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Understanding cross-communication between aboveground and belowground tissues via transcriptome analysis of a sucking insect whitefly-infested pepper plants

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ABSTRACT

Plants have developed defensive machinery to protect themselves against herbivore and pathogen attacks. We previously reported that aboveground whitefly (*Bemisia tabaci* Genn.) infestation elicited induced resistance in leaves and roots and influenced the modification of the rhizosphere microflora. In this study, to obtain molecular evidence supporting these plant fitness strategies against whitefly infestation, we performed a 300 K pepper microarray analysis using leaf and root tissues of pepper (*Capsicum annuum* L.) applied with whitefly, benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), and the combination of BTH + whitefly. We defined differentially expressed genes (DEGs) as genes exhibiting more than 2-fold change (1.0 based on $\log_2$ values) in expression in leaves and roots in response to each treatment compared to the control. We identified a total of 16,188 DEGs in leaves and roots. Of these, 6685, 6752, and 4045 DEGs from leaf tissue and 6768, 7705, and 7667 DEGs from root tissue were identified in the BTH, BTH + whitefly, and whitefly treatment groups, respectively. The total number of DEGs was approximately two-times higher in roots than in whitefly-infested leaves subjected to whitefly infestation. Among DEGs, whitefly feeding induced salicylic acid and jasmonic acid/ethylene-dependent signaling pathways in leaves and roots. Several transporters and auxin-responsive genes were upregulated in roots, which can explain why biomass increase is facilitated. Using transcriptome analysis, our study provides new insights into the molecular basis of whitefly-mediated intercommunication between aboveground and belowground plant tissues and provides molecular evidence that may explain the alteration of rhizosphere microflora and root biomass by whitefly infestation.

1. Introduction

Under natural conditions, plants continuously face an onslaught of insect herbivores and pathogens. To overcome these critical attacks from invaders, plants have developed defensive mechanisms to protect themselves [1]. As similar to plant–pathogen interactions, plants have also been proposed a variety of defense responses against insect infestation [2]. Infested plants exhibit increasing levels of Ca^{2+} ion fluxes, activation of mitogen-activated protein kinases (MAPKs), increasing levels of plant defense hormones, jasmonic acid (JA), ethylene (ET), and salicylic acid (SA), production of reactive oxygen species (ROS), and increased volatile emissions [2]. In addition, effector-triggered immunity (ETI) was modulated by plant resistance (*R*) proteins including three *R* genes, including *Mi-1.2*, *Vat*, and *Bph14*. *Mi-1.2* in tomato confers resistance to potato aphid and two whitefly biotypes. *Vat* and *Bph14* increase plant resistance against melon-cotton aphid and rice brown hopper, respectively [3–5]. In general, ETI is highly specific and is often accompanied by the hypersensitive response (HR), which is likely to induce programmed cell death to arrest pathogen growth in infected plant sites [6,7]. When plant defense responses are activated in local infection sites, systemic defense responses are subsequently triggered throughout the whole plants to protect themselves from serial invasions of pathogens. This long-lasting, broad-spectrum response is referred to as systemic acquired resistance (SAR) [8–10]. In addition to induction of SAR by pathogen attack, systemic responses have been observed in distal (systemic) parts of plants after infestation by insect herbivores [11–14]. In general, SA signaling triggers plant resistance to biotrophic and hemibiotrophic pathogens and sucking insects, whereas JA/ET signaling contributes to the elicitation of resistance against necrotrophic pathogens and chewing insect herbivores [15].

To date, approximately 1500 species of whitefly (*Bemisia tabaci* Genn.) have been reported in the warmer, tropical, and subtropical

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regions. In addition to affecting plant primary production, whitefly may produce secondary damage by encouraging mold development, blocking sunlight, and reducing photosynthesis [16–18]. Like pathogens, whitefly also induces plant defense responses that are dependent on the SA and JA/ET pathways [19,20]. In *Arabidopsis*, SA-responsive genes (*PR1*, *BGL2*, *PR5*, *SID2*, *EDS5*, and *PAD4*) are upregulated in whitefly-infested local leaves, among which the transcripts of three genes *PR1*, *BGL2*, and *PR5* are systemically accumulated in distal leaves. On the other hand, the expression levels of the JA/ET-dependent genes including *PDF1.2*, *VSP1*, *HEL*, *THI2.1*, *FAD3*, *ERS1*, and *ERF1* are repressed in whitefly-infested leaves [21]. In addition, pathogen-related (PR) proteins are expressed in plants in response to *B. tabaci* infestation [22–24]. For example, several PR proteins including *β-1,3-glucanase*, *chitinase*, *peroxidase*, *PR2*, and *PR4* are highly upregulated in whitefly-infested tomato leaves and systemic leaves compared with control plants [25]. In addition, *SLW* (*silverleaf whitefly*)*1* and *SLW3* are locally and systemically inducible after whitefly infestation in squash. Specifically, the transcripts of *SLW1* and *SLW3* are activated by nymph feeding but not by adult feeding. In addition, this study suggests the existence of novel signaling pathway(s) that are not regulated by SA or JA [26].

