage-based plant defense responses share similar gene expression profiles, but that direct damage induced much stronger responses in these genes. Soybean (*Glycine max*) is an important crop because its seed is rich in protein and oil. Both the completion of the soybean genome sequencing and technical advances in transcriptome sequencing analysis have allowed for the large-scale study of soybean gene expression (Schmutz *et al*., 2010). In this study, we systematically monitored aphid- or volatiles-induced variations of soybean gene transcription levels using RNA sequencing (RNA-seq) transcriptome analysis. The results revealed many soybean leaf transcriptome changes, with gene expression changes of at least 13.61% and 12.33% of genes observed in aphid and volatiles responses, respectively. Among these genes, 6.99% with altered transcriptome profiles were common to both aphid-infested leaves and receiver leaves, with altered expression of ten genes confirmed using quantitative PCR (qPCR). Gene ontology (GO) term enrichment analysis of soybean genes by aphid and volatiles-regulated revealed numerous biological process response genes with functions assigned to 'hormone responses', 'pathogen-related', and 'oxidative stress' functions. These results provide new insight into the roles of genes involved in soybean responses to aphid infestation and volatiles exposure.

Materials and Methods

Plant and aphid materials: Soybean seeds were provided by Shuzhen Zhang (Northeast Agricultural University). Plants were grown in plastic pots (18-cm diameter, 16-cm depth) in light incubator (25°C, 50% relative humidity and 16-h light/8-h dark conditions). Two-weeks-old plants with two fully expanded primary leaves were used for all experiments. Soybean aphids (*Aphis glycines*) were obtained from soybean plants reared in laboratory under the same conditions as those described above.

Plant treatments: Soybean leaf transcriptome changes in response to aphid attack and volatiles exposure were measured. Soybean plants were infested with aphids by transferring 40 nymphs and apterous adults to each plant as the emitter leaves. In order to prevent the escape of aphids from infested emitter leaves, we spread the net on the surface of each plastic pot before placing infested plants together with uninfested receiver plants in a light incubator. The experimental device was maintained at 25°C, 50% relative humidity and 16-h light/8-h dark conditions for 24 h. Ten uninfested plants were maintained alone in a separate light incubator for 24 h as negative controls. The infested emitter leaves, uninfested receiver leaves and control leaves were used for the total RNA extraction. Three biological replicates were harvested for each group of samples.

Total RNA extraction and library construction: Total RNAs from leaves were extracted using TRIzol reagent (Invitrogen) and digested with RNase-free DNase I (Promega) to remove genomic DNA. The quality and integrity of the total RNA extracted were detected with NanoDrop (Thermo) and 1 % agarose gel electrophoresis. Before the RNA-seq library construction, the oligo (dT)-linked magnetic beads were used to purify and concentrate the mRNAs. The purified mRNAs were fragmented and subjected to 5' adaptor ligation, and then reverse transcripted with random hexamer primers and RT primers with 3' adaptor. PCR products of 150bp-200bp were obtained from the purified cDNAs. Meanwhile, RNA-seq libraries were constructed and submitted to Illumina Genome Analyzer for sequencing by GENEWIZ Inc. (Suzhou, China).

Functional annotation and differential expression: Sequencing reads were mapped to the soybean genome using the HISAT2 (v2.0.1) tool. BLAST searches were used to annotate genes to maximize the accuracy of sequence recognition (Kent 2002). These genes were compared with those in the databases of Kyoto Encyclopedia of Genes and Genomes (KEGG) and National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). GO annotations were obtained using the Gene Ontology database. Expression levels of genes were evaluated and normalized using RPKM (reads per kilobase of exon model per million mapped reads) (Mortazavi *et al*., 2008). Differentially expressed genes (DEGs) were identified using DESeq2 (V1.6.3) (Love *et al*., 2015) and edgeR (V3.4.6) software (Robinson). Significantly differentially expressed gene sequences exhibiting at least a two-fold change in RPKM values between the two libraries (with adjusted P values < 0.001) were subjected to analysis in this work.

qPCR analysis: Soybean mRNA isolates (CK1, HI1, and VI1) prepared for RNA-seq were reverse-transcribed into cDNA then were validated for gene transcript abundance via qPCR using Power SYBR Green Master Mix (TaKaRa) and an Applied Biosystems 7500 Real-Time PCR System. For qPCR, 22 primers were used and their sequences are shown in (Table 1). The tubulin gene was used as an internal quantitative reference and all samples were processed in triplicate. The ΔΔCT method was used to normalize the CT value of each gene with that of the reference gene to determine the relative fold change of each sample (Livak & Schmittgen, 2001).

Results and Discussion

Soybean (*G. max*) transcriptome analyses: Soybean transcriptomes were analyzed by RNA-seq Analyzer II. Total RNA was prepared from plants that were grown at 25°C for 2 weeks then infested with aphids. For 24 h, aphid-damaged leaves (the emitter leaves) generated volatiles that interacted with uninfested receiver leaves, then total RNA was separately extracted from emitter and receiver leaves and analyzed. Three biological replicates were harvested for each group of samples (emitter, receiver, negative control). More than 467 million raw reads were produced that generated about 52 million clean reads per sample (Table 2). We aligned the clean reads against the whole soybean reference genome using the HISAT2 (v2.0.1). 78.22%–88.14% of reads were uniquely mapped to a single genomic location, while 4.18%–7.65% of reads were filtered into multiple-mapped reads.

Differential expression in response to aphid damage and volatiles: In order to study the effects of aphids and volatiles on soybean gene expression, the transcriptional abundance of each gene was evaluated by RPKM. For comparison of transcriptomes, a heat map was used to show the transcriptional abundance of all DEGs. The results indicated that after 24 hours of exposure to aphids and volatiles, a series of transcriptome changes occurred in soybean.

