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ORIGINAL PAPER

Root nematode infection enhances leaf defense against whitefly in tomato

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Abstract The foliar response to different herbivores sharing the same hosts is an important topic for the study of plant-insect interactions. Plants evolve local and systemic resistant strategies to cope with herbivores. Many researchers have characterized the mechanisms of leaf responses to insect infestation; however, the fact that roots serve as systemic resistance modulators to leaf herbivores has been widely ignored. Here, we report that tomato (*Solanum lycopersicum*) plants infected with southern root-knot nematodes (*Meloidogyne incognita*)—which feed on the roots to form nodules—enhanced leaf defenses against aboveground attackers, specifically, the whitefly (*Bemisia tabaci*). Our results show that nematode infection reduced the whitefly population abundance because of conferring a stronger SA-dependent defense pathway against whitefly than in tomato plants without nematode infection. Meanwhile, nematode-infected tomato plant also activated the foliar JA-dependent defense pathway at 4 h after whitefly infestation. However, the foliar JA-dependent defense under whitefly infestation alone was suppressed, with the JA content being nearly 30 % lower than that in tomato plants co-infected with nematodes and whiteflies. Furthermore, nematode infection significantly decreased the plant nitrogen concentration in leaves and roots. As a result, nematode infection reduced the number of whiteflies by enhancing foliar SA-dependent defense, activating JA-dependent defense and decreasing nitrogen nutrition. Our results suggest that underground nematode infection significantly enhances the defense ability of tomato plants against whitefly.

Keywords Southern root-knot nematode · *Bemisa tabaci* · Jasmonic acid · Salicylic acid · Nitrogen

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Introduction

In terrestrial ecosystems, plant-herbivore interactions are a pivotal subject of terrestrial ecology. Classical plant–insect studies tend to focus on leaf-insect interactions, and ignore the fact that roots serve as a modulator of systemic resistance to foliar insects. Roots, under the cover of earth, sustain plant growth and development. Roots associated with soil organisms influence foliar primary and secondary metabolism, which affect plant-insect interactions (Soler et al. 2012; Bezemer et al. 2005). Therefore, it is crucial to emphasize the role of roots in leaf resistance responses to insect attacks in order to fully understand the systemic defense aspects of plant-insect interactions.

That roots play an important role in regulating plant resistance to foliar herbivores is supported by growing evidence. One review proposes general patterns of root to foliar influence on herbivores that depend on the herbivores' feeding strategy, in which root-chewing insects influence leaf-chewing insects negatively and leaf-sucking insects positively (Soler et al. 2013). Two widely accepted hypotheses are used to explain the output of root to shoot signals. The nutritional-stress hypothesis, proposed by Masters et al. (1993), posits out that the improved performance of foliar insects accompanied by root insects is

caused by the insects feeding on leaves with elevated nutrition (Johnson et al. 2012). Roots with insect infestation are less able to absorb water and mineral nutrients, which leads to higher amino acid concentrations in leaves (Huberty and Denno 2004). Another is the induced defense hypothesis, which suggests that a plant with root insect infestation or one with root pathogen infection enhances foliar defenses against future damage by activating defense signaling (Liu et al. 2007; Jung et al. 2012; Annapurna et al. 2013; Weller et al. 2012). Further, roots with insect infestation not only affect the synthesis of foliar defense compounds such as nicotine and alkaloids (Hol et al. 2004), but also affect foliar phytohormone-dependent defense signaling (Erb et al. 2009a, 2012; Kaplan et al. 2008b).

Root-parasitic nematodes, which are one of the dominant tomato root pests, are free-living until the second larval stage and then finish their life cycle by parasitizing plant roots. Second-stage juvenile (J2) nematodes penetrate the root tips and construct feeding-sites called giant cells serving as a nutrient resource for nematodes (Davis et al. 2004). Microarray analysis shows that many different root metabolisms change during plant-nematode interactions, including cell wall metabolism, nutrient allocation and defense responses (Jammes et al. 2005). Nematode infection induces numerous defense protein syntheses such as pathogen-related proteins (PRs) and proteinase inhibitors (PIs), among others (Gheysen and Mitchum 2011). However, the role ascribed to phytohormone-dependent signaling in plant-nematode interactions is contradictory. Some studies indicate that nematode infection increases the accumulation of salicylic acid (SA) in root and the expression of *PR* genes around the infection site (Molinari and Loffredo 2006). Overexpression of *PR* genes reduces nematode infection rates in *Arabidopsis thaliana*. However, nematode infection occurs at a similar rate in NahG tomato plants, which are deficient in SA signaling (Bhattarai et al. 2008).

During the infestation period, current evidence shows that changes in plant metabolism occur not only at the root but also at distal tissues, such as leaves, that are far from the initial infection site (Kyndt et al. 2012). Metabolome analysis demonstrates that nematode infection influences the metabolism composition in systemic tissues, which increases shoot organic acid and sugar accumulation and reduces the amino acid level in *A. thaliana* (Hofmann et al. 2010). In addition to primary metabolism, nematode infection also affects foliar defense compounds such as glucosinolates, phenolics, phenylalanin ammo-nialyase (PAL) and foliar defense-related genes (Hamamouch et al. 2011). In turn, these changes to foliar metabolism caused by nematode infection affect foliar insects. Foliar herbivores' responses to root-feeding nematodes are variable; they depend not only on plant susceptibility but also on the types of insect mouthparts. The effect of root nematodes on foliar chewing insects is diverse, including positive, neutral and negative (Kaplan et al. 2008a, b; Wurst and Van der Putten 2007; van Dam et al. 2005). In contrast to chewing insects, most sucking insects, such as aphids, are negatively affected (Hol et al. 2010; Kaplan et al. 2011). The underlying mechanism through which nematode negatively affects phloem-feeding aphids is unclear. Recently, scientists have proposed the sink competition hypothesis and the induced defense hypothesis (Bezemer et al. 2005; Soler et al. 2012). The induced defense hypothesis proposes that nematode infection activates foliar phytohormone-dependent defense signals. This has been confirmed for systems involving in nematode and chewing insect interactions (Erb et al. 2008, 2009b); however, there is no experimental evidence that nematode infection reduces aphid populations by activating foliar phytohormone-dependent defense signaling.

