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Original article

Identification and characterization of microRNA during *Bemisia tabaci* infestations in *Solanum lycopersicum* and *Solanum habrochaites*

Q1 *Ketao Wang*[a,b,1], *Xiaomei Su*[a,1], *Xia Cui*[a], *Yongchen Du*[a], *Shuaibin Zhang*[a,*], *and Jianchang Gao*[a,*]

[a] *Key Laboratory of Biology and Genetic Improvement of Horticultural Crops of the Ministry of Agriculture, Sino-Dutch Joint Laboratory of Horticultural Genomics, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China*
Q2 [b] *College of Horticulture, China Agricultural University, Beijing 100193, China*

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A B S T R A C T

MicroRNAs (miRNAs) are a class of small non-coding RNAs that can regulate target gene expression during many plant growth and development processes. In recent years, several studies identified the miRNAs involved in fruit development, leaf development, and abiotic responses in tomato. However, little is known about the miRNAs that respond to insect attack. Here, miRNAs were identified by high-throughput sequencing at different stages after infections by the whitefly *Bemisia tabaci* in *Solanum lycopersicum* and *Solanum habrochaites*, which are susceptible and resistant to whitefly, respectively. A total of 44 known miRNA families were identified, and 33 were shared between the two species. Among these, 13 miRNA families were newly reported in tomato. After strict filtering, some novel miRNAs were also discovered. The global expression patterns of the miRNAs exhibited were different between the two species, reflecting their different responses and resistance levels to whitefly attack. Some of the predicted target genes of differentially expressed miRNAs may be involved in responding to, and defending against, diseases and insects. Thus, plant miRNAs are important in the responses to, and resistance against, insects and provide a useful resource for further investigations into the mechanism of miRNA-mediated plant–insect interactions.

Keywords: miRNAs; Whitefly infection; *Solanum lycopersicum*; *Solanum habrochaites*; *Bemisia tabaci*; High-throughput sequencing

1. Introduction

Tomato is a greatly distributed vegetable crop worldwide that suffers from attack by a broad range of pathogens and insects. Among them, *Bemisia tabaci*, which is also known as the whitefly, is a serious pest, causing severe damage. The whitefly affects tomato production directly through phloem feeding or indirectly by transmitting more than 100 plant viruses, such as Tomato yellow leaf curl, Tomato mottle (Jones, 2003), and African cassava mosaic (Mehta et al., 1994; Moriones and Navas-Castillo, 2000; Maruthi et al., 2001). Exploiting host-plant resistance is a promising way to reduce pest-associated damage because of the whitefly's resistance to many insecticides.

Presently, transcriptome analyses in plants, especially in *Arabidopsis*, have indicated that a large number of genes are induced by insect attack. For example, LRR protein kinases and wall-associated kinases are highly expressed after induction by different insects (Dangl and Jones, 2001). The calcium-signaling

* Corresponding authors.
E-mail addresses: zhangshuaibin@caas.cn; gaojianchang@caas.cn
[1] These authors contributed equally to this work.
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pathway plays a significant role in *Arabidopsis*-phloem-feeding-insect (PFI) interactions. Genes encoding calcium-binding proteins, calmodulin (CaM)–like proteins, CaM-binding proteins, calcium-dependent protein kinases, calreticulins, and calcium-transporting ATPases are more abundant in leaves attacked by PFIs (Foyer et al., 2015). In addition, the numbers of genes responding to auxin, ethylene, jasmonic acid, and salicylic acid were significantly differentially expressed after insect attack (Kunkel and Brooks, 2002). Although numerous insect-responding genes have been identified, less is known about how they are regulated.

Gene expression is regulated at the transcriptional or post-transcriptional levels. Transcriptional regulation is mainly accomplished by transcription factors (TFs). The expression levels of 82 TFs belonging to 30 families were altered after infection of *Medicago truncatula* by the blue green aphid (*Acyrthosiphon kondoi*) (Gao et al., 2010). Additionally, microRNAs (miRNAs), a class of small non-coding RNAs, can regulate gene expression in transcriptional and post-transcriptional manners. MiRNAs play important roles in insect-related responses in plants. In *Arabidopsis*, aphid fecundity is significantly reduced in the mutants of DCL1 and ARGONAUTE1 (AGO1), which participate in miRNA processing, suggesting that the miRNA pathway is involved in *Arabidopsis* resistance to aphids (Kettles et al., 2013). In rice, hundreds of miRNAs are differentially expressed between the lines resistant and susceptible to the brown plant hopper (Wu et al., 2017). Among these differentially expressed miRNAs, Osa-miR531 was reported to target the mitogen-activated protein kinase family of genes, which are involved in plant innate immunity (Raghuram et al., 2014).