A threshold minimum of a 2-fold change in abundance between any two groups of samples was used to define DEGs during the analysis. Gene expression profiles of *G. max* leaf tissues after aphid infestation and exposure to volatiles significantly differed from control leaf tissue profiles, with aphid infestation resulting in 2413 up- and 3905 down-regulated genes and volatiles exposure of leaves resulting in 2153 up- and 3572 down-regulated genes (Fig. 1; Table 3). Among these DEGs, 3247 were observed in both infested leaves and receiver leaves. The results showed that gene expression were differences between soybean responses to aphid damage and volatiles exposure. These results align with results obtained for other plant species, as several previous studies have revealed volatiles-responsive genes in lima bean plants, although these studies did not focus on suppressor genes due to limitations of microarray analysis (Arimura *et al*., 2000a). Here, analysis of suppressor genes in leaves infested by aphids or exposed to induced volatiles was important to increase understanding of signal transduction. To verify RNA-seq results, expression profiles of several genes implicated using RNA-seq were also analyzed using qRT-PCR. The qRT-PCR results demonstrated expression patterns similar to patterns revealed using RNA-seq (Table 1).

Table 1. RNA-Seq and qPCR results of 10 genes.

Gene ID	RNA-Seq		qPCR		Primer (forward/reverse)
	CK-VS-HI	CK-VS-VI	CK-VS-HI	CK-VS-VI	
Glyma.07G007000	-	Ups	0.76	1.19	CCTTACCAGCCCTCAAGGAA/TGTGGGATCAAGTGTGCTCT
Glyma.06G123800	-	Ups	0.61	2.22	CAGCCTAAACGGAAGGAAGC/CGTGGGAATGTTGTCGTGAA
Glyma.18G195200	-	Ups	1.85	2.74	GCTGTGAAGGGTGTTGGAAG/GGAGTTGCACTCCACTCCTT
Glyma.15G268800	-	Ups	1.29	1.2	CACCACAAAGGCGGATACTG/GACACGGAGGATTGGAGCTA
Glyma.01G063000	Down	-	1.15	1.11	ATCCCGACAATCCGAAACCT/CCGAGGATGCGATTGATGTC
Glyma.18G062000	Down	-	1.01	3.22	TCGCGACTATGGTTGGATCA/CCTTTGCTGCTTTAGTCGCT
Glyma.17G128900	Down	Down	1.57	0.96	GACGGGTTGTTGTGTTTGGA/AACCCACACCTCTCCGTATC
Glyma.12G073100	Down	-	0.23	0.68	AGCTACGCCGATTTCTACCA/GGCTCAGGTTTGTCCTCTCT
Glyma.19G068300	Down	-	0.89	0.79	TGGATGCTGATGGGAATGGT/AGCAGCAGAGATGAACCCAT
Glyma.14G205200	-	Down	1.4	0.87	GTGAACCACCCAGAGATCCA/ATGTGTGGGACAAGGAGAGG

CK: control sample. HI: emitter leaves infested by aphid. VI: uninfested receiver leaves exposed to volatiles. The CK-VS-HI and CK-VS-VI represent differentially expressed genes in response to aphid damage and volatiles exposure, respectively

Table 2. Summary of RNA-Seq datasets and mapped results for the nine libraries.

Samples	length	Total clean reads	Total clean nucleotides (nt)	Q20 percentage	Q30 percentage	GC percentage	Total mapped	Multiple mapped	Uniquely mapped
CK1	148.70	57,601,490	8,565,536,730	96.77	91.54	49.11	89.44%	7.65%	81.79%
CK2	148.62	58,627,092	8,713,387,660	96.49	90.95	48.79	90.79%	6.62%	84.16%
CK3	147.52	52,028,944	7,675,059,437	94.88	87.50	44.79	87.67%	5.32%	82.34%
HI1	148.79	43,626,394	6,491,088,114	97.31	92.88	46.37	86.74%	5.72%	81.02%
HI2	148.73	42,223,660	6,279,823,896	97.46	93.26	44.17	83.57%	5.34%	78.22%
HI3	147.52	68,271,664	10,071,573,726	95.18	88.11	43.87	89.12%	4.47%	84.65%
VI1	148.74	40,987,348	6,096,531,662	97.20	92.62	46.52	90.64%	5.98%	84.65%
VI2	148.62	38,567,534	5,731,945,162	96.59	91.00	44.70	92.32%	4.18%	88.14%
VI3	147.67	65,414,922	9,659,991,090	95.37	88.53	43.91	89.54%	4.66%	84.88%

CK1, CK2 and CK3: control sample (three independent biological replicates). HI1, HI2 and HI3: emitter leaves infested by aphid (three independent biological replicates). VI1, VI2 and VI3: uninfested receiver leaves exposed to volatiles (three independent biological replicates)