Until now, numerous studies of above- and belowground insect interactions have focused on model insects or on native species. Many invasive and non-model insects that are of economic importance are widely ignored. Whitefly, a sucking insect that is an invasive tomato pest, is becoming the dominant pest in agricultural production in China and is already regarded as the most destructive agricultural invasive tomato pest (Dalton 2006). To develop populations in new habitats, the whitefly modulates the host plant's defenses, initially increasing the expression of the SA defense and suppressing the expression of jasmonic acid (JA) defense, which is an effective defense against whitefly. It also takes advantage of mutualistic relationships with geminivirus-tomato yellow leaf curl virus to usurp host resistance (Zarate et al. 2007; Zhang et al. 2012; Li et al. 2014).

In the field, root nematodes always occur together with whiteflies; the two organisms share the different parts of the tomato plants. Therefore, it is important to determine whether and how nematode infection influences whitefly infestation. To answer the question how root nematodes affect leaf-attacking herbivores-whiteflies, we choose the tomato as the host plant, the southern root-knot nematode as the root insect and the whitefly as the foliar insect to study how nematode-infected roots modulate leaf responses to leaf attacker-whiteflies. We proposed the following two hypotheses: (1) root nematodes reduce foliar nitrogen concentration; (2) root nematodes enhance leaf defenses, resulting in decreased whitefly fitness.

Materials and methods

Whitefly

The Q whitefly population was provided by Prof. Youjun Zhang (Department of Plant Protection, Institute of Vegetables and Flowers, Chinese Academy of Agricultural

Sciences, Beijing 100081, China). Whiteflies were initially transferred to cotton plants to maintain the population in separated cages in a greenhouse at 25 ± 2°C and 75 ± 10% relative humidity, with a photoperiod of 16: 8 h (L: D). The purity of the colony was controlled by sampling 30 adults and sequencing the *mtCO1* (mitochondrial cytochrome oxidase I) gene, which is a molecular marker that distinguishes the *B. tabaci* groups (De Barro et al. 2011). The cotton plants were grown in the greenhouse using the same conditions under which the herbivores were reared.

Host plants and nematode inoculation

Tomato seeds (Ying Fen) were purchased from the Beijing Vegetable Research Center. The seeds were placed in petri dishes containing 0.75% agar and kept under natural lighting at 25 °C for 2 days until germination. The buds were sown in small pots with an approximate volume of 1.5 l (1 plant per pot). The host plants were cultivated until the 2–3 leaf stage for the experiments.

Southern root-knot nematodes were collected from soil from tomato fields in Shandong, China. The nematode eggs were isolated using a sieve. A pure nematode culture was maintained on tomato plants in a glasshouse. After a 40-day incubation, we isolated the adult nematodes using the shallow-dish method (Hooper et al. 2005). The males and juveniles of root-knot nematode, which were killed and fixed by triethanolamine formalin solution, and the females, which were fixed by 2% formalin, were attached to semi-permanent slides for observing under microscopes. According to the morphological features (Eisenback, Hirschmann and Triantaphyllou 1980; Taylor and Netscher 1974), we identified the *M. incognita* using microscopes. The nematode suspension was collected after approximately 48 h. The 3–4 leaf tomato plants were inoculated with 15 ml of suspension containing approximately 2000 second-stage *M. incognita* juveniles per plant or mock inoculated with water. The nematode infection level was estimated by counting the galls and calculating the number of galls per gram of fresh root weight. After 2-week nematode infection, the nematode-infected tomato plants were used for the next experiments.

Whitefly population

Ten tomato plants with and without nematodes were infested with 5 pairs of newly emerging whiteflies. The plants with these adults were individually caged (using 80-mesh gauze). Ten other whitefly non-infested plants with and without nematode infection were also caged and served as controls. After a 24-h infestation, we swept the adults and left the eggs. After 25 days, the population on each plant was counted for all whitefly stages, including eggs, 1–4 nymphs and adults.

Time course experiment of whitefly infestation

For the time course experiment, tomato leaves were collected after 2, 4, 8, 12 and 24 h whitefly infestation on nematode infected and non-infected tomato plants. Thirty tomato plants with and without nematode infection were randomly selected for these experiments, and each was infested with five pairs of newly emerging whiteflies; six biological replicates were used in this experiment. Tomato leaves at 0 h were collected from six other whitefly-non-infested plants with and without nematode infection.

Hormone analysis

After whitefly infestation for 2, 4, 8, 12 and 24 h, leaves from each of the four replicates for each treatment were sampled. Approximately 300 mg of fresh leaves and roots was used to quantify the hormone content according to a modified method that was described previously (Guo et al. 2013). Plant tissues were homogenized in liquid nitrogen, and approximately 300 mg of fresh leaves was sealed in a 10-ml tube. Extraction buffer (0.5 ml) was added to each sample. Samples were agitated for 30 min at 4°C. Subsequently, 1 ml of dichloromethane was added, and the samples were agitated for another 30 min at 4 °C. The samples were then centrifuged at 13,000*g* for 10 min. After centrifugation, two phases formed, with the plant debris was located in the middle of the two layers. The aqueous phase was discarded, and approximately 1.5 ml of the lower layer was collected. Then, the samples were concentrated in a dry machine and re-solubilized in 200 µl of MeOH. Before transfer to a glass tube, the sample was filtered through a 5-mm filter. Next, 5 µl of the sample was injected into a column for analysis. The concentrations of the hormones were estimated using standard curves, which were constructed based on a gradient dilution of the reference standard.