In tomato, some of the wild species, such as *Solanum pennellii*, *Solanum habrochaites*, *Solanum pimpinellifolium*, and *Solanum galapagense*, are resistant to whitefly. However, the regulatory mechanism of resistance to PFIs, especially the role that miRNAs play, is still unknown. In recent years, miRNAs responding to pathogens and other biotic stresses were identified in tomato using small RNA deep sequencing, and they indicated the important roles of miRNA in plant defense systems (Jin and Wu, 2015). Nevertheless, the kinds of miRNAs in plants that respond to attacks by insects, especially the whitefly, are poorly characterized. Here, a population of miRNAs and their expression profiles were identified using small RNA deep sequencing after whitefly attacks on *S. lycopersicum* and *S. habrochaites*, which are susceptible and resistant to whitefly, respectively. Our study will aid in better understanding the roles of miRNAs and their target genes in whitefly resistance and provide new genetic resources for breeding whitefly-resistant cultivars.

2. Materials and methods

2.1. Plant materials

Cultivated tomato (*S. lycopersicum*) line '9706' (abbreviated as 9706) and *S. habrochaites* accession 'PI 134,417' (abbreviated as PI) were planted in a greenhouse at 24 °C/18 °C (day/night) with 12 h of light per day. The plants were placed into a cage after budding. At least four plants were used in each treatment. The samples treatment was based on the previous study (Estrada-Hernández et al., 2009; Gao, 2011). In brief, the fifth fully expanded leaves of all plants in each group were harvested at 21 d after budding. For the 8-h samples, the plants were exposed to adult whiteflies for 8 h 21 d after budding. Then, the insects were removed, and the leaves were collected. For the 2-d samples, the plants were exposed to the adult whiteflies 19 d after budding. The whiteflies were removed after 8 h, and the leaves were harvested after 48 h. For the 21-d samples, the plants were exposed to adult whiteflies just after budding. The whiteflies were removed 8 h after treatment, and the plants were grown for 21 d without any change in the conditions. Then, the leaves were collected. For the control, the plants were grown under the same conditions but without insect exposure, and the leaves were harvested 21 d after budding. Samples were immediately frozen in liquid nitrogen and stored at − 80 °C until use.

2.2. Small RNA library construction and deep sequencing

To explore the roles of small RNAs during the entire whitefly (*Bemisia tabaci*) infection process, high-throughput sequencing was performed to detect small RNAs in the susceptible and resistant tomato species '9706' and 'PI', respectively. Leaves of the two tomato species were collected at 8 h (8H), 48 h (48H) and 21 d (21D) after whitefly infection to extract small RNAs and construct sequencing libraries. Leaves of uninfected plants of both species were used as controls (CKs). Total RNA was isolated using the RNAisoreagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. The quality and quantity of RNA were detected by denaturing agarose gel and optical density measurement, respectively. The small RNA fragments of 18–28 nucleotides (nt) were separated on 15% denaturing polyacrylamide gels, purified and then sequentially ligated to 5′ and 3′ adaptors. The RNA was subsequently converted to complementary DNA by RT-PCR. Finally, the purified DNA products were sequenced on a SOLEXA sequencer (Illumina) following the manufacturer's instructions, in the Beijing Genomics Institute (Shenzhen, China). The raw data were deposited in the NCBI sequence read archive under accession number SRP110555.

2.3. Bioinformatics analysis of small RNA sequences

The removal of low quality tags, trimming of adaptor sequences, and removal of contaminants formed by adaptor self-ligation were carried out first. The reads of 18–30 nt were chosen for further analysis. The clean small RNAs were filtered by removing sequences that matched known noncoding RNAs (tRNAs, rRNAs, snRNAs, and snoRNAs) in the Rfam (http://rfam.xfam.org) and SOL Genomics Network (http://solgenomics.net/) databases. To detect the miRNAs, the unique reads from all of the samples after filtering were combined into one library and then mapped to the tomato genome (SL2.50) using the Burrows–Wheeler Aligner tools software (Li and Durbin, 2009). The mapped sequences were used for further analyses.

2.4. Identification of known and novel miRNAs

Candidate miRNAs were predicted by MIREAP (http://sourceforge.net/projects/mireap/) with default parameters based on the plant miRNA's prediction criteria as described by Meyers et al., (2008). The maximal space between miRNA and miRNA* was 300 bp, and the minimum

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