As described above, accumulating data reveal the potential functions of certain genes in the plant defense responses and signaling pathways of diverse plant species interacting with whitefly. Despite the tremendous efforts focused on elucidating the communication between plants and whitefly, molecular mechanism of pepper (*Capsicum annuum* L.) in response to whitefly infestation is not well understood yet. In current study, in order to fill the current gap of this knowledge between aboveground whitefly feeding on leaves significantly enhanced the plant defense responses in roots as well as altered pepper fitness in our previous research [12] and molecular evidence, we analyzed transcript both in leaf and root by using a pepper 300 k microarray in response to whitefly infestation, benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), and the combination of BTH + whitefly on leaves when compared with water control. Whitefly infestation induced SA and JA/ET dependent pathways in leaves and roots. Notably, several transporters and auxin-responsive genes were upregulated in roots when compared to the water control, suggesting the potential physiological mechanism that facilitates the root biomass.

2. Materials and methods

2.1. Pepper plant growth and BTH and whitefly treatments

Pepper (*C. annuum* L. cv. Bukwang) was used in this study as followed previously [9]. Pepper seeds were surface sterilized with 6% sodium hypochlorite, washed at least five times to remove the remaining sodium hypochlorite, and incubated at 25–28 °C on MS agar plates until germination. One-week old germinated pepper seeds were transplanted to natural pepper field soil containing sand and silt loam soil obtained from the KRIBB greenhouse facility, Daejeon, South Korea. The transplanted pepper plants were grown at 25–28 °C with a 12 h light/dark photoperiod under controlled conditions in a growth chamber (7000 L × light intensity).

Two-week-old pepper seedlings were drenched with either 10 ml of 0.5 mM BTH (Syngenta, Research Triangle Park, NC, USA) or sterile water as a control. Each pepper plant was then incubated in a transparent plastic cylinder with a diameter of 15 cm and a height of 50 cm; each end of the plastic cylinder was covered with a nylon stocking. For whitefly infestation, 2-week-old pepper plants were placed into plastic cylinders as described above, but the cylinders were not covered with nylon stockings, and the plants were exposed to constant whitefly infestation. Approximately 20 of *B. tabaci* per leaf were infested and 0.5 mM BTH was treated for a week. In addition, 10 pepper plants were subjected to a combination of BTH treatment and whitefly infestation as described above. All treatments were performed for 1 week prior to analysis.

2.2. Whitefly effects on root biomass

To assess whether whitefly infestation in aboveground affected the plant fitness in belowground, root dry weight was measured 7 days after infestation of whitefly with the same conditions as followed above. The experiment was repeated three times with 10 replications.

2.3. Total RNA extraction and cDNA synthesis

The pepper plants were harvested, immediately frozen in liquid N_2, and ground with a sterilized mortar and pestle. Total RNA was isolated from leaves and roots treated with whitefly, BTH, BTH + whitefly, and water control. Total RNA was isolated followed the protocol described by Yang et al. [9] and Yi et al. [27]. Briefly, total RNA was treated with 1 U of RNase-free DNase (Promega, Madison, WI, USA) for 10 min at 37 °C, and the RNA was then subjected to a second round of purification with the TRI reagent. First-strand cDNA was synthesized from 1 μg DNase-treated total RNA using oligo-dT primers and Moloney murine leukemia virus reverse transcriptase (MMLV-RT; Enzynomics, Daejeon, South Korea).

2.4. Quantitative (q)-RT-PCR

Before carrying out qRT-PCR for gene expression profiling of candidate genes, to ensure that equal amounts of RNA were analyzed in each sample and each experiment, *CaActin1* (GenBank accession No. AY572427) was amplified, and the PCR product was separated by 2% agarose gel electrophoresis. The qRT-PCR was conducted in a Chromo4 Real-time PCR System (Bio-Rad, Hercules, CA, USA). Each 10 μl reaction mixture used for qRT-PCR contained 5 μl of 2 × Brilliant SYBR Green QPCR Master Mix (Bio-Rad), cDNA, and 0.5 μM of each primer. Amplification conditions for each gene were as follows: 95 °C for 10 min and 44 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. The relative expression of each candidate gene was calibrated and normalized to that of *CaACT1*. All primers used in this study are listed in Supplementary Table 1.