After 2-week nematode infection, tomato leaves and roots with and without nematode infection were sampled. The hormone content in nematode-infected and nematode-uninfected tomato leaves and roots was quantified following the above method.

RNA extraction and real-time quantitative PCR (qPCR) analysis

After 2-week nematode infection, tomato leaves and roots with and without nematode infection were sampled for RNA extraction. After whitefly infestation for 2, 4, 8, 12 and 24 h, leaves from each treatment were also sampled for RNA extraction. All samples were stored at −70 °C for the following analysis.

Gene expression was measured using qPCR. Each treatment was replicated with four biological repeats and four

technical repeats. The RNA easy Mini Kit (Qiagen, Hilden, Germany) was used to isolate total RNA from the leaves or roots (0.05 g from samples stored at −70 °C), and 1 μg of RNA was used to generate cDNA. We determined the mRNA levels according to a modified method that has been described previously (Guo et al. 2013). Specific primers for each gene were designed from the expressed sequence tag sequences using PRIMER5 software (Table 1). The qPCR reactions were performed using the following protocol: a 20-μl total reaction volume including 10 μl of 2× SYBR Premix EX Taq^{TM} (Qiagen, Hilden, Germany) master mix, 5 mM of each gene-specific primer and 1 μl of cDNA template. Reactions were carried out using the Mx 3000P detection system (Stratagene, American), with the parameters set as described previously (Guo et al, 2013). We used *actin2* as the internal qPCR standard; every target gene's expression level was normalized to the tomato *actin2* gene (Yan et al. 2013). The fold changes in target gene expression were calculated using the $2^{-\Delta\Delta Ct}$ normalization method.

Statistical analyses

All statistical analyses were performed with the statistical package IBM SPSS Statistics 21.0. The nitrogen concentration in roots and leaves was analyzed using two-factor ANOVA. Effects of the sample date (time course), nematode and interaction of both (time × nematode) on plant hormones and marker genes were tested with repeated measures of the general lineal model. Other analyses were performed using t-tests.

Results

Nematode infection decreases the population abundance of whiteflies

Tomato plants without nematode infection were more favorable for the whitefly populations. Nematode infection reduced the number of whiteflies on the tomato plants by nearly 40 % compared with plants without nematode infection (Fig. 1, $P = 0.002$).

Nematode infection negatively affects the growth traits and nitrogen concentration of tomato plants

Nematode infection significantly reduced the plant height. The height of nematode-infected plants was 6 cm shorter than that in nematode-uninfected plants (Fig. 2a, $***P < 0.001$). Nematode infection also decreased the fresh weight of shoots by 33.6% (Fig. 2b, $P < 0.001$). The tomato plant photosynthetic rate was also decreased in nematode-infected plants (Fig. 2c, $P < 0.001$).

Furthermore, nematode infection significantly decreased the nitrogen concentration of roots in tomato plant not infested by whiteflies. This value was approximately 20 % lower than the nitrogen concentration of plants without nematode infection. However, the root nitrogen concentration increased to 1.77 ng/g in whitefly and nematode co-infested plants. Leaf nitrogen concentration was also reduced by nematode infection alone and whitefly infestation alone. The lowest leaf nitrogen concentration was found in plants co-infected with nematodes and whiteflies (Fig. 3).

Nematode infection stimulates the phytohormone-dependent induced defense of tomato plants

After nematode infection, the SA content in roots was elevated by nearly 50 % (Fig. 4a, $P = 0.010$). Expression of the marker gene *PR* in roots also increased nearly twofold over plants without nematode infection. The JA content and expression of *PI* in roots were similar between nematode-infected and nematode-uninfected tomato plants, which indicates that nematode infection alone was unable to activate the root JA-dependent signaling pathway (Fig. 4b, c).

Nematode infection increased the foliar SA content significantly. Without whitefly infestation (0 h), nematode infection increased the foliar SA content from 10 to 12 ng/g. We analyzed the foliar SA content at different time points (2, 4, 8, 12, 24 h) after whitefly infestation on nematode-infected and nematode-non-infected tomato plants. Whitefly infestation increased the foliar SA content and reached its peak after 8 h whitefly infestation and then

Table 1 Primers for the experiment

PI-II	Forward primer 5′-GAAAATCGTTAATTTATCCCACCG-3′
	Reverse primer 5′-ACATACAAACTTTCCATCTTTACCA-3′
PR-1	Forward primer 5′-GAGGGCAGCCGTGCAA-3′
	Reverse primer 5′-CACATTTTTCCACCAACACATTG-3′
LOX-D	Forward primer 5′-GACTGGTCCAAGTTCACGATCC-3′
	Reverse primer 5′-ATGTGCTGCCAATATAAATGGTTCC-3′
MYC-2	Forward primer 5′- AGCAGGAGCATCGGAAGAA-3′
	Reverse primer 5′-CCAAATCGGGCTGGAACTA-3′

Table 2 The effects of hour, nematode, and hour × nematode interaction on the tomato plant foliar defenses