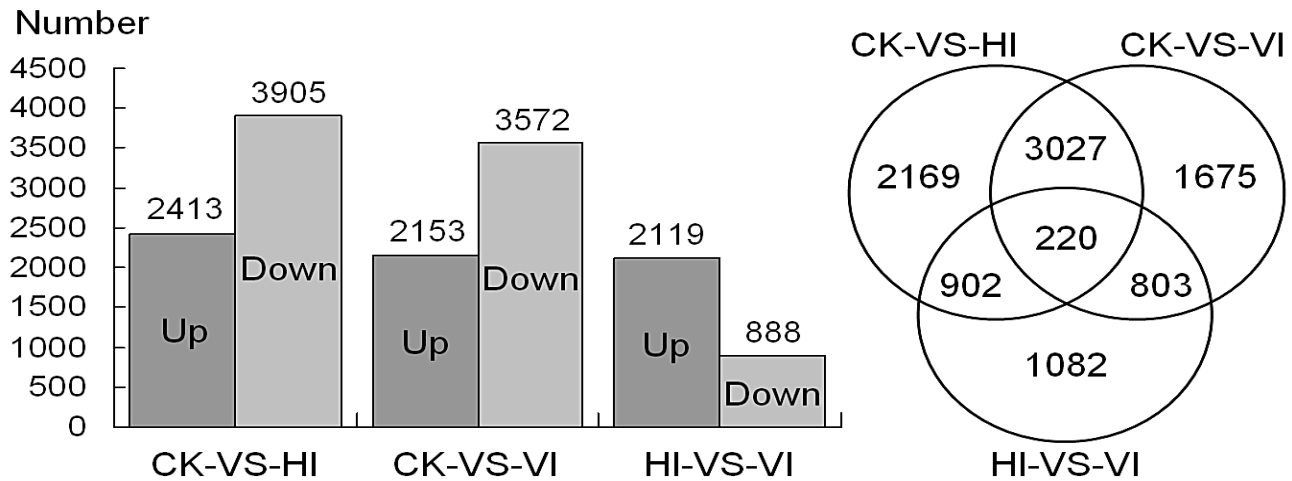

Fig. 1. 2-fold differentially expressed genes of *Glycine max* in response to aphid damage and volatiles exposure. CK: control sample. HI: emitter leaves infested by aphid. VI: uninfested receiver leaves exposed to volatiles. The CK-VS-HI and CK-VS-VI represent differentially expressed genes in response to aphid damage and volatiles exposure, respectively.

GO term enrichment and KEGG analysis: In order to elucidate the obvious changes of biological processes in response to aphid damage and volatiles exposure in soybean, DEGs were subjected to GO and KEGG enrichment analysis (Figs. 2 and 3). The results indicated that these genes were associated with broad functions, including amino sugar metabolism, biosynthesis of amino acids, carbon metabolism, glycine metabolism, nucleotide sugar metabolism, photosynthesis, serine metabolism, starch metabolism, sucrose metabolism, and threonine metabolism.

Previous studies have demonstrated that hormones, as signaling molecules, play an important role in regulating gene expression in response to herbivory and volatiles exposure (Ruther & Kleier, 2005; Ton *et al*., 2010; Li *et al*., 2022; Huang *et al*., 2022; Ye *et al*., 2022; Karssemeijer *et al*., 2022). RNA-seq results revealed altered expression of many genes associated with hormone signaling functions after aphid infestation and exposure to volatiles. For example, we found more than ten auxin-induced protein genes, such as Glyma. 13G091100, Glyma. 06G123500, Glyma. 16G084300, Glyma. 06G278400, Glyma. 19G161000, Glyma. 04G250600, Glyma. 12G124500, Glyma. 10G031800, Glyma. 10G031900, Glyma. 13G142900, Glyma. 10G180100, Glyma. 02G142500, Glyma. 02G142600, Glyma. 09G221600, Glyma. 15G012800, Glyma. 13G189700, and Glyma. 06G025500. Of these, some genes were up-regulated in response to aphid damage or volatiles, while others were down-regulated (Table 4). Notably, an ACX (acyl-CoA oxidase) gene involved in jasmonic acid (JA) biosynthesis (Schilmiller *et al*., 2007) aligns with a soybean gene identified here that encodes an ACX gene (Glyma.11G035200) which showed decreased transcript abundance after aphid infestation (Table 4). In another study, microarray analysis showed up-regulation of a tyrosine aminotransferase gene and allene oxide synthase (AOS) gene of aphid-infested *A. thaliana* (Kusnierczyk *et al*., 2007). Here, our data confirmed increased transcripts of the soybean tyrosine aminotransferase gene (Glyma.06G235500) in response to aphid damage and volatiles (Table 4). By contrast, two AOS genes (Glyma.17G246500 and Glyma.14G078600) exhibited decreased transcript abundance in soybean after aphid infestation (Table 4).

In our RNA-seq analysis of soybean transcriptomes, we also examined the expression of known plant defense genes revealed in previous studies. Of these genes, several were up-regulated, including callose synthase 7-like (Glyma.18G107900) (in response to aphids), disease resistance protein (in response to volatiles) (Glyma.18G195200), callose synthase 2-like (Glyma.15G268800) (in response to volatiles), and regulatory protein NPR3-like (Glyma.02G283300) (in response to volatiles). Meanwhile, several genes with previously established roles in pathogen-induced plant immunity were down-regulated during aphid infestation, including alpha-1,4-glucan-protein synthase (Glyma.01G063000) and microsomal omega-3 fatty acid desaturase (Glyma.18G062000) (Table 4) (Asai *et al*., 2002; Lai *et al*., 2008; Lu *et al*., 2011; Wang 2009; Will and van *et al*., 2006; MacWilliams *et al*., 2023).

Because microarray results had previously demonstrated up-regulation of other plant defense DEGs in wheat (Smith *et al*., 2010), we examined expression profiles of their soybean counterparts here. Our results showed increased transcript abundance in soybean of genes encoding glutathione S-transferase GST 6 (Glyma.07G140100) (after infestation by aphids and exposure to volatiles), heat stress transcription factor Hsf-21 (Glyma.10G029600) (after infestation by aphids), and glutathione S-transferase GST 7 (Glyma.07G140000) (after exposure to volatiles) (Table 4). However, our results showed decreased transcript abundance reflecting down-regulation of expression of soybean genes encoding putative calcium-binding protein (Glyma.17G128900) and nitrate reductase [NADH] 2 (Glyma.14G165000) after aphid infestation and volatiles exposure (Table 4).