2.5. Microarray sample preparation and data analysis

For microarray analysis, 10 individual samples per each treatment from both leaf and root were collected and used for further analysis as described previously [9]. This study employed a 300 K pepper cDNA microarray manufactured by NimbleGen Systems Inc. (http://www.nimblegen.com/). The microarray was generated from 29580 unigenes, among which the orientations of 24417 genes were known, and six probes were designed for each gene. Further information on this microarray including statistical analysis should be found at http://www.ggbio.com (GreenGene Biotech, Korea). The expression data were normalized using quantile normalization as described previously [28]. The Robust Multi-Chip Analysis (RMA) using a median polish algorithm implemented in NimbleScan software (NimbleGen, CA, USA) was used to produce call files [29]. Functional categories were analyzed using DAVID (http://david.abcc.ncifcrf.gov/tools.jsp) and KEGG (http://www.genome.jp/kegg2.html). The microarray data reported in this study

are available from the NCBI Gene Expression Omnibus (GEO), accession number GSE52661.

2.6. Statistical analysis

Analysis of variance for experimental datasets was performed using JMP software v5.0 (SAS Institute, Cary, NC, USA) for Fig. 3. Significant effects of treatment were determined by the magnitude of F value ($P = 0.05$). When a significant F test was obtained, separation of means was accomplished by Fisher's protected least significant difference (LSD) at $P = 0.05$.

3. Results and discussion

3.1. cDNA microarray analysis of gene expression in leaves and roots of pepper

We previously showed that plant defense was enhanced after *B. tabaci* feeding both in the aboveground and belowground parts of pepper plants. *B. tabaci* feeding also resulted in an increase in root biomass and the attraction of Gram-positive bacteria and fungi [12]. To elucidate how *B. tabaci* affects induced resistance and increases root biomass at the molecular level, we applied whitefly, BTH, BTH + whitefly, and control (water) treatments to pepper leaves for 7 d, harvested the leaves and roots separately, and performed transcriptome analysis using a pepper cDNA microarray. To identify significantly altered transcript levels of genes after *B. tabaci* infestation, we considered genes that were differentially expressed by a level of more than 2-fold (1.0 based on $\log_2$ values) compared to the control treatment to be differentially expressed genes (DEGs); these included both up- and downregulated genes. Of the DEGs identified, the expression of 16188 DEGs in leaves and roots was significantly altered by treatment, among which 8253 and 8800 genes were initially annotated based on comparisons with previously identified *Arabidopsis* and tomato/potato genes, respectively. The 8253 annotated genes from *Arabidopsis* were highly overlapping with those in pepper compared with the annotated genes from tomato/potato.

In leaves, 6685, 6752, and 4045 DEGs were identified in BTH-, BTH + whitefly-, and whitefly-treated samples, respectively (Supplementary Table 2). Among these, 2094, 1648, and 569 genes were specifically up- or downregulated by BTH, BTH + whitefly, and whitefly, respectively. The expression of 2613 genes was altered by all three treatments (Fig. 1A). In roots, 6768, 7705, and 7667 DEGs were identified in BTH-, BTH + whitefly-, and whitefly-treated samples, respectively (Supplementary Table 2). Among these, 924, 2010, and 1469 genes were specifically regulated by BTH, BTH + whitefly, and whitefly treatments, respectively. There were as many as 4037 genes that were classified as DEGs that were regulated by all three treatments (Fig. 1B). The total number of DEGs was 190% higher in roots than in *B. tabaci*-infested leaves (Fig. 1A and B). In addition, the number of genes that were specifically differentially expressed in *B. tabaci*-infested roots was significantly higher (approximately 2.5-times) than that of *B. tabaci*-infested leaves (Fig. 1A and B).

3.2. Clustering of DEGs in leaves and roots

To investigate the expression patterns of DEGs in leaves or roots in all groups, we performed clustering analysis; 10 individual groups were categorized (Fig. 2). In leaves, transcripts of clusters 2, 3, and 7 were significantly upregulated in response to the treatments, whereas genes belonging to clusters 5, 6, and 8 were downregulated by the treatments. In roots, higher expression levels were observed in clusters 4 and 8, but a repression pattern was clearly observed in clusters 1, 7, and 9 (Fig. 2). The largest group, which included 3395 genes, was cluster 4, while the smallest cluster, cluster 10, included only 73 genes. The repression pattern of transcripts in both leaves and roots was represented in clusters 1, 6, and 9. By contrast, an upregulation pattern of transcripts was detected in clusters 2, 3, and 4. Clusters 5, 7, and 8 exhibited the opposite expression patterns between leaves and roots. Among the 10 clusters, clusters 1 and 4 genes, which were downregulated and upregulated by all treatments, respectively, comprised approximately 39% of the DEGs.

