J. Agr. Sci. Tech. (2022) Vol. 24(1): 71-82

Systemic Induced Resistance to the Root-Knot Nematode in Tomato by Chemical Inducers

H. Charehgani[1*], A. Karegar[1], M. Djavaheri[1], and A. Niazi[2]

ABSTRACT

Systemic Acquired Resistance (SAR) as a management strategy for plant parasitic nematode is a state of resistance increased after a previous infection of plant to a biotic pathogen. Induction of SAR is accompanied by local and systemic enhancement of Salicylic Acid (SA). SA increase in plant is concomitant with PR1 expression. We examined the effect of three chemicals including SA, Abscisic Acid (ABA), and DL-*β*-Amino-*n*-Butyric Acid (BABA) on the root-knot nematode *Meloidogyne incognita* on tomato plants. The expression of *PR1* genes and 9-Cis-Epoxycarotenoid Dioxygenase (*NCED*) as markers for SAR and ABA-related activity genes was investigated in growth chamber conditions. Results showed that all elicitors reduced the population of nematode as compared to the control. Shoot length, shoot fresh and dry weight of nematode infected tomatoes pre-treated with BABA increased by 20, 25, and 8 % and number of eggs, galls, egg masses and reproduction factor decreased by 33, 18, 18, and 20%, respectively. All elicitors increased the expression of *PR1* and *NCED* genes in nematode infected tomato. These data suggest that SA, BABA and ABA activate similar defenses in tomato plants, which is partly SA- and ABA-related. SA, BABA, and ABA pretreated tomatoes infected with *M. incognita* trigger a SAR-response and lead to the control of the nematode under controlled conditions.

Keywords:, Abscisic acid, DL-β-amino-n-butyric acid, Gene expression, *Meloidogyne incognita*, Salicylic acid.

INTRODUCTION

Various types of plant resistance such as non-host resistance, *R*-gene mediated resistance and basal resistance have been mentioned for plant resistance against plant pathogens. Basal resistance is dependent on some plant hormones such as Ethylene (ET), Jasmonic Acid (JA) and Salicylic Acid (SA) (Verhagen *et al.*, 2006). Induced Resistance (IR) is part of basal resistance, which is not available in healthy plant. IR can be induced by limited pathogen infection, avirulent pathogens, beneficial non-pathogenic bacteria and fungi, and certain chemicals (Walters and Fountaine, 2009). One of the well-studied examples of IR is Systemic Acquired Resistance (SAR), which occurs following a localized infection in plants. Other types of IR are Induced Systemic Resistance (ISR) and *β*-Aminobutyricacid-IR (BABA-IR), which involves JA/ET. Following localized infection, SAR expands systemically and occurs away from the infection sites (Buonaurio *et al.*, 2009). Synthetic chemicals such as SA, JA, Acibenzolar-S-Methyl (ASM), 2,6-dichloroisonicotinic Acid (INA), Ethylene (ET), BABA or many other chemicals at levels that are not toxic can lead to the induction of local and systemic resistance

[1] Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.
[2] Biotechnology Research Center, College of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.
*Corresponding author; e-mail: H.charehgani@yu.ac.ir

[DOR: 20.1001.1.16807073.2022.24.1.11.1]

(Ryals *et al.*, 1996; Kuc 2001; Sanz-Alferez *et al.*, 2008). SAR is accompanied by local and systemic increase in SA. Expression of some pathogenesis-related genes such as *PR1*, *PR2* and *PR5* has been observed associated with SA-dependent defense responses (Van Loon, 1997; Mauch-Mani and Mauch, 2005). Hence, changes in the levels of SA affect *PR1* expression (Van Loon, 1997).

BABA is a synthetic amino acid, which induces resistance in plants (BABA-IR). Natural defenses and internal mechanisms of plant are used by this type of resistance (Buonaurio *et al.*, 2009). In this process, Abscisic Acid (ABA) and SA-dependent signaling pathways play an important role. ABA augmented formation of callose against pathogenic fungi and oomycetes (Ton *et al.*, 2005). Moreover, ABA leads to stomatal closure and improves plant tolerance to drought and salinity stress. *9-Cis-Epoxycarotenoid Dioxygenase* (*NCED*) gene involved in ABA biosynthesis. BABA-IR is effective against broad-spectrum of biotrophic, necrotic or hemibiotrophic pathogens, and even abiotic stress such as drought and salinity stress (Buonaurio *et al.*, 2009).