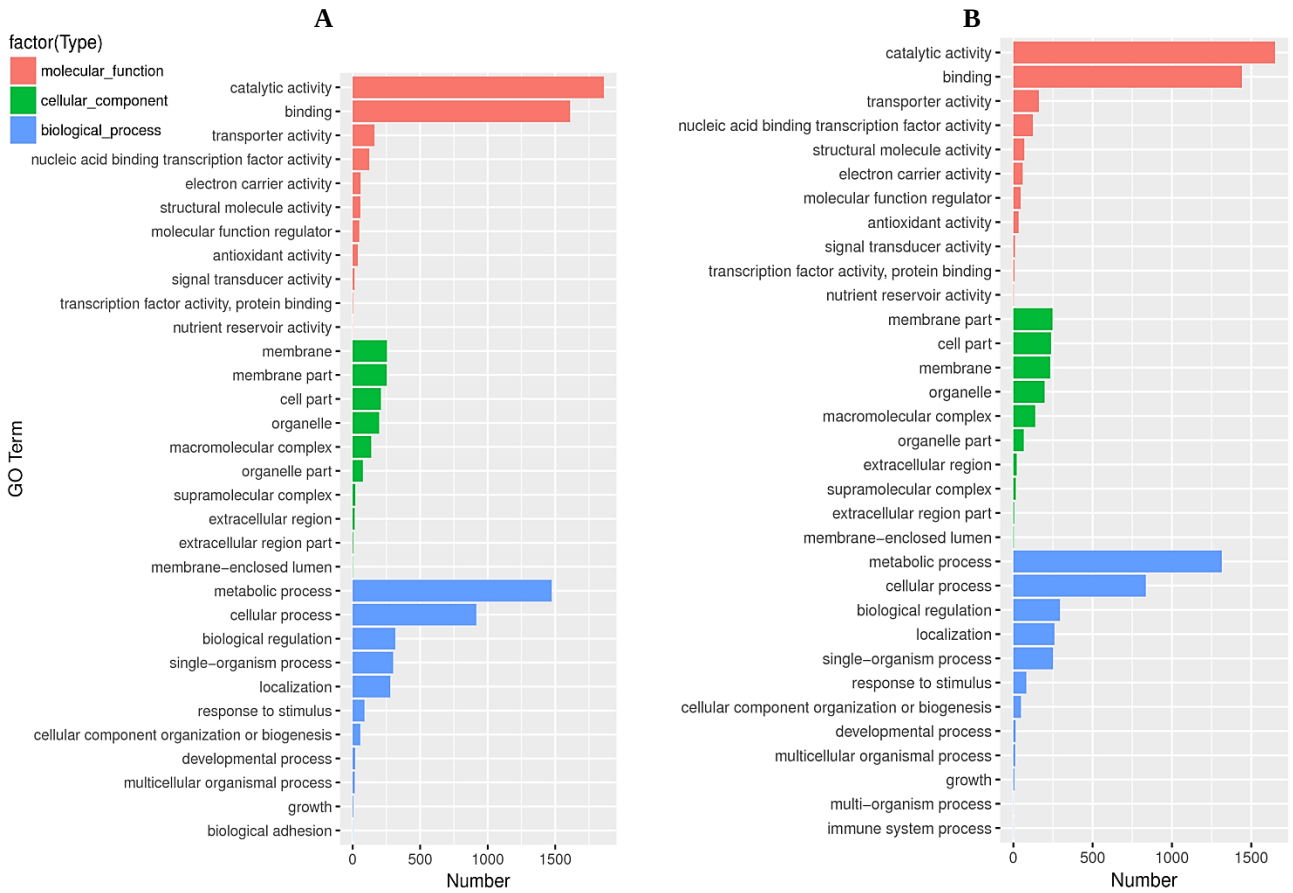

Fig. 2. Functional categories of the GO terms of all *Glycine max* genes in response to aphid (A) and volatiles (B). The red, green, and blue color represents molecular function, cellular component, and biological process, respectively.