3.3. Defense-related signaling genes in leaves and roots

Along with using KEGG information to help us analyze the functional categories of the DEGs, we investigated whether the expression levels of the genes implicated in defense-related plant hormone signaling were altered in leaves and roots subjected to whitefly, BTH, and BTH + whitefly treatments compared with the control. We examined the expression levels of genes in response

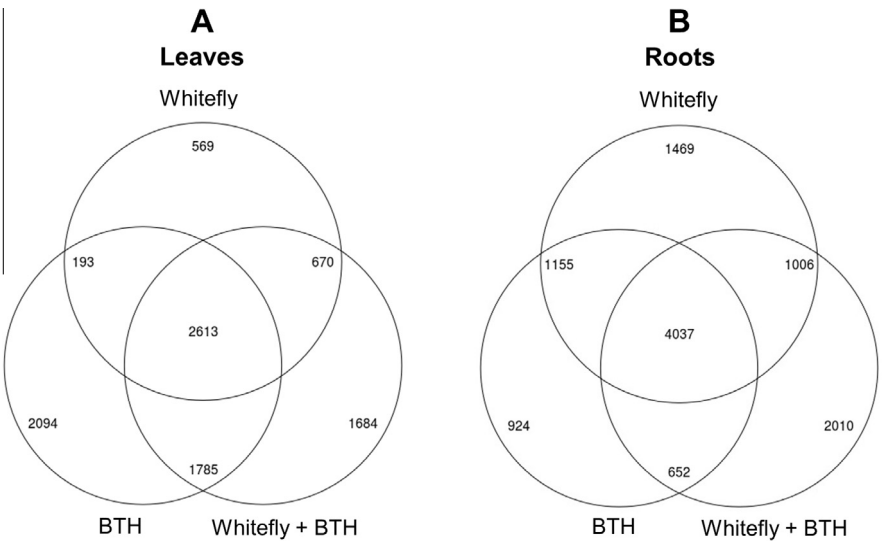

Fig. 1. Venn diagram representing the distribution of differentially expressed genes (DEGs) in leaves and roots. Genes exhibiting more than a 2-fold (1.0 based on $\log_2$ values) change in transcript levels compared to the control were selected in leaves (A) and roots (B) in response to application of whitefly, BTH, and whitefly + BTH.

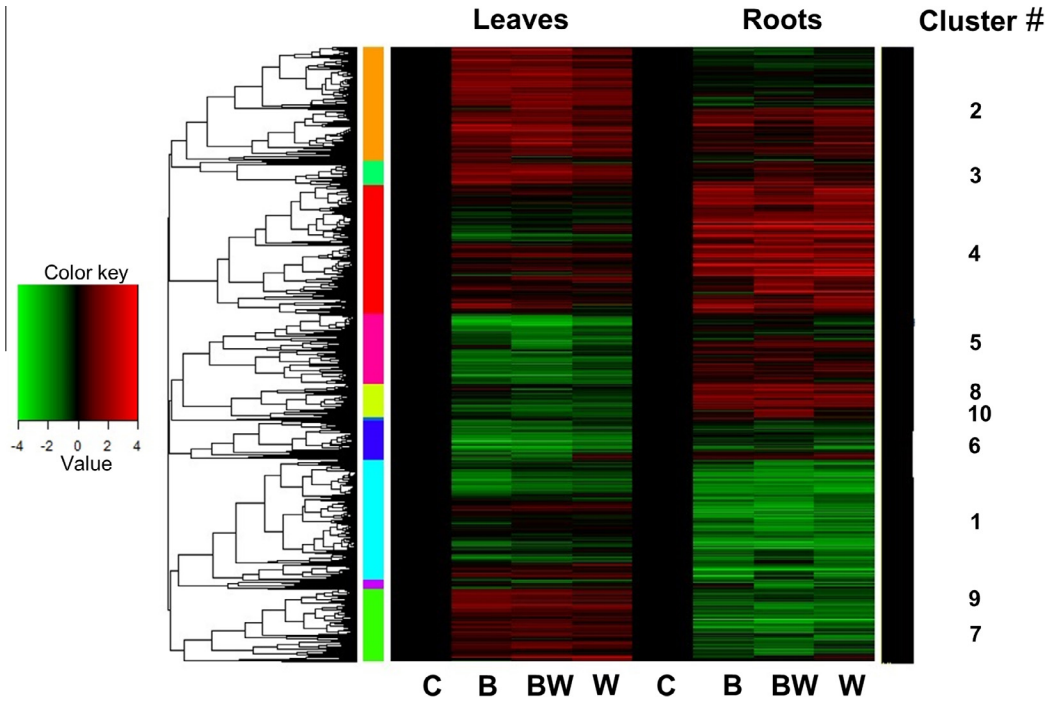

Fig. 2. Clustering of differentially expressed genes (DEGs) in leaves and roots. A total of 16188 genes were identified as DEGs in leaves or roots. Hierarchical clustering of the DEGs in leaves and roots subjected to whitefly infestation (W), BTH (B), and whitefly + BTH (BW) compared to the control treatment (C). The colors represent the gene induction ratios expressed as $\log_2$ values, as shown in the color key (left). The specifically upregulated genes are indicated by red coloring. The number of each figure on right side indicates the number of genes belonging to each cluster.

