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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