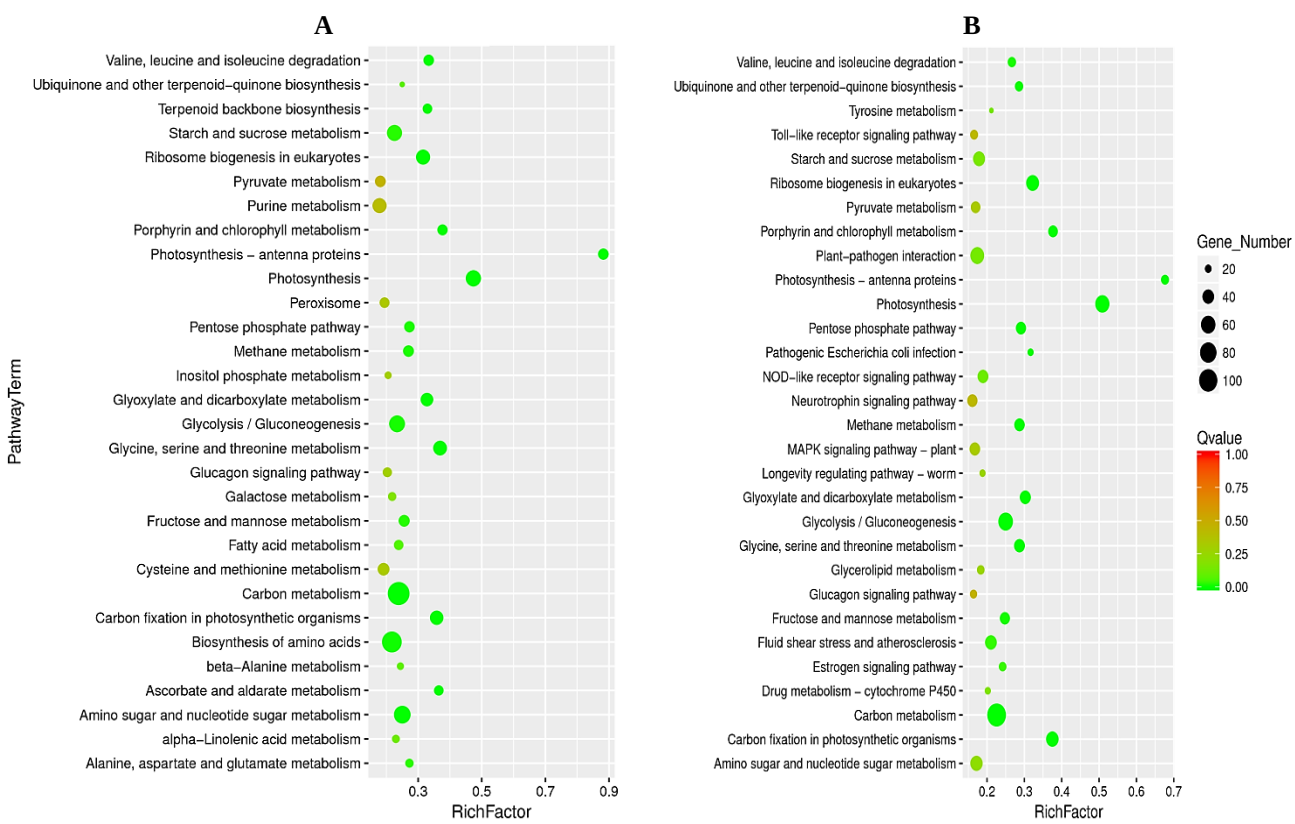

Fig. 3. The KEGG pathway terms of all *Glycine max* genes in response to aphid (A) and volatiles (B). The size represented gene number and the color represented Q-value.

Table 3. Highly up- and down-regulated genes of *Glycine max*.

Condition	Up		log2 Fold Change	Down		log2 Fold Change
	Gene ID	Description		Gene ID	Description	
CK-HI	Glyma.09G080100	uncharacterized	6.55	Glyma.08G138200	inositol-3-phosphate synthase	-8.00
	Glyma.17G044300	alpha-aminoadipic semialdehyde synthase-like	5.92	Glyma.06G044400	peamaclein	-6.94
	Glyma.19G227200	importin subunit alpha-4-like	5.21	Glyma.10G021300	uncharacterized	-6.37
	Glyma.13G242100	stem-specific protein TSJT1-like	5.18	Glyma.08G069300	uncharacterized	-6.12
	Glyma.06G120300	2-oxoisovalerate dehydrogenase subunit alpha 2	5.12	Glyma.09G021100	polygalacturonase-like	-6.03
	Glyma.02G059500	uncharacterized	5.10	Glyma.19G007700	carbonic anhydrase, chloroplastic	-5.90
	Glyma.17G128000	malate synthase	5.01	Glyma.14G164900	nitrate reductase [NADH] 2	-5.56
	Glyma.13G349300	beta-fructofuranosidase, cell wall isozyme-like	4.97	Glyma.16G165200	chlorophyll a-b binding protein of LHCII type 1	-5.44
	Glyma.14G168100	uncharacterized	4.91	Glyma.05G160900	uncharacterized	-5.39
	Glyma.15G023800	beta-galactosidase 1	4.88	Glyma.10G094600	uncharacterized	-5.23
CK-VI	Glyma.15G186100	uncharacterized	6.18	Glyma.06G044400	peamaclein	-8.89
	Glyma.01G077000	cytokinin induced message	4.94	Glyma.01G216000	dehydration-responsive element-binding protein 1E	-6.60
	Glyma.09G032100	transcription factor MYB108-like	4.56	Glyma.09G021100	polygalacturonase-like	-6.57
	Glyma.03G183900	transcription factor RAX2	4.46	Glyma.07G212400	uncharacterized	-6.39
	Glyma.13G349300	beta-fructofuranosidase, cell wall isozyme-like	4.37	Glyma.08G171000	uncharacterized	-6.32
	Glyma.05G141200	protein TIFY 5A-like	4.30	Glyma.07G038500	uncharacterized	-6.18
	Glyma.04G049200	branched-chain-amino-acid aminotransferase 2, chloroplastic-like	4.30	Glyma.17G047300	dehydration-responsive element binding protein 3	-5.47
	Glyma.08G011500	squamosa promoter-binding-like protein 13A	4.25	Glyma.17G046500	uncharacterized	-5.45
	Glyma.04G108500	18.1 kDa class I heat shock protein-like	4.24	Glyma.17G212200	uncharacterized	-5.43
	Glyma.07G048800	caffeic acid 3-O-methyltransferase-like	4.18	Glyma.05G160900	uncharacterized	-5.43

CK: control sample. HI: emitter leaves infested by aphid. VI: uninfested receiver leaves exposed to volatiles. CK-HI: regulated genes after infestation by aphids in comparison with controls. CK-VI: regulated genes after exposure to volatiles in comparison with controls

Table 4. Identified genes expressed in *Glycine max* leaves.