Root-knot nematodes (*Meloidogyne* spp.) have been known as the most important plant pathogenic nematodes all over the world. They are one of the main obstacles to provide enough food in many developing countries. Based on different reports, root-knot nematodes cause reduction in tomato (*Lycopersicon esculentum* Mill.) yield over 50% (Natarajan *et al.*, 2006).

In different studies, foliar spray and soil-drenching with chemical inducers such as SA, INA, MeJA, Gamma-Aminobutyric Acid (GABA), BABA, ASM and Methyl Salicylate (MeSA) have been done in plants infected with root-knot nematodes. Molinari *et al*. (2014) showed *PR1* gene was up-regulated in roots and shoots of SA-treated tomato plants and infected with *M. incognita*. Foliar spray of SA on cowpea infected with *M. incognita* significantly reduced the reproduction factor of nematode and induced expression and accumulation of PR1 protein in the leaves (Nandi *et al*., 2002). Meller *et al*. (2018) showed *PR1* gene was up-regulated in leaves of potato plants pre-treated with BABA. Foliar spray of BABA inhibited development of *M. javanica* on oat (Oka *et al*., 1997). In many cases, they reduced nematode population (Fatemy *et al.*, 2012), increased induced resistance (Mohamed, 2010; Sanz-Alférez *et al.*, 2008) and increased the activity of Reactive Oxygen Species (ROS) scavenging enzymes (Sahebani and Hadavi, 2009; Sahebani *et al.*, 2011). Brueske (1980) showed an increase in Phenylalanine Ammonia-Lyase (PAL) and polyphenol oxidase activities in tomato plants infected with *M. incognita*. PAL plays a key role in regulation of phenylpropanoid production in plants. Increases in the H_2O_2 accumulation, induction of defense enzymes involved in the phenylpropanoid pathway (Nandi *et al*., 2003), and scavenging reactive oxygen species i.e., guaiacol peroxidase, polyphenol oxidase, catalase (Sahebani and Hadavi, 2009), and accumulation of phenolics and PR proteins would have contributed to the control of root knot nematodes (Anita *et al*., 2004). PAL has been considered as a part of defense mechanism in plants against biotic and abiotic stress (Peiser *et al*., 1998).

The objective of the present study was to investigate the effect of SA, ABA and BABA on the root-knot nematode *M. incognita* on tomato plants, and expression of *PR1* and *NCED* genes as markers for SAR and ABA-related activity genes.

MATERIALS AND METHODS

Plant and Nematode Materials

To obtain *M. incognita* second stage Juveniles (J2s), the nematode population originally from Khorasan Province, Iran (identified by Katooli *et al*. (2020) based on the study of perineal pattern and Inc-14 primer), was maintained in a greenhouse in Shiraz (Iran) on susceptible tomato (cv.

[DOR: 20.1001.1.16807073.2022.24.1.11.1]

Early-Urbana) at 27±5°C. To do this, the infected roots were washed with water, cut into small pieces (2-3 cm) and mixed with 0.5% NaOCl in a blender to cover the roots. Roots were chopped in a blender for 30 sec at low speed, followed by passing through a series of sieves including 80, 200 and 500 mesh inch^{-1}. Eggs on the 500 mesh sieve were gently washed by cold tap water to free them from NaOCl and collected into a Petri dish (Hussey and Barker, 1973). The eggs were stored in incubator at 28°C for four days to hatch (Baghaee Ravari and Mahdikhani Moghaddam, 2015).