Variable	Source	*F* value	*P* value
Foliar SA	Hour	211.068	<0.001***
	Nematode	26.145	<0.001***
	Hour × nematode	8.877	<0.001***
Foliar JA	Hour	2.489	0.760
	Nematode	106.683	<0.001***
	Hour × nematode	17.077	<0.001***
Foliar *PI*	Hour	51.675	<0.001***
	Nematode	808.437	<0.001***
	Hour × nematode	57.295	<0.001***
Foliar *PR*	Hour	413.109	<0.001***
	Nematode	134.202	<0.001***
	Hour × nematode	11.966	<0.001***
Foliar *LOX-D*	Hour	35.993	<0.001***
	Nematode	522.110	<0.001***
	Hour × nematode	82.240	<0.001***
Foliar *MYC-2*	Hour	3.014	0.021**
	Nematode	112.644	<0.001***
	Hour × nematode	8.027	<0.001***

Hour (0 h, 2 h, 4 h, 8 h, 12 h, and 24 h)

Nematode (nematode infection, no nematode infection)

*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

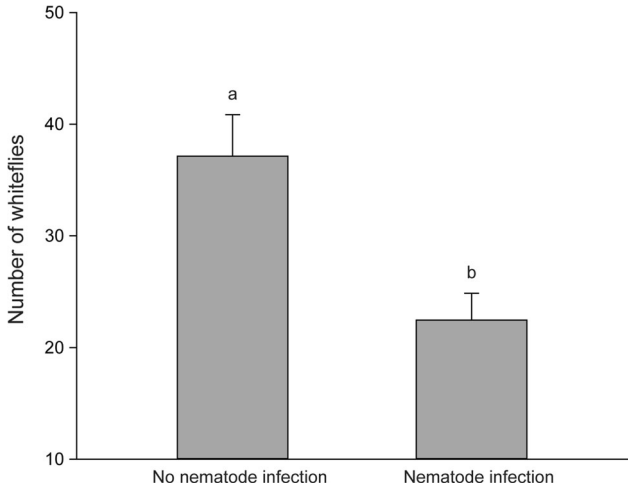

Fig. 1 Population abundance of whiteflies fed on tomato plants with and without nematode infection. Each value represents the average (±SE) of 23 replicates. Different *lowercase letters* indicate significant differences between the nematode infection groups

showed a slight decrease on two-type tomato plants. Moreover, foliar SA content at 2 and 24 h increased significantly more in nematode-infected tomato plants than that in nematode-uninfected plants. Expression of *PR*, which is a marker gene for the SA-dependent defense

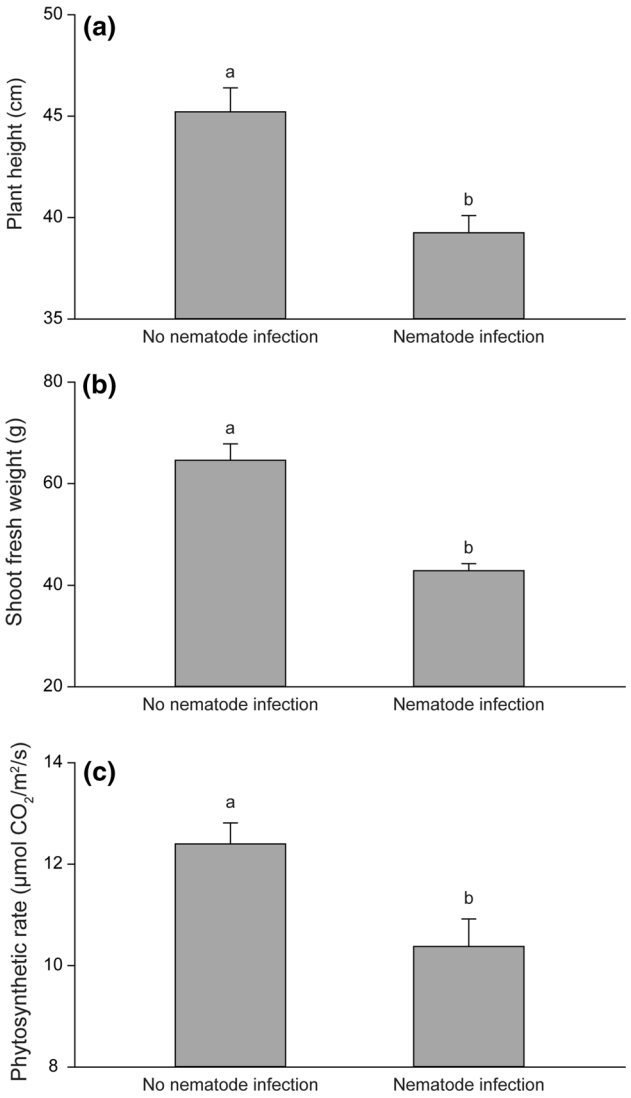

Fig. 2 Physiological traits of tomato plants grown with or without nematode infection: **a** plant height, **b** above-ground fresh weight and **c** photosynthesis. Each value represents the average (±SE) of 15 replicates. Different *lowercase letters* indicate significant differences between the nematode infection groups

pathway, was equivalent in nematode infected and nematode-non-infected tomato plants without whitefly infestation (0 h). However, whitefly infestation induced the expression of *PR* in nematode-non-infected tomato plants. The level of *PR* in nematode-infected tomato plants is nearly two-fold higher than that in nematode-non-infected plants. Therefore, whitefly infestation initiates stronger SA-dependent defense in nematode-infected tomato plants than in nematode-non-infected tomato plants (Figs. 5a, 6a; Table 2).