Categorizations	Description	Gene ID	log2 Fold change	
			CK-HI	CK-VI
Hormone responses	auxin-induced protein 5NG4-like	Glyma.13G091100	3.40	2.31
	auxin-induced protein 5NG4-like	Glyma.06G123500	2.31	1.79
	auxin-induced in root cultures protein 12	Glyma.16G084300	-2.14	
	auxin-induced protein 10A5	Glyma.06G278400	1.70	
	auxin-induced protein 22E-like	Glyma.19G161000	1.21	
	auxin-induced protein 5NG4-like	Glyma.04G250600	1.78	
	auxin-induced protein 6B-like	Glyma.12G124500	1.88	
	auxin-induced protein 22D-like	Glyma.10G031800	1.03	
	auxin-induced protein AUX28-like	Glyma.10G031900		-1.08
	auxin-induced protein X15-like	Glyma.13G142900		-2.64
	auxin-induced protein ali50 (SLR1)	Glyma.10G180100		-1.09
	auxin-induced protein AUX28-like	Glyma.02G142500		-1.04
	auxin-induced protein 22D-like	Glyma.02G142600		-1.25
	auxin-induced protein 10A5-like	Glyma.09G221600		-1.94
	auxin-induced protein 22C-like	Glyma.15G012800		-2.08
	auxin-induced protein 5NG4-like	Glyma.13G189700		1.56
	auxin-induced protein 6B-like	Glyma.06G025500		-1.81
	acyl-CoA oxidase	Glyma.03G056400	2.07	
	acyl-CoA oxidase	Glyma.11G035200	-1.38	
	allene oxide synthase	Glyma.17G246500	-1.95	
	allene oxide synthase	Glyma.14G078600	-2.11	
	lipoxygenase	Glyma.07G007000		1.07
	lipoxygenase 3	Glyma.13G239000	-2.80	
	12-oxophytodienoate reductase 2-like	Glyma.19G057500	-1.53	
	12-oxophytodienoate reductase 2	Glyma.01G235600	-1.78	
	chlorophyllase-1	Glyma.07G096900	-1.34	-1.13
	tyrosine aminotransferase	Glyma.06G235500	3.16	1.52
	tryptophan synthase beta chain 2	Glyma.13G049500	-1.47	
	anthranilate synthase alpha subunit 1, chloroplastic-like	Glyma.18G028900	-1.49	-1.88
Pathogen-related	pathogen-related protein-like	Glyma.06G123800		2.69
	disease resistance protein	Glyma.18G195200		1.98
	callose synthase 2-like	Glyma.15G268800		1.20
	callose synthase 7-like	Glyma.18G107900	1.13	
	alpha-1,4-glucan-protein synthase	Glyma.01G063000	-1.30	
	regulatory protein NPR3-like	Glyma.02G283300		1.59
	microsomal omega-3 fatty acid desaturase	Glyma.18G062000	-4.16	
	putative calcium-binding protein CML19	Glyma.17G128900	-2.41	-3.29
	nitrate reductase [NADH] 2	Glyma.14G165000	-5.05	-3.35
	heat stress transcription factor Hsf-21	Glyma.10G029600	2.25	
	glutathione S-transferase GST 6	Glyma.07G140100		1.81
	glutathione S-transferase GST 7	Glyma.07G140000		1.75
Oxidative stress	L-ascorbate peroxidase 2	Glyma.12G073100	-2.18	

Table 4. (Cont'd.).

Categorizations	Description	Gene ID	log2 Fold change	
			CK-HI	CK-VI
	peroxidase-like protein	Glyma.14G177200	-2.18	
	polyubiquitin	Glyma.20G141600	-1.34	-1.22
	polyubiquitin	Glyma.10G251900	-1.24	
	phenylalanine ammonia-lyase 1	Glyma.03G181600	-1.88	
	phenylalanine ammonia-lyase 1	Glyma.19G182300		1.91
	phenylalanine ammonia-lyase 1	Glyma.03G181700		2.27
	phenylalanine ammonia-lyase	Glyma.10G058200	-1.24	
	polygalacturonase inhibitor 1-like protein	Glyma.15G209300	-2.07	
	polygalacturonase inhibitor protein	Glyma.08G079200		-2.14
Cell wall hemicellulose metabolism	beta-D-xylosidase 1	Glyma.15G143700	1.68	
Chaperones	PREDICTED: Glycine max luminal-binding protein 5	Glyma.08G025700	-1.41	1.34
	PREDICTED: Glycine max luminal-binding protein	Glyma.05G219600	-1.41	1.25
Secondary messengers	PREDICTED: Glycine max calmodulin-like	Glyma.03G022800	2.29	1.48
	Glycine max calmodulin (SCAM-3)	Glyma.19G068300	-1.26	
	Glycine max calmodulin (SCAM-3)	Glyma.05G079700	-1.31	-1.55
	PREDICTED: Glycine max calmodulin-like protein 3	Glyma.13G344200	-1.25	-1.65
	calmodulin-like protein 5-like	Glyma.13G159600		-1.55
	calmodulin-like protein 1-like	Glyma.01G185600		-1.62
	calmodulin-like protein 4	Glyma.17G242700		1.04
	calmodulin-like protein 3	Glyma.17G112000		-1.54
	calmodulin-like protein 1	Glyma.11G056500		-1.35
	calcium-dependent protein kinase 33-like (CDPK)	Glyma.14G023500	1.25	1.13
	calcium-dependent protein kinase 26-like (CDPK)	Glyma.10G239600	1.1	
	calcium-dependent protein kinase 3-like (CDPK)	Glyma.08G019700	1.61	
	calcium-dependent protein kinase 3-like (CDPK)	Glyma.05G213200		1.38
	calcium-dependent protein kinase 2-like (CDPK)	Glyma.18G096500		1.88
	calcium-dependent protein kinase 33-like (CDPK)	Glyma.02G291300		1.35
	calcium-dependent protein kinase 24-like (CDPK)	Glyma.11G128900		-2.06
Protein/peptide degradations	cysteine proteinase RD21a-like	Glyma.14G085600	-3.92	-3.67
	xylem cysteine proteinase 2-like	Glyma.06G014700	-2.02	-1.76
Photosynthesis	trans-cinnamate 4-monooxygenase-like	Glyma.14G205200		-1.61
Others	asparagine synthetase	Glyma.02G228100	-3.61	-2.82
	asparagine synthetase	Glyma.11G170300	2.03	
	asparagine synthetase 1 (AS1)	Glyma.18G061100	1.92	
	asparagine synthetase 2 (AS2)	Glyma.14G195000	-2.37	
	polyphenol oxidase A1	Glyma.13G183200	-1.66	-1.78
	polyphenol oxidase I	Glyma.07G193300	-2.29	
	polyphenol oxidase, chloroplastic-like	Glyma.06G270400		2.12
	polyphenol oxidase A1	Glyma.13G183500		2.10
	polyphenol oxidase A1	Glyma.13G183000		2.03