Table 1
Selected top 10 defense-related genes in leaves and roots subjected to *B. tabaci* infestation.

Category	SEQ ID	Functional annotation	Microarray ($\log_2$ FC)			qRT-PCR ($\log_2$ FC)		
			WF	BTH	BTH + WF	WF	BTH	BTH + WF
Leaf								
SA/JA	PEPPERS0009686	Glucan endo-1,3-beta-D-glucosidase	3.07	2.27	4.23			
SA	PEPPERS0009346	Pathogenesis related gene 1 (PR1)	2.98	2.20	2.93	1.63	1.07	2.56
JA	PEPPERS0000030	Iaa-amino acid hydrolase 11	2.67	1.01	0.50			
SA	PEPPERS0002982	Osmotin (PR5)	2.45	3.11	3.67	1.68	1.25	1.60
JA	PEPPERS0001199	Glycosyltransferase	2.42	3.12	2.90	0.99	0.15	0.76
SA	PEPPERS0017836	Sulfotransferase family protein	2.33	0.28	1.53	3.91	1.47	2.57
JA	PEPPERS0006011	Tryptophan synthase beta chain	2.26	1.42	0.53			
JA	PEPPERS0012323	Catalase isozyme 2	2.05	0.44	0.64			
SA	PEPPERS0003607	Hin1	1.96	0.57	1.07	0.78	0.00	1.45
SA	PEPPERS0006470	Osmotin (PR5)	1.95	2.99	2.70			
Root								
JA	PEPPERS0005248	Glutathione S-transferase	6.38	6.41	6.78			
JA	PEPPERS0008903	Pectinesterase 4	3.40	3.94	2.87			
SA/JA	PEPPERS0009686	Glucan endo-1,3-beta-D-glucosidase	2.72	1.02	−0.12	6.68	3.97	2.39
ET	PEPPERS0018807	ACC synthase	2.54	2.00	2.10	1.71	0.45	1.28
JA/SA	PEPPERS0011206	Indole-3-acetic acid-amido synthetase GH3.3	2.53	1.47	2.26	2.27	0.30	1.17
JA	PEPPERS0004082	UDP-glycosyltransferase-like protein	2.49	1.25	1.31	3.54	1.65	1.27
JA	PEPPERS0012817	17.6 kD class I small heat shock protein	2.41	2.50	2.45			
SA	PEPPERS0010575	Thaumatin (PR5)	2.40	0.34	0.39	5.59	2.26	2.94
SA	PEPPERS0009932	WRKY transcription factor-30	2.32	1.88	1.95	2.88	1.20	2.00
JA	PEPPERS0001200	Glycosyltransferase	2.17	2.45	2.25			

FC, fold change.
The numbers on the table indicate the fold change ratio both in leaf and root treatments as compared to the control treatment using $\log_2$ value.

to *B. tabaci* infestation in both leaves and roots; the 10 genes with the highest fold change levels when compared with water control are listed in Table 1. In leaves, both SA and JA signaling genes were highly induced by whitefly, among which the transcripts PEPPERS0009686 and PEPPERS0009346 were the most significantly upregulated. These genes represent *endo-1,3-β-glucosidase* and *pathogenesis-related gene 1* (*PR1*), respectively. Although different probes were used to identify two *osmotin* (*PR5*) genes, these genes were also listed among the top 10 genes. Among the top 10 genes, pathogenesis-related genes and SA signaling genes were predominant in leaves (Table 1, top). We performed qRT-PCR analysis of an independent set of RNA samples to validate the microarray results. Of the five selected genes, the expression ratios were similar to those of the microarray data (Table 1, top). In the current study,

Fig. 3. Effects of *B. tabaci* infestation on root growth. This representative photograph was taken 7 days after treatment with the control water treatment, whitefly, BTH, and whitefly + BTH. *B. tabaci* infestation resulted in an increase in root biomass. The number below the figure indicated the dry weight (DW) of roots and was adapted from a previous study [12]. The different letters below number indicated significant differences ($P = 0.05$). Data shown are means ± SE of samples conducted in 10 replications.

we identified the top 10 genes based on the fold induction ratio in leaves in response to whitefly infestation compared to the control. Among the top 10 genes, both SA and JA signaling-related genes were highly expressed in leaves in response to whitefly infestation compared to the control. Taken together, the data suggest that both SA and JA signaling pathways are induced in *B. tabaci*-infested leaves, which was agreeable to the previous studies [19,20].