In contrast to SA resistance, foliar JA content did not increase after nematode infection. Furthermore, it is interesting that foliar JA signaling showed an absolutely

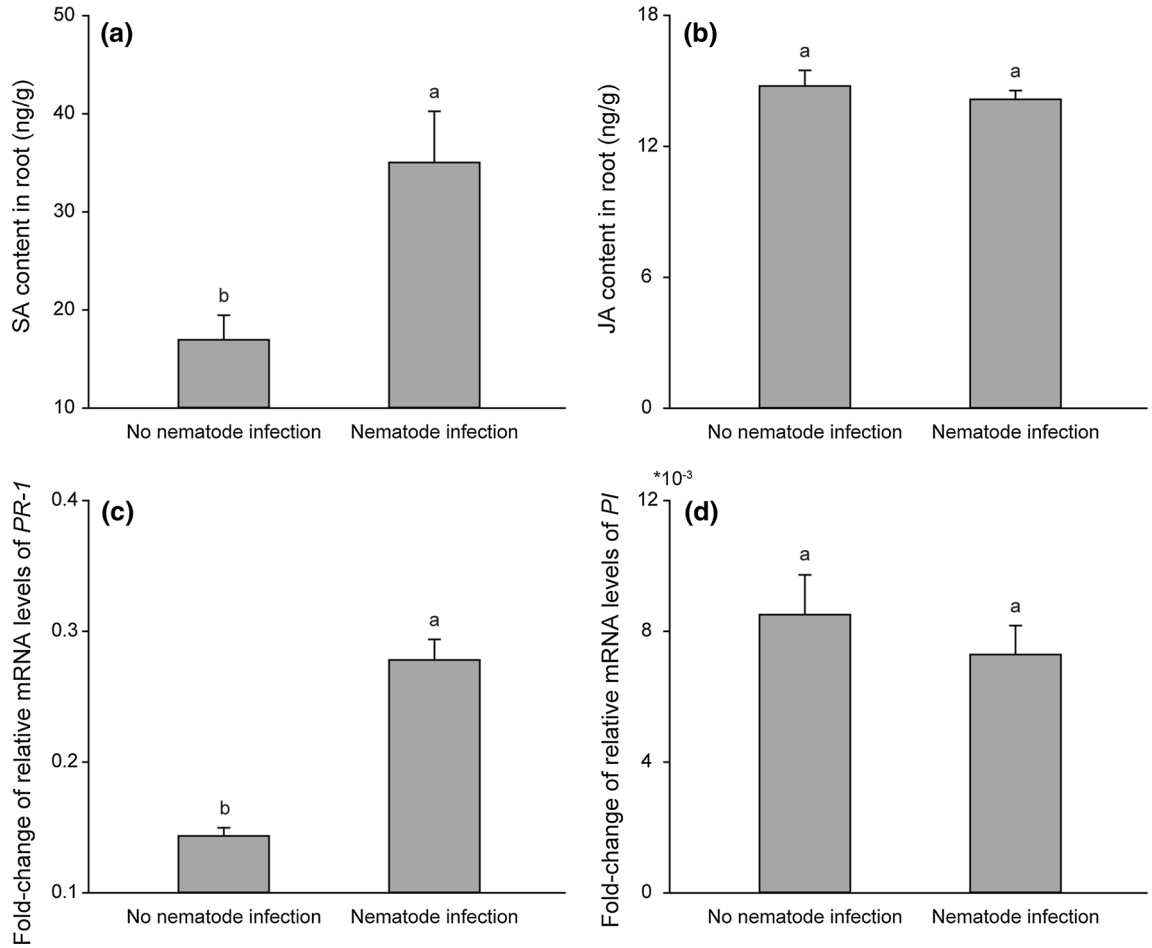

Fig. 3 Foliar and root nitrogen concentrations with and without nematode infection. Each value represents the average (±SE). 'Whitefly-' means tomato plants without whitefly infestation, and 'whitefly+' means tomato plants with whitefly infestation. Different *uppercase letters* indicate significant differences between the nematode infection groups. Different *lowercase letters* indicate significant differences between the whitefly infestation groups ($P < 0.05$)

opposite tendency in nematode-infected and nematode-uninfected plants with whitefly infestation. Whitefly infestation decreased the JA content nearly two fold at 24 h when compared with that at 0 h in nematode-non-infected tomato plants. Whitefly infestation, however, activated the foliar JA-dependent defense at 4 h in nematode-infected plants. Foliar JA content increased from 12 to 14 ng/g in nematode-infected plants, but dropped from 12 to 10 ng/g in nematode-non-infected plants. JA content in nematode-infected plants was approximately 30% higher than that in nematode-uninfected plants after 12 h whitefly infestation. *Lipoxygenase D* (*LOX-D*) and *PI*, which are marker genes of the JA defense pathway, were suppressed by whitefly infestation alone, but whitefly infestation induced the expression of foliar *LOX-D* and *PI* in nematode-infected plants, which were approximately 15- and 10-fold higher than those in nematode-non-infected plants, respectively (Figs. 5b, 6b–d; Table 2). Expression of foliar *MYC-2* differed from the expression of *LOX-D* and *PI* and increased significantly with nematode infection. Whitefly infestation significantly enhanced the expression of foliar *MYC-2* in nematode-infected plants compared with nematode-uninfected plants (Fig. 6d; Table 2). Hence, co-infection also activated foliar JA-dependent defense pathway.