CK: control sample. HI: emitter leaves infested by aphid. VI: uninfested receiver leaves exposed to volatiles. CK-HI: regulated genes after infestation by aphids in comparison with controls. CK-VI: regulated genes after exposure to volatiles in comparison with controls

Conclusions

After 24 hours of aphid infection and exposure to volatiles, a series of transcriptome changes occurred in soybean. Soybean aphid infested plants exhibited 2413 up-regulated and 3905 down-regulated genes, while leaf exposure to volatiles resulted in 2153 up-regulated and 3572 down-regulated genes. These genes were endowed with a wide range of functions including 'hormone responses', 'pathogen-related', and 'oxidative stress' functions. The results ultimately demonstrated that aphid damage and volatiles exposure elicited drastic metabolic changes in soybean leaves. These results also emphasize that RNA-seq analysis of repressed genes should contribute to our understanding of signal transduction in aphid-infested leaves, as well as in leaves exposed to herbivory-induced volatiles.

Acknowledgments

We thank Shuzhen Zhang (Northeast Agricultural University) for supplying the soybean seeds of *G. max* in the experiments. This work was financially supported by the Special Fund for Modern Agro-industry Technology Research System (CARS-04) to K. Zhao; the National Key R&D Program of China (2017YFE0105000), and the National Natural Science Foundation of China (31872120) to A. Wang; and the Doctoral Starting up Foundation of Guizhou University of Traditional Chinese Medicine (2020-19) to H. Chen.

References

Aartsma, Y., F.J.J.A. Bianchi, V.D.W. Wopke, E.H. Poelman and M. Dicke. 2017. Herbivore - induced plant volatiles and tritrophic interactions across spatial scales. *New Phytol.*, 216: 1054-1063.

Arimura, G., K. Tashiro, S. Kuhara, T. Nishioka, R. Ozawa and J. Takabayashi. 2000a. Gene responses in bean leaves induced by herbivory and by herbivore-induced volatiles. *Biochem. Bioph. Res. Co.*, 277: 305-310.

Arimura, G., R. Ozawa, T. Shimoda, T. Nishioka, W. Boland and J. Takabayashi. 2000b. Herbivory-induced volatiles elicit defence genes in lima bean leaves. *Nature*, 406: 512-515.

Asai, T., G. Tena, J. Plotnikova, M.R. Willmann, and W.L. Chiu, L. Gomez-Gomez, T. Boller, F.M. Ausubel and J. Sheen. 2002. Map kinase signalling cascade in arabidopsis innate immunity. *Nature*, 415: 977-983.

Bruin, J., M. Dicke and M.W. Sabelis. 1992. Plants are better protected against spider-mites after exposure to volatiles from infested conspecifics. *Experientia*, 48: 525-529.

Dicke, M., M.W. Sabelis, J. Takabayashi, J. Bruin and M.A. Posthumus. 1990a. Plant strategies of manipulating predatorprey interactions through allelochemicals: Prospects for application in pest control. *J. Chem. Ecol.*, 16: 3091-3118.

Dicke, M., R. Gols, D. Ludeking and M.A. Posthumus. 1999. Jasmonic acid and herbivory differentially induces carnivore-attracting plant volatiles in lima bean plants. *J. Chem. Ecol.*, 25: 1907-1922.

Dicke, M., T.A.V. Beek, M.A. Posthumus, N.B. Dom, H.V. Bokhoven and A.D. Groot. 1990b. Isolation and identification of volatile kairomone that affects acarine predatorprey interactions involvement of host plant in its production. *J. Chem. Ecol.*, 16: 381-396.

Godard, K.A., R. White and J. Bohlmann. 2008. Monoterpene-induced molecular responses in *Arabidopsis thaliana*. *Phytochemistry*, 69: 1838-1849.

Heil, M. and J.C.S. Bueno. 2007. Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proc. Natl. Acad. Sci. USA*, 104: 5467-5472.

Huang, X., H. Zhang, H. Li, M. Wang, X. Guo, E. Liu, X. Han, C. Zhen, A. Li, W. Shi and Y. Zhang. 2022. Functional characterization of a terpene synthase responsible for (E)-b-ocimene biosynthesis identified in *Pyrus betuleafolia* transcriptome after herbivory. *Front. Plant Sci.*, 13: 1077229.

Karban, R., I.T. Baldwin, K.J. Baxter, G. Laue and G.W. Felton. 2000. Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. *Oecologia*, 125: 66-71.

Karssemeijer, P.N., K.A. de Kreek, R. Gols, M. Neequaye, M. Reichelt, J. Gershenzon, J.J.A. van Loon and M. Dicke. 2022. Specialist root herbivore modulates plant transcriptome and downregulates defensive secondary metabolites in a brassicaceous plant. *New Phytol.*, 235: 2378-2392.