In roots, two JA-related genes, PEPPERS0005248 (*glutathione S-transferase*) and PEPPERS0008903 (*pectinesterase 4*), were the most significantly upregulated by whitefly infestation. PEPPER0009686 (*glucan endo-1,3-β-D-glucosidase*) was identified in both roots and leaves. In addition, the *PR5* thaumatin type gene was induced in roots by *B. tabaci* infestation (Table 1, bottom). To confirm the microarray data, six selected genes from roots were examined by qRT-PCR. The qRT-PCR data presented that the fold-change ratio pattern between treatments was similar to the microarray results (Table 1, bottom). The top 10 genes in roots, which were similar to those in leaves, are listed in Table 1. Since tobacco plants exhibiting the hypersensitive response to *Tobacco mosaic virus* (TMV) were first used to identify defense-related proteins, 17 families of pathogenesis-related (PR) proteins/genes have been identified in plants responding to fungi, oomycetes, bacteria, viruses, viroids, nematodes, and insect feeding [30]. PEPERS0009686, which encodes a *glucan endo-1,3,-β-D-glucosidase* (*β-1,3-endoglucanase*), was upregulated in both roots and leaves subjected to whitefly feeding (Table 1). The *β-1,3-glucanase* gene is a typical member of the *PR2* gene family. In addition, *PR3*, *PR4*, *PR8*, and *PR11* encode endo-chitinase. Both *β-1,3-glucanase* and *chitinase* exhibit antifungal activity in plants [31,32] and these proteins exhibit synergistic inhibitory effects on fungal growth [33]. Our result suggests that *B. tabaci* infestation can elicit induced resistance to oomycetes and fungal pathogens in pepper roots via overexpression of *β-1,3-glucanase*. As shown in Table 1, two *PR5* genes encoding osmotin were significantly upregulated by whitefly infestation, BTH, and whitefly + BTH. By contrast, downregulated expression of these genes was elicited by these treatments in roots (Supplementary Tables 3 and 4). However, the pattern of fold-change for thaumatin (a *PR5* gene) differed from the expression pattern of osmotin (a *PR5* gene; Table 1). *PR5* family proteins are divided into three subclasses including acidic, basic, and neutral proteins. The acidic class proteins are likely secreted into the extracellular spaces, and members of the basic class can be found in plant cell vacuoles [34]. Therefore, it is possible that osmotin-like *PR5* and thaumatin-like *PR5* are differentially regulated in pepper leaves and roots by *B. tabaci* infestation. Our data suggest that both the SA and JA signaling pathways are induced by *B. tabaci* infestation in both roots and leaves.

3.4. Promotion of root biomass after B. tabaci infestation

In order to investigate belowground physiological changes after *B. tabaci* feeding in aboveground, we determined to monitor the root morphology and root biomass. The data showed that whitefly infestation in leaves leaded to increasing root biomass (Fig. 3). In contrast, BTH treatment resulted in the inhibition of root biomass compared with the control. Moreover, BTH + whitefly treatment caused as similar level as the control (Fig. 3). The data prompted us to investigate the relationship between the increasing number of DEGs in roots (Fig. 1) subjected to *B. tabaci* infestation compared with the control and the positive effect of *B. tabaci* infestation on root biomass. We hypothesize that the increase in root biomass by *B. tabaci* infestation involves the allocation of active nutrients from leaves to roots and/or from soil to roots. In fact, foliar herbivores can elicit the allocation of nutritional reserves (i.e., sugars) to

Table 2
Highly upregulated transporter and auxin-response genes in roots subjected to *B. tabaci* infestation.

Category	SEQ ID	Functional annotations	Microarray (log_2 FC)			qRT-PCR (log_2 FC)		
			WF	BTH	WF + BTH	WF	BTH	WF + BTH
Root								
Transporter	PEPPERS0013216	ATP-binding cassette (ABC) transporter	3.06	0.45	2.09	5.72	1.33	3.98
	PEPPERS0016401	Peptide transporter	2.46	1.51	2.36	3.20	1.03	0.56
	PEPPERS0016508	Zinc transporter protein	1.95	0.40	1.40	3.88	0.25	1.75
	PEPPERS0002518	Phosphate transporter	1.95	1.11	1.09	3.06	1.54	1.18
Auxin	PEPPERS0009964	Auxin-responsive GH3-like	1.88	0.63	0.68	3.13	0.82	1.30
	PEPPERS0018818	Auxin-responsive protein	1.62	0.95	1.38	2.23	0.60	1.32

FC, fold change.
The numbers on the table indicate the fold change ratio by each treatment when compared with control treatment using log_2 value.

roots, improve the quality of roots, and facilitate the attraction of phytoparasitic nematodes to the plant [35].