Discussion

Plants evolve local and systemic resistance to insect attacks. Different parts of the plant, even when separated spatially, can perceive damage because of changes in plant physiology. Here, we show that nematodes contribute to anti-whitefly resistance in leaves as the whitefly population abundance was lower in tomato plants infected with southern root-knot nematodes. We prove that nematode-

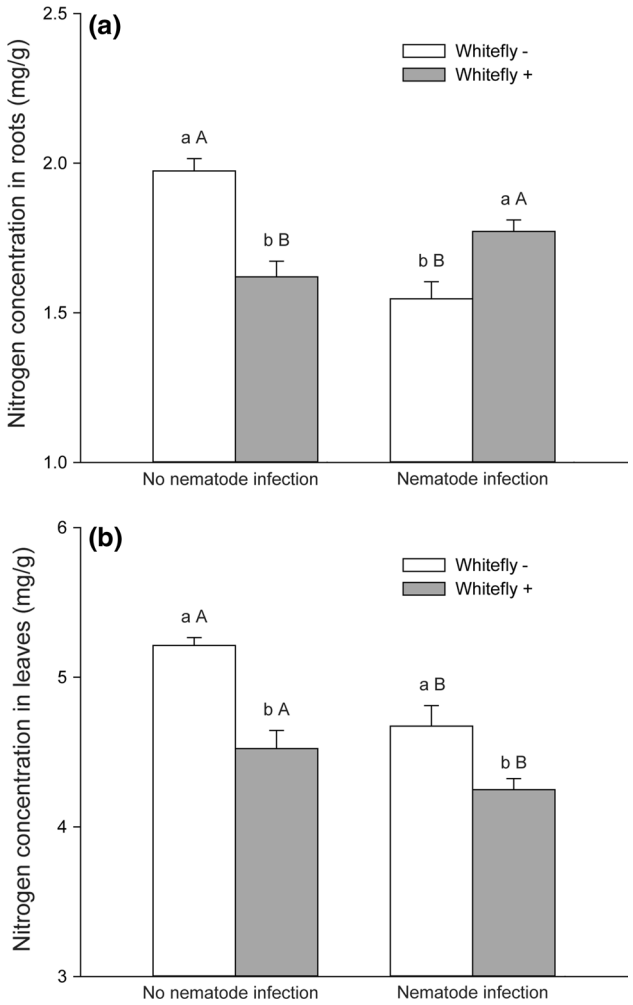

Fig. 4 Effect of nematode infection on root salicylic acid (SA) and jasmonic acid (JA) defense signaling in tomato plants: **a** SA content, **b** JA content, **c** PR and **d** PI. *Each value* represents the average (±SE). Different *lowercase letters* indicate significant differences between the nematode infection groups ($P < 0.05$)

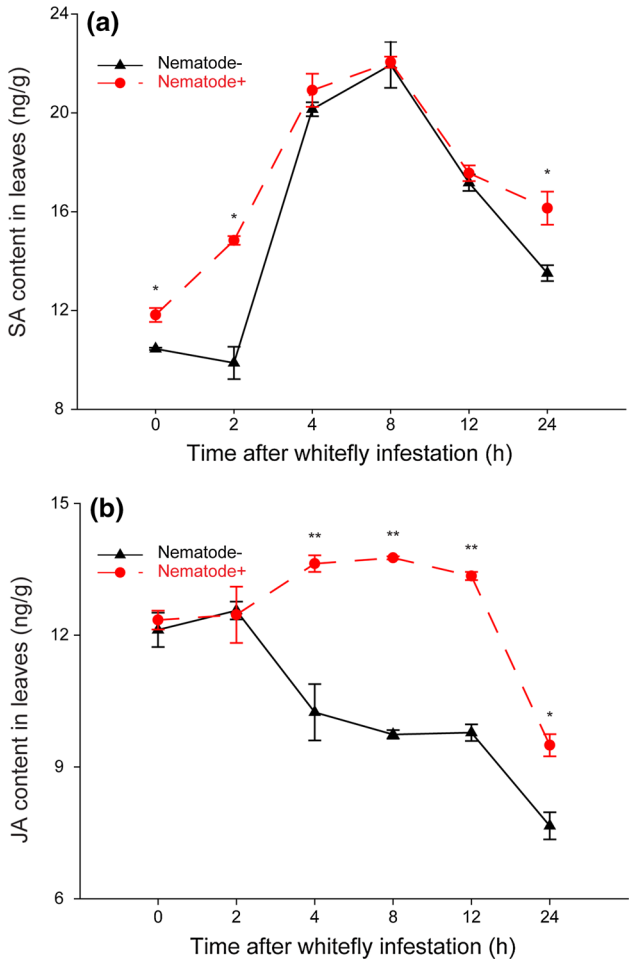

Fig. 5 Effect of whitefly infestation on foliar SA and JA levels in tomato plants with and without nematode infection. 'Nematode-' means tomato plants without nematode infection, and 'nematode+' means tomato plants with nematode infection. *Each value* represents the average (±SE). *Asterisks* indicate significant differences between the nematode infection groups ($P < 0.05$)

infected roots serve as activators of leaf resistance to whitefly infestation by reducing plant nitrogen nutrition and activating leaf phytohormone-dependent systemic defense signaling. Furthermore, nematode infection activated two different types of phytohormone-dependent systemic defense signaling—SAR and ISR. First, nematode infection enhanced the accumulation of SA in root and leaf simultaneously. SA-dependent defense in tomato plants co-infected with whiteflies and nematodes was higher than that in plants infected with whiteflies alone. Second, nematode infection alone did not stimulate the accumulation of JA in roots and leaves. However, whitefly infestation activated JA-dependent defense signaling in nematode-infected tomato plants—a response that is suppressed by whitefly infestation in nematode-uninfected tomato plants. These results indicate that roots serve as modulators of systemic resistance to foliar insects.