Kent, W.J. 2002. BLAT-the BLAST-like alignment tool. *Genome Res.*, 12(4): 656-664.

Kusnierczyk, A., P. Winge, H. Midelfart, W.S. Armbruster, J.T. Rossiter and A.M. Bones. 2007. Transcriptional responses of arabidopsis thaliana ecotypes with different glucosinolate profiles after attack by polyphagous myzus persicae and oligophagous brevicoryne brassicae. *J. Exp. Bot.*, 58: 2537-2552.

Lai, Z., K.M. Vinod, Z. Zheng, B. Fan and Z. Chen. 2008. Roles of Arabidopsis WRKY3 and WRKY4 transcription factors in plant responses to pathogens. *BMC Plant Biol.*, 8: 68.

Li, A., M. Wang, Z. Chen, C. Qin, F. Liao, Z. Wu , W. He, P. Lakshmanan, Y. Pan and D. Huang. 2022. Integrated transcriptome and metabolome analysis to identify sugarcane gene defense against fall armyworm (*Spodoptera frugiperda*) herbivory. *Int. J. Mol. Sci.*, 23: 13712.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C(T)) method. *Methods*, 25: 402-408.

Love. M.I., S. Anders and W. Huber. 2015. Differential analysis of count data-the DESeq2 package. (DESeq2).

Lu, B., W. Sun, S. Zhang, C. Zhang, J. Qian, X. Wang, R. Gao and H. Dong. 2011. HrpNEa-induced deterrent effect on phloem feeding of the green peach aphid Myzus persicae requires AtGSL5 and AtMYB44 genes in *Arabidopsis thaliana*. *J. Biosci.*, 36: 123-137.

MacWilliams, J.R., P.D. Nabity, K.E. Mauck and I. Kaloshian. 2023. Transcriptome analysis of aphid-resistant and susceptible near isogenic lines reveals candidate resistance genes in cowpea (*Vigna unguiculata*). 2023. *BMC Plant Biol.*, 23: 22.

Moraes, C.M.D., W.J. Lewis, P.W. Paré, H.T. Alborn and J.H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature*, 393: 570-573.

Mortazavi, A., B.A. Williams, K. McCue, L. Schaeffer and B. Wold. 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods*, 5: 621-628.

Rhoades, D.F. 1983. Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheromonal sensitivity of willows. *Plant Resistance to Insects*, 208: 55-68.

Robinson, M.D., D.J. McCarthy and G.K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26: 139-140.

Ruther, J. and S. Kleier. 2005. Plant-plant signaling: ethylene synergizes volatile emission in *Zea mays* induced by exposure to (z)-3-hexen-1-ol. *J. Chem. Ecol.*, 31: 2217-2222.

Schilmiller, A.L., A.J. Koo and G.A. Howe. 2007. Functional diversification of acyl-coenzyme a oxidases in jasmonic acid biosynthesis and action. *Plant Physiol*, 143: 812-824.

Schmutz, J., S.B. Cannon, J. Schlueter, J. Ma, T. Mitros, W. Nelson, D.L. Hyten, Q. Song, J.J. Thelen, J. Cheng, D. Xu, U. Hellsten, G.D. May, Y. Yu, T. Sakurai, T. Umezawa, M.K. Bhattacharyya, D. Sandhu, B. Valliyodan, E. Lindquist, M. Peto, D. Grant, S. Shu, D. Goodstein, K. Barry, M. Futrell-Griggs, B. Abernathy, J. Du, Z. Tian, L. Zhu, N. Gill, T. Joshi, M. Libault, A. Sethuraman, X.C. Zhang, K. Shinozaki, H.T. Nguyen, R.A. Wing, P. Cregan, J. Specht, J. Grimwood, D. Rokhsar, G. Stacey, R.C. Shoemaker and S.A. Jackson. 2010. Genome sequence of the palaeopolyploid soybean. *Nature*, 463: 178-183.

Smith, C.M., X. Liu, L.J. Wang, X. Liu, M. Chen, S. Starkey and J. Bai. 2010. Aphid feeding activates expression of a transcriptome of oxylipin-based defense signals in wheat involved in resistance to herbivory. *J. Chem. Ecol.*, 36: 260-276.

Takabayashi, J., M. Dicke and M.A. Posthumus. 1994. Volatile herbivore-induced terpenoids in plant-mite interactions: variation caused by biotic and abiotic factors. *J. Chem. Ecol.*, 20: 1329-1354.

Ton, J., M. D'Alessandro, V. Jourdie, G. Jakab, D. Karlen, M. Held, B. Mauch-Mani and T.C. Turlings. 2010. Priming by airborne signals boosts direct and indirect resistance in maize. *Plant J.*, 49: 16-26.

Turlings, T.C., J.H. Tumlinson and W.J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, 250: 1251-1253.

Wang, X. 2009. The arabidopsis ataf1, a NAC transcription factor, is a negative regulator of defense responses against necrotrophic fungal and bacterial pathogens. *Mol. Plant Microb. Interact.*, 22: 1227-1238.

Will, T. and A.J. van Bel. 2006. Physical and chemical interactions between aphids and plants. *J. Exp. Bot.*, 57: 729-737.

Ye, W., C. Bustos-Segura, T. Degen, M. Erb, and T.C.J. Turlings. 2022. Belowground and aboveground herbivory differentially affect the transcriptome in roots and shoots of maize. *Plant Direct.*, 6(7): e426.

Zhang, S., J. Wei and L. Kang. 2012. Transcriptional analysis of *Arabidopsis thaliana* response to lima bean volatiles. *Plos One* 7: e35867

(Received for publication 22 January 2022)