Inducer Treatment and Pathogen Inoculation

Seeds of tomato (cv. Moneymaker) were sown in a mixture of one part sand and one part peat moss in 1 kg plastic pots. Plants were grown at 30/25°C the day/night temperatures with 16 hours light in a growth chamber. Tomato seedlings at four-leaf stage were sprayed (≈1 mL per plant) with 0.5 mM BABA or SA, 0.1 mM ABA or water as a control. These concentrations were selected according to biological effect on *M. incognita*, based on Charehgani *et al.* (2014) studies. After 24 hours, the seedlings were inoculated with ≈1,300 J2s in 1 mL of water. The experiments were carried out in completely randomized designs with five replications. Five replicates of each treatment were harvested 60 days after inoculation, nematode indices including eggs/root system as described by Hussey and Barker (1973), galls and egg masses/root system as described by Taylor and Sasser (1978), and reproduction factor and host growth indices including fresh shoot and root weight, dry shoot weight and shoot height were determined. The reproduction factor was calculated by dividing the final population density of the nematode by the initial nematode population density. Twenty-four, 48, and 72 hours after induction (0, 24 and 48 hours after nematode inoculation (hai)), leaf samples were collected. For this purpose, five samples of each plant, treated by SA, BABA, ABA, SA+Nematode, BABA+Nematode, ABA+Nematode and water (as control), at each time point were collected, frozen immediately in liquid nitrogen, and stored at −80°C. Total RNA was obtained with the total RNA isolation kit (DENAzist Asia. Co., Mashhad, Iran), following the manufacturer's instructions, after tissue extraction. Total RNA was isolated from each sample and random primers (Table 1) were used in the cDNA synthesis using the cDNA synthesis Kit (Fermentas Inc., Vilnius, Lithuania). The cDNA was used as the template for the qRT_PCR reaction to determine the expression of the *PR1* and *NCED* genes. For qRT_PCR reaction, SYBR Green qRT_PCR kit (BioEasy) was used. The quantification was accomplished using the Elongation factor1-alpha (*ef-1*) as an endogenous control. For qRT_PCR data, the relative expression of target gene was calculated based on the Threshold Cycle (CT) method. The CT for each sample was calculated using the Line-gene K software and the method by Larionov *et al.* (2005). When replicate PCRs are run on the same sample, it is more appropriate to average CT data before performing the $2^{\Delta\Delta CT}$ calculation. All qRT_PCR reactions were done in triplicate, on cDNA from two independent

Table 1. Specific primers employed in qRT_PCR reactions.

Primers	Sequences of oligonucleotides	
PR1-1b	Forward: *5´-GCC AGA CTA TAA CTA CGC TAC C-3´*	Reverse: *5´-GAA CCA CCA CCC ATT GTT GC-3´*
NCED	Forward: *5´-GCT TAT TTG GCT ATC GCT GAA C-3´*	Reverse: *5´-CGT CTT CTT CCT TGC TGT TGG-3´*
ef1	Forward: *5´-GGT TAA GAT GAT TCC CAC-3´*	Reverse: *5´-GAC AAC ACC AAC AGC AAC-3´*

[DOR: 20.1001.1.16807073.2022.24.1.11.1]

biological replicates and two technical replicates.

The determined mean CT values for both the target and internal control genes were used in equation: ΔT = CT (target gene-housekeeping gene) at Time x–CT (target gene-housekeeping genes) at Time 0. Time x represents time point after nematode treatment and Time 0 represents time point before treatment. The fold change ratios of target (*PR1* and *NCED*) genes were normalized to internal control (*ef-1*) gene and were calculated relative to the expression at time zero.

STATISTICAL ANALYSIS

Experiments were repeated twice in a Completely Randomized Design (CRD) with five replications for each treatment. The data of both trials were analyzed and, if the results were similar, data from the two trials were combined for statistical analysis. Data of plant growth parameters were subjected to a 4×2 (Inducers×Nematode) factorial Analysis Of Variance (factorial ANOVA). The data of nematode indices were subjected to a simple Analysis Of Variance (ANOVA) and the data of gene expression were subjected to a 2×2×3 (Inducers×Nematode×Time) factorial analysis of variance using SAS statistical software (SAS Institute, Cary, NC). Where the *F*-test showed significance difference at P< 0.01, treatment means were compared using Duncan's multiple range test.