Leaves from tomato plants with southern root-knot nematode infection contain less nitrogen than those without nematode infection (Maung 1959). The results of the present study also show that nematodes reduce the plant nitrogen concentration, resulting in reduced height and fresh shoot weight. The N-relative quality of plants has been considered as a limiting factor of herbivore development and reproduction. The nitrogen concentration of leaves is particularly important for sucking insects such as aphids and whiteflies, which ingest their diets from phloem sap, an N-deficient tissue (Byrne and Miller 1990). The intrinsic rate of increase is increased for aphids feeding on barley plants with 8 mol m^{-3} nitrogen (Ponder et al. 2000). The plant nitrogen concentration also influences host resistance and susceptibility to herbivores. Tomato plants fertilized with various nitrogen concentrations sustain more whitefly eggs and attract more whiteflies for feeding

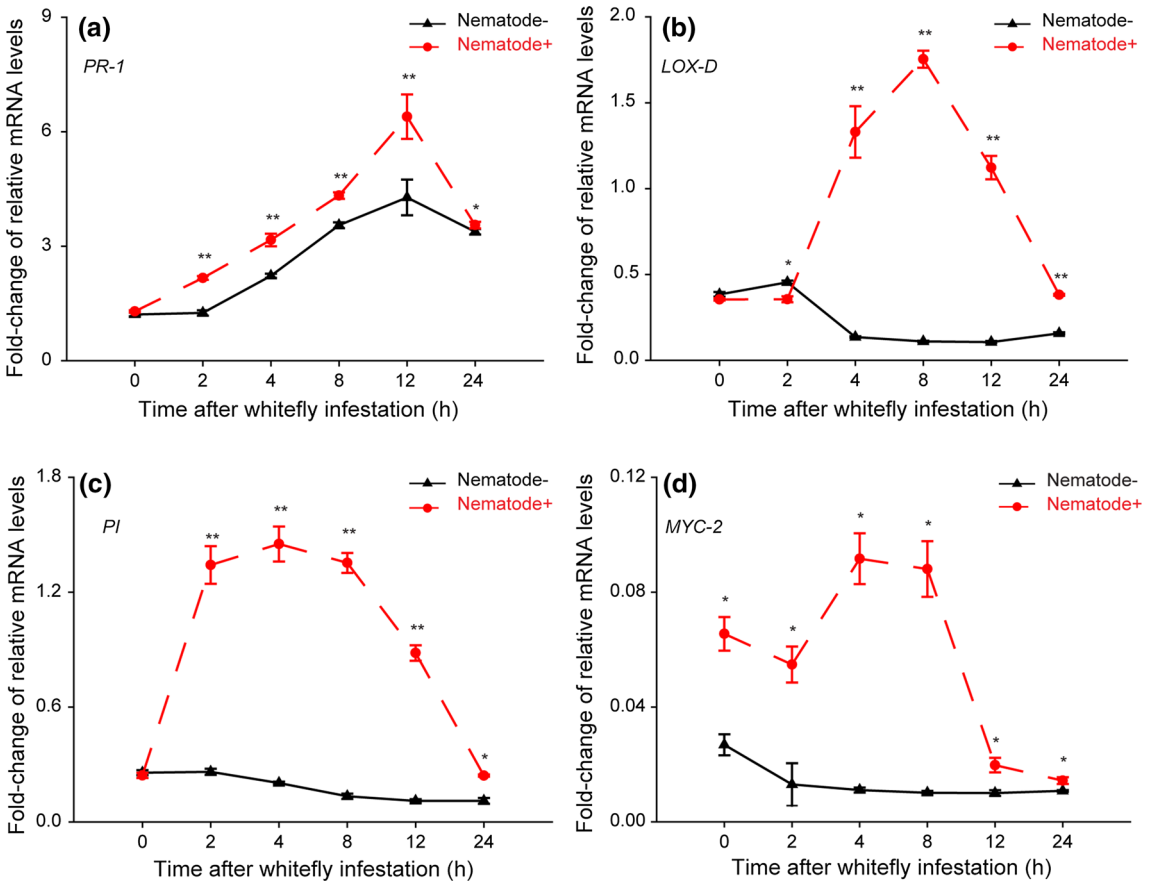

Fig. 6 Effect of whitefly infestation on foliar defense signaling marker genes in tomato plants with and without nematode infection: **a** *PR*, **b** *LOX-D*, **c** *PI* and **d** *MYC-2*. 'Nematode-' means tomato plants without nematode infection, and 'nematode+' means tomato plants with nematode infection. *Each value* represents the average (±SE). *Asterisks* indicate significant differences between the nematode infection groups ($P < 0.05$)

(Crafts-Brandner 2002). These results indicate that the reduction in nitrogen concentrations is an important aspect of the enhanced leaf resistance to whitefly infestation modulated by nematode infection.

To cope with insect attacks, plants have evolved a series of phytohormone-dependent defense signals that include SA-dependent signaling and JA-dependent signaling. We analyzed the phytohormone-dependent defense signals for both roots and leaves after nematode infection. In our experiments, nematode infection activated the root SA-dependent signaling and increased the expression of root *PR*. The phytohormone-dependent defense response to nematodes is limited (Nahar et al. 2011; Valerie and Cynthia 2003). A gene expression assay indicates that root nematodes upregulate many PR proteins such as chitinase and α-1,3-endoglucanase in root giant cells (Williamson and Hussey 1996; Gheysen and Fenoll 2002). This indicates that increased SA is required to alleviate tomato root nematode infection. The phytohormone-dependent defenses are not only induced locally but also systemically (Heil and Ton 2008). Nematode infection also enhanced foliar SA content and expression of the marker gene *PR* in our experiments. This response is similar to systemic acquired resistance (SAR), which enhances systemic foliar SA-dependent defense after local infections. SA undoubtedly is important in systemic leaf responses that modulate and enhance SA-dependent insect resistance (Vlot et al. 2009). SAR requires MeSA, which serves as a mobile signal from infected tissue to systemic tissue, to stimulate defense (Heil and Ton 2008; Park et al. 2007). MeSA transforms along the path from root to leaf, converting to SA, which is a basal defense mechanism to fight against whitefly infestation on healthy plants. Whitefly infestation induces foliar PR proteins such as β-1,3-glucanase, chitinase and peroxidase. In *A. thaliana* and whitefly interactions, many SA-dependent response genes (*PR1*, *PR5* and *PAD4*) accumulate locally (Inbar and Gerling 2008). In our study, whitefly infestation activated a stronger SA-dependent defense signaling with nematode infection than without nematode infection. This result agrees with SAR, which heightens SA-dependent defenses to subsequent attacks on systemic tissues. Therefore, an induced defense modulated by SA provides an underlying

bridge linking the two insects. Our results show that nematode infection decreased whitefly populations via two aspects: it not only upregulated the SA-dependent defense signal but also elicited the shoot SAR signal in foliage.