To help explain why *B. tabaci* and BTH application affect root biomass and recruitment of beneficial microbes, we focused on plant hormones that regulate root growth (i.e., auxin) and plant transporters that modulate the levels of any nutrient from our microarray datat. We searched for genes that exhibited high fold-change ratios in response to *B. tabaci* and lower fold-change ratios in response to BTH treatment and were categorized as auxin-response and transporters. We identified six such genes, which are listed in Table 2. Four transporters, including ATP-binding cassette (ABC) transporter, peptide transporter, zinc transporter, and phosphate transporter were chosen based on their fold-change patterns. Additionally two auxin-responsive genes were selected. These six genes exhibited quite high fold-change levels in response to *B. tabaci* infestation and low fold-change levels in response to BTH. As expected, an intermediate level of fold-change was observed by BTH + whitefly treatment for all six genes (Table 2). The fold-change patterns for each candidate gene were confirmed by qRT-PCR (Table 2, far right column). Although beneficial microbes are attracted to whitefly-infested roots, the key molecules or signals in root exudates that function in this process are still unknown. Overall, our data indicate that aboveground *B. tabaci* infestation may lead to the accumulation of nutrient molecules (i.e., minerals, phosphate, and peptides) and plant hormone in roots from aboveground plant parts and/or from the soil environment by strongly inducing the overexpression of transporters, leading to an increase in root biomass.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.11.105.