RESULTS

Effect of Elicitors on Tomato and Nematode Development

Sixty days after foliar spray of the tomato plants with elicitors, no phytotoxic symptom on the leaves nor measurable differences on either shoot fresh and dry weight or shoot length of inoculated and non-inoculated tomato plants was observed, except for tomato plants treated by BABA and inoculated with nematodes, which showed significantly higher foliar height and weight than the inoculated controls (Table 2). There were fewer eggs, galls and egg masses on roots of plants sprayed with SA, BABA or ABA as compared with non-treated control plants. The reduction in mean number of eggs, galls, egg masses and nematode reproduction factor in the infected tomatoes pre-treated with BABA was up to 33, 18, 18, and 20%, respectively, 10, 10, 16, and 16% reduction with SA pre-treatment and 5, 6, 10, and 12% reduction with ABA pre-treatment, respectively, when compared with non-treated control plants (Table 3).

Table 2. Effect of foliar spray with different inducers on growth indices of non-inoculated and inoculated tomato plants (cv. Moneymaker) with *Meloidogyne incognita.*[a]

Inducers	Tomato plants	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)
Control	Non-inoculated	51.3 ± 2.4b	29 ± 1.2ab	4.2 ± 0.26a	4.12 ± 0.11bc
	Inoculated	49 ± 1.6b	26 ± 0.8b	3.6 ± 0.34b	4.26 ± 0.08abc
SA (0.5 mM)	Non-inoculated	53.6 ± 2ab	28.5 ± 1.4ab	4.3 ± 0.65a	3.8 ± 0.13c
	Inoculated	52 ± 2.2ab	31.6 ± 2.2ab	4.2 ± 0.14a	4.4 ± 0.05a
BABA (0.5 mM)	Non-inoculated	49.4 ± 1.8b	28.4 ± 1.6ab	4.1 ± 0.22a	4.1 ± 0.1bc
	Inoculated	61.5 ± 3.5a	34.5 ± 2.5a	3.9 ± 0.38a	4.3 ± 0.09ab
ABA (0.1 mM)	Non-inoculated	52.4 ± 2.6ab	32 ± 1.1ab	4.3 ± 0.44a	4.32 ± 0.1ab
	Inoculated	55.2 ± 3ab	30 ± 1.8ab	4.1 ± 0.42a	4.29 ± 0.12ab

[a] Data are presented as the mean±standard deviation of two independent trials with five replicates. Means in a column followed by the same letter(s) are not different according to Duncan's multiple range test (P< 0.01).

[DOR: 20.1001.1.16807073.2022.24.1.11.1]

Table 3. Effect of foliar spray with different inducers on number of eggs, galls and egg masses/root and reproduction factor of inoculated tomato plants (cv. Moneymaker) with *Meloidogyne incognita.*[a]

Inducers	Eggs/Root system	Galls/Root system	Egg masses/Root system	Reproduction factor
Control	9600 ± 240a	245 ± 12a	170 ± 9a	5 ± 0.2a
SA (0.5 mM)	8650 ± 320bc	210 ± 16b	148 ± 6b	4.2 ± 0.2bc
BABA (0.5 mM)	7400 ± 160c	200 ± 10b	140 ± 14b	4 ± 0.34c
ABA (0.1 mM)	9100 ± 360b	230 ± 9ab	153 ± 15b	4.4 ± 0.16b

[a] Data are presented as the mean±standard deviation of two independent trials with five replicates. Means in a column followed by the same letter(s) are not different according to Duncan's multiple range test *(P< 0.01).*