In addition to SA-dependent defense signaling, we also considered that the JA-dependent defense signaling modulates southern root-knot nematode and whitefly interactions. JA is traditionally regarded as a long-distance signaling candidate that triggers systemic protection (Schilmiller and Howe 2005; Wasternack et al. 2006; Lee and Howe 2003). Many root and leaf chewing insect interactions show that root caterpillars influence foliar herbivores by systemically enhancing the JA-dependent defense. JA-dependent defense is also involved in the plant response to whitefly infestation in leaves. Two JA-dependent defense genes, *SLW1* (silverleaf whitefly-induced 1) and *SLW3* (silverleaf whitefly-induced 3), are induced by whitefly infestation in squash (Van Wees et al. 2000). In *A. thaliana*, JA-deficient mutants accelerate the silverleaf whitefly development, while JA-activated mutants slow the silverleaf whitefly development (Zarate et al. 2007). In nematode-infected tomato plants, nematode infection did not induce the accumulation of JA in roots or leaves. Whitefly infestation stimulated JA-dependent defense signaling in nematode-infected tomato plants. In contrast, JA-dependent defense signaling was suppressed by whitefly infestation alone in nematode-non-infected tomato plants. This response is similar to induced systemic resistance (ISR), in which the JA-dependent defense is not elicited locally or systemically until the appearance of an attacker. In contrast to SAR, plant defenses are not activated in advance. However, defense signaling responses accumulate more quickly and strongly when the plant is infested by foliar insects (Van Wees et al. 2008; Conrath et al. 2006; Berendsen et al. 2012; Pieterse et al. 2014; Frost et al. 2008; Liu et al. 2007; Jung et al. 2012; Annapurna et al. 2013; Weller et al. 2012). Recently, the transcription factor MYC-2 has been found to play an important role in modulating ISR in systemic tissues. Pozo et al. (2008) indicate that promoters of ISR-primed genes enrich the MYC-2 binding site motif. In JIN1 plants, which are MYC-2 deficient, *Pseudomonas fluorescens* (*P. fluorescens*) is unable to activate ISR against *Pseudomonas syringae* (*P. syringae*) *pv. tomato DC3000* (Pozo et al. 2008). Whitefly infestation also suppress the JA-dependent signaling by interacting with MYC-2, which is a strategy used by phloem-feeding insects such as aphids and whiteflies to avoid effective plant resistance (Zarate et al. 2007; Zhang et al. 2013). In our studies, the expression of *MYC-2* differs from that of the other two genes (*LOX-D* and *PI*). Nematode infection induced the expression of *MYC-2* even without whitefly infestation in our experiment. This result indicated that MYC-2 could be an important functional element for regulating foliar ISR resistance to whitefly infestation accompanied by nematode infection.

Many cases show reciprocally antagonistic action between the JA-dependent and SA-dependent pathway (Koornneef and Pieterse 2008; Robert-Seilaniantz et al. 2011; Thaler et al. 2012; Alba et al. 2015). The antagonistic interaction between these two hormones seems to be dependent on the species, concentration of the two hormones and duration time of the two hormones' pathways (Mur et al. 2006; Koornneef et al. 2008; Wondafrash et al. 2013). Several works also suggest the synergistic effects between these two defense pathways, especially in the priming mechanism against *P. syringae* (Devadas et al. 2002; Halim et al. 2009; Mur et al. 2006; Scalschi et al. 2013). Priming means that primed plants, which are pre-infected with pathogens or herbivores, respond more quickly and strongly to secondary infection than do non-primed plants (Scalschi et al. 2013; Conrath 2011; Conrath et al. 2002; Van Wees et al. 2000). Hexanoic acid promotes the tomato resistance against *P. syringae* by priming the JA-dependent and SA-dependent pathways together. Meanwhile, in *A. thaliana*, Van Wees et al. (2000) proved that two types of priming mechanisms, the SAR (SA-dependent pathway) and ISR (JA-dependent pathway), are induced simultaneously by the foliar pathogen *P. syringae pv. tomato,* and there is no significant cross-talk between these two pathways. Moreover, combining SAR and ISR together improves the activity of disease control (Van Wees et al. 2000). This was consistent with our results showing that the SA and JA defenses were important for enhancing plant resistance against whitefly in nematode-infected tomato plants, where the whitefly population abundance was obviously decreased. Nematode pre-infection enhanced the leaf SA-dependent defense first. Moreover, the JA-dependent defense, which was suppressed under whitefly infestation only, was also initiated by whitefly infestation in tomato plants with nematode infection.

In the present experiment, we found that roots infected with southern root-knot nematode reduce whitefly fitness. Nematode infection activated two different types of phytohormone-dependent systemic defense signaling: the SA-dependent systemic defense (SAR), which was enhanced in leaves, and the JA-dependent systemic defense (ISR), which was activated after whitefly infestation. Furthermore, nematode infection reduced foliar nitrogen nutrition. These findings show that nematode-infected roots take part in regulating leaf defense strategies against foliar insects. These results also suggest that future crop pest control efforts should pay increased attention to interactions between insects that share the same host.

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