References

[1] C.M.J. Pieterse, A. Leon-Reyes, Ent. S. Van der, S.C.M. Van Wees, Networking by small-molecules hormones in plant immunity, Nat. Chem. Biol. 5 (2009) 308–316.
[2] J. Wu, I.T. Baldwin, New insights into plant responses to the attack from insect herbivores, Annu. Rev. Genet. 44 (2010) 1–24.
[3] M. Rossi, F.L. Goggin, S.B. Milligan, I. Kaloshian, D.E. Ullman, V.M. Williamson, The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid, Proc. Natl. Acad. Sci. USA 95 (1998) 9750–9754.
[4] C.L. Casteel, L.L. Walling, T.D. Paine, Behavior and biology of the tomato psyllid, *Bactericerca cockerelli*, in response to the Mi-1.2 gene, Entomol. Exp. Appl. 121 (2006) 67–72.
[5] L.L. Walling, Avoiding effective defenses: strategies employed by phloem-feeding insects, Plant Physiol. 146 (2008) 859–866.
[6] K.S. Mysore, C.-M. Ryu, Nonhost resistance: how much do we know?, Trends Plant Sci 9 (2004) 97–104.
[7] D. Zeidler, U. Zähringer, I. Gerber, I. Dubery, T. Hartung, W. Bors, P. Hutzler, J. Durner, Innate immunity in *Arabidopsis thaliana*: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes, Proc. Natl. Acad. Sci. USA 101 (2004) 15811–15816.
[8] W.E. Durrant, X. Dong, Systemic acquired resistance, Annu. Rev. Phytopathol. 42 (2004) 185–209.
[9] H.-S. Yi, J.W. Yang, H.K. Choi, S.-Y. Ghim, C.-M. Ryu, Benzothiadiazole-elicited defense priming and systemic acquired resistance against bacterial and viral pathogens of pepper under field conditions, Plant Biotechnol. Rep. 6 (2012) 373–380.
[10] G.C. Song, C.-M. Ryu, Two volatile organic compounds trigger plant self-defense against a bacterial pathogens and a sucking insect in cucumber under open field conditions, Int. J. Mol. Sci. 14 (2013) 9803–9819.
[11] A.J. Koo, X. Gao, A.D. Jones, G.A. Howe, A rapid wound signal activates the systemic synthesis of bioactive jasmonates in *Arabidopsis*, Plant J. 59 (2009) 974–986.
[12] J.W. Yang, H.-S. Yi, H. Kim, B. Lee, S. Lee, S.-Y. Ghim, C.-M. Ryu, Whitefly infestation of pepper plants elicits defense responses against bacterial pathogens in leaves and roots and changes the below-ground miroflora, J. Ecol. 99 (2011) 46–56.
[13] H.-S. Yi, J.W. Yang, S.-Y. Ghim, C.-M. Ryu, A cry for help from leaf to root: recruiting rhizosphere microbes by aboveground insect attack leading to plant self-protection, Plant Signal. Behav. 6 (2011) 1192–1194.
[14] B. Lee, S. Lee, C.-M. Ryu, Foliar aphid feeding recruits rhizosphere bacteria and primes plant immunity against pathogenic and non-pathogenic bacteria in pepper, Ann. Bot. 110 (2012) 281–290.
[15] J. Glazebrook, Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens, Annu. Rev. Phytopathol. 43 (2005) 205–227.
[16] W.L. Yee, N.C. Toscano, C.C. Chu, T.J. Henneberry, R.L. Nichols, *Bemisia argentifolii* (Homoptera: Aleyrodidae) action thresholds and cotton photosynthesis, Environ. Entomol. 25 (1996) 1267–1273.
[17] T.J. Henneberry, L.F. Jech, D.L. Hendrix, T. Steele, *Bemisia argentifolii* (Homoptera: Aleyrodidae) honeydew and honeydew sugar relationships to sticky cotton Southwest, Entomology 25 (2002) 1–14.
[18] J. Chen, H.J. McAuslane, R.B. Carle, S.E. Webb, Impact of *Bemisia argentifolii* (Homoptera: Auchenorrhyncha: Aleyrodidae) infestation and squash silverleaf disorder on zucchini yield and quality, J. Econ. Entomol. 97 (2004) 2083–2094.
[19] L.L. Walling, The myriad plant responses to herbivores, J. Plant Growth Regul. 19 (2000) 195–216.
[20] I. Kaloshian, L.L. Walling, Hemipterans as plant pathogens, Annu. Rev. Phytopathol. 43 (2005) 491–521.
[21] S.I. Zarate, L.A. Kempema, L.L. Walling, Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses, Plant Physiol. 143 (2007) 866–875.
[22] R.T. Mayer, M. Inbar, C.L. McKenzie, R. Shatters, V. Borowicz, U. Albrecht, C.A. Powell, H. Doostdar, Multitrophic interactions of the silverleaf whitefly, host plants, competing herbivores, and phytopathogens, Arch. Insect Biochem. Physiol. 51 (2002) 151–169.
[23] L.A. Kempema, X. Cui, F.M. Holzer, L.L. Walling, Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids, Plant Physiol. 143 (2007) 849–865.
[24] D.P. Puthoff, R.M. Holzer, T.M. Perring, L.L. Walling, Tomato pathogenesis-related protein genes are expressed in response to *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B feeding, J. Chem. Ecol. 36 (2010) 1271–1285.
[25] M. Inbar, H. Doostdar, G.L. Leibee, R.T. Mayer, The role of plant rapidly induced responses in asymmetric interspecific interactions among insect herbivores, J. Chem. Ecol. 25 (1999) 1961–1979.
[26] W.T.G. van de Ven, C.S. LeVesque, T.M. Perring, L.L. Walling, Local and systemic changes in squash gene expression in response to silverleaf whitefly feeding, Plant Cell 12 (2000) 1409–1423.
[27] J.W. Yang, S.H. Yu, C.-M. Ryu, Priming of defense-related genes confers root-colonizing *Bacilli*-elicited induced systemic resistance in pepper, Plant Pathol. J. 25 (2009) 389–399.
[28] B.M. Bolstad, R.A. Irizarry, M. Astrand, T.P. Speed, A comparison of normalization methods for high density oligonucleotide array data based on variance and bias, Bioinformatics 19 (2003) 185–193.
[29] R.A. Irizarry, B.M. Bolstad, F. Collin, L.M. Cope, B. Hobbs, T.P. Speed, Summaries of Affymetrix GeneChip probe level data, Nucleic Acids Res. 31 (2003) e15.
[30] L.C. van Loon, M. Rep, C.M.J. Pieterse, Significance of inducible defense-related proteins in infected plants, Annu. Rev. Phytopathol. 44 (2006) 135–162.
[31] F.B. Abeles, R.P. Bosshart, L.E. Forrence, H.W. Habig, Preparation and purification of glucanase and chitinase from bean leaves, Plant Physiol. 47 (1971) 129–134.
[32] E. Mauch, B. Mauch-Mani, T. Boller, Antifungal hydrolases in pea tissue, Plant Physiol. 88 (1988) 936–942.
[33] Y.J. Kim, B.K. Hwang, Isolation of a basic 34-kilodalton beta-1,3-glucanase with inhibitory activity against *Phytophthora capsici* from pepper stems, Physiol. Mol. Plant Pathol. 50 (1997) 103–115.
[34] S. Anzlovar, M. Dermastia, The comparative analysis of osmotins and osmotin-like PR-5 proteins, Plant Biol. 5 (2003) 116–124.
[35] I. Kaplan, R. Halitschke, A. Kessler, B.J. Rehill, S. Sardanelli, R.F. Denno, Physiological integration of roots and shoots in plant defense strategies links above- and belowground herbivory, Ecol. Lett. 11 (2008) 841–851.