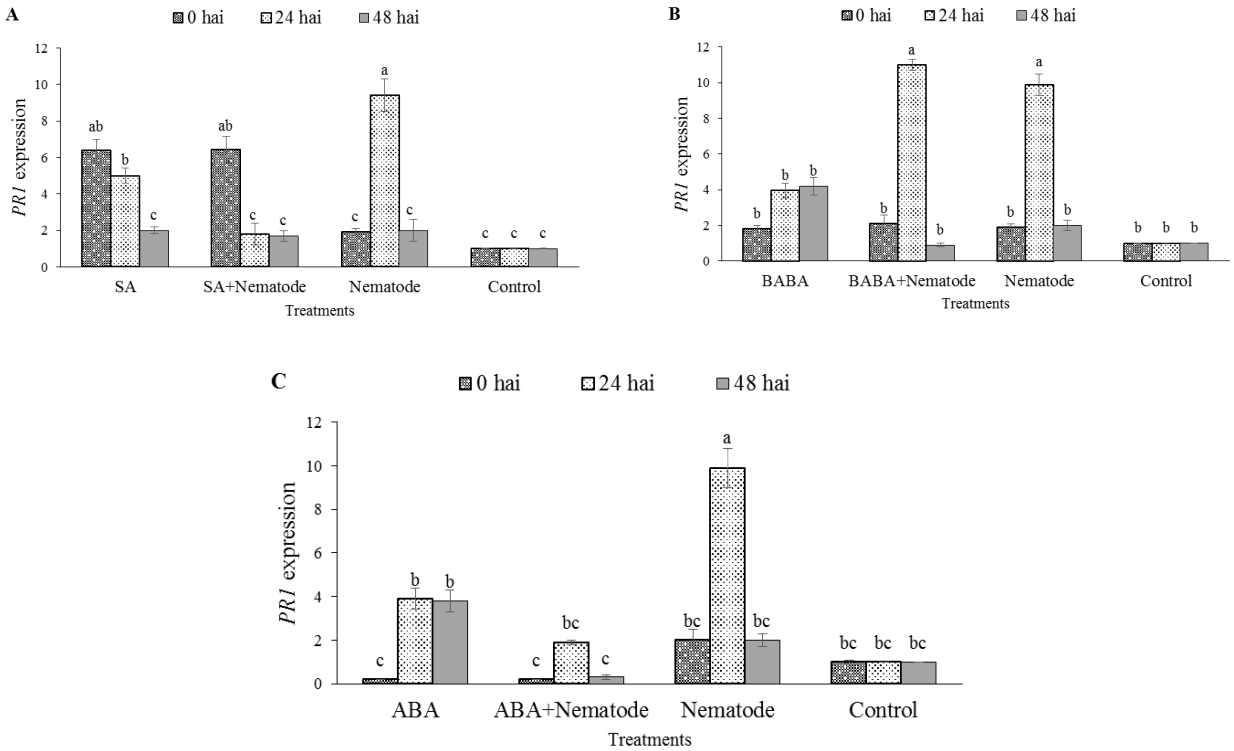

Figure 1. The *PR1* expression in the leaves of non-inoculated as well as inoculated tomato plants with *Meloidogyne incognita*, pretreated with (A) 0.5 mM Salicylic Acid (SA), (B) 0.5 mM DL-*β*-Amino-*n*-Butyric Acid (BABA) and (C) 0.1 mM Abscisic Acid (ABA) at 0, 24 and 48 hours after inoculation (hai). Bars represent the mean and standard error of mean of two independent trials with five replicates. Different letters indicate statistically significant differences (Duncan's multiple range test (P< 0.01).

Expression of *PR1* and *NCED* Genes

In the non-treated plants with elicitors, *PR1* and *NCED* expressions were induced on leaves at 24 hours after the nematode inoculation, and significantly decreased at 48 hours (Figure 1). *PR1* expression level increased after foliar spray of SA at the first 24 hours and gradually decreased at the 48 and 72 hours (Figure 1-A). The *PR1*

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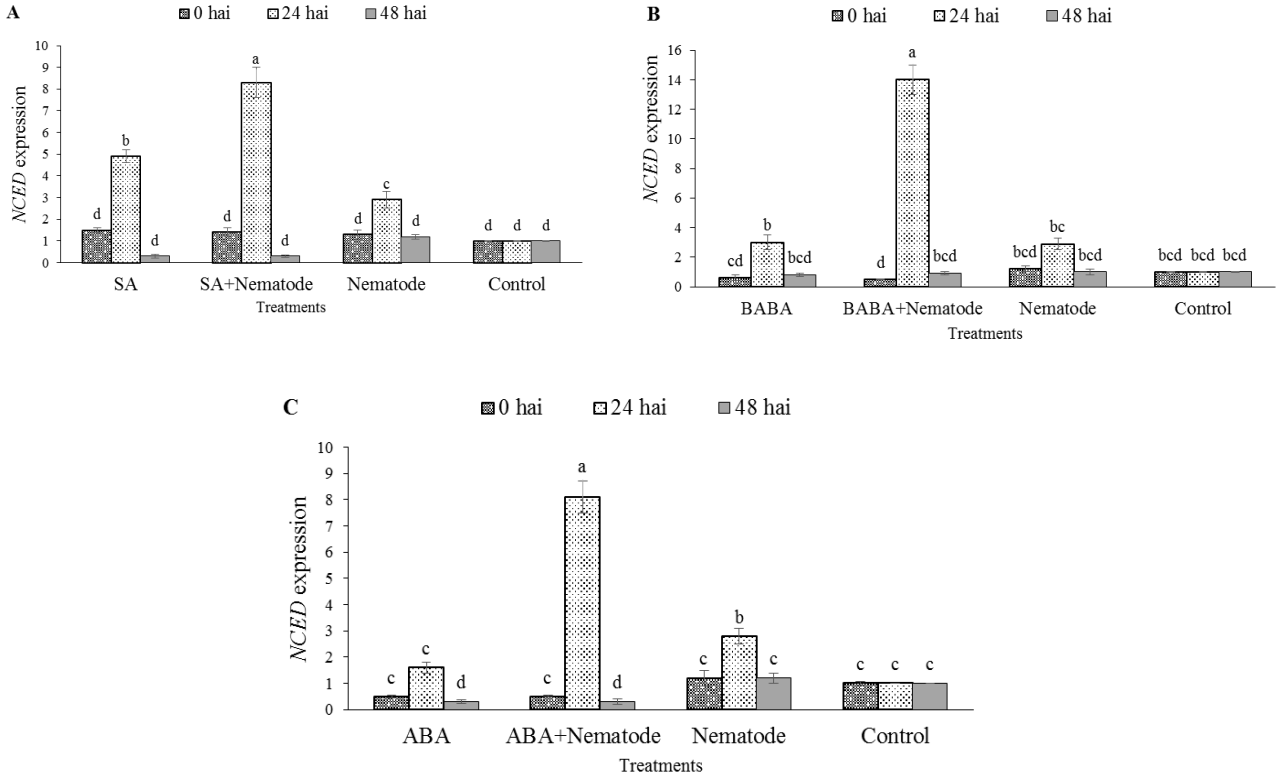

Figure 2. The *NCED* expression in the leaves of non-inoculated as well as inoculated tomato plants with *Meloidogyne incognita*, pretreated with (A) 0.5 mM Salicylic Acid (SA), (B) 0.5 mM DL-*β*-Amino-*n*-Butyric Acid (BABA) and (C) 0.1 mM Abscisic Acid (ABA) at 0, 24 and 48 hours after inoculation (hai). Bars represent the mean and standard error of mean of two independent trials with five replicates. Different letters indicate statistically significant differences (Duncan's multiple range test (P< 0.01).

expression was induced in BABA pre-treated (Figure 1-B) and ABA pre-treated plants (Figure 1-C) at 24 hours and significantly decreased at 48 hours after nematode inoculation. On the contrary, level of *PR1* expression decreased at the first 24 hours and significantly increased at the 48 and 72 hours in non-inoculated plants with ABA-pre-treatment (Figure 1-C). In nematode-inoculated plants treated with SA, BABA and ABA, level of *PR1* expression increased 6.3, 1.8 and 1.7-fold, 2, 11 and 0.9-fold, and 0.1, 1.8 and 0.2-fold at 0, 24, and 48 hours after the nematode inoculation, respectively, as compared to the control plants (Figure 1). According to Figure 2, level of *NCED* expression increased after foliar spray of the elicitor at 48 hours after treatment and decreased at 72 hours after treatment. In nematode inoculated plant treated with SA, BABA and ABA, level of *NCED* expression increased 1.5, 8.5 and 0.4-fold, 0.4, 14.2 and 0.9-fold, and 0.5, 8.2 and 0.3-fold at 0, 24, and 48 hours after the nematode inoculation, respectively, as compared to the control plants (Figure 2).

DISCUSSION

Several studies have shown that application of high concentrations of chemical resistance inducers on plants lead to phytotoxicity and even plant death. The reason is imposing additional burden to plant by activation of plant resistance

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responses. This problem reduces the overall fitness of plant (Molinari and Baser, 2010). As well as in some cases, low concentrations of chemical resistance inducers do not induce resistance in plants (Molinari and Baser 2010; Zhu and Tian, 2012). In this experiment, toxicity was not observed when the inducers were tested. Numerous studies have shown that high concentrations of chemical resistance inducers decrease plant fitness, cause toxicity, and decrease plant growth indices (Molinari and Baser, 2010). The present study revealed that foliar spraying with inducers caused reduction in nematode indices including the number of galls, eggs and egg masses per root system, the number of eggs in egg masses and nematode reproduction factor in comparison with the controls. In most cases, treated plants with BABA showed better effect on reducing nematode populations over the other two inducers. These results were consistent with other studies (Sahebani and Hadavi, 2009; Molinari and Baser, 2010; Mohamed, 2010). Previous studies have shown that protection against some plant pathogens by BABA functions via priming for Salicylic Acid (SA)-inducible defense mechanisms (Zimmerli *et al.*, 2000; Flors *et al.*, 2008). Ji *et al.* (2015) demonstrated that BABA treatment of rice plants inhibited *M. graminicola* penetration and led to delayed nematode and giant cell development. Resistance through ABA signaling pathways induced by BABA treatment causes increase of callose formation and ultimately increases resistance against pathogens (Jakab *et al.*, 2005; Ji *et al.*, 2015). Expression patterns of marker genes for the SA and ABA pathways in other pathogens indicate that both pathways always will be active following BABA treatment (Slaughter *et al.*, 2012). Present study revealed that level of *PR1* expression increased 9.75-fold at 48 hours after the nematode inoculation as compared to the control plants. This result suggests that salicylic acid accumulated in the early stages of *M. incognita* infected tomato and then increase in *PR1* expression occurred in the leaves tissue. Infection of plants by plant parasitic organisms including fungi, bacteria, viruses, nematodes, parasitic plants, and even insect herbivores nematodes leads to accumulation of SA in different parts of plants (Tripathi *et al.*, 2019). In the present study, it was demonstrated that nematode infection Increased expression of *PR1* in inoculated plants with different pathogens have been reported in other studies. Mohr and Cahill (2007) showed that SA induced and increased expression of *PR1* gene in *Pseudomonas syringae* pv. *syringae*-infected Arabidopsis. (Mohr and Cahill, 2007). They showed that SA induced and increased expression of *PR1* gene in *Pseudomonas syringae* pv. *syringae*-infected Arabidopsis. It has been recently revealed that *PR1* protein binds and sequesters host sterols that are required by the pathogens for their growth. The sterol-binding activity of *PR1* protein reveals the mode of action of an antimicrobial protein (Gamir *et al.*, 2017). The results of present study showed that *PR1* expression level decreased approximately 4.7-fold on the third day after inoculation with nematode over second day after nematode inoculation. It seems that the nematode suppressed the plant defense responses. These results also have been demonstrated in other studies (Puthoff *et al.*, 2003; Jammes *et al.*, 2005; Sanz-Alférez *et al.*, 2008).

Present study revealed that in nematode non-inoculated plants, level of *PR1* expression increased significantly in SA-pretreated plants over control plants at 48 and 72 hours after treatment. However, *PR1* expression showed downtrend at 48, 72 and 96 hours after foliar spraying with SA, such that there were no significant differences among SA-pretreated plants at 96 hours as compared with the control. According to results of Fan *et al.* (2009), level of *PR1* expression increased after SA treatment in Arabidopsis in the first and second days. Then, it gradually decreased in the third and fourth days. Molinari *et al.* (2014) showed up-regulation of *PR1* gene in shoots and

[DOR: 20.1001.1.16807073.2022.24.1.11.1]

roots of SA-treated tomato one day before *M. incognita* infection.

Level of *PR1* expression increased significantly in inoculated SA-pretreated plants over the control at the first 24 hours after inoculation (48 hours after SA-pretreatment). It seems that increased expression of *PR1* gene, which was induced by SA in the early stages of plant infection by nematode, was not suppressed. In contrast, level of *PR1* expression decreased significantly in the following day, and it reached the same level as the control. The reason can be attributed to suppression of *PR1* expression by effector proteins. These proteins are produced by nematode after deploying root tissue and early stages of giant cells formation (Smant and Jones, 2011; Dehghanian *et al.*, 2020).

Results of the present study revealed that BABA will increase markedly *PR1* expression and will activate SAR resistance pathways if the plant is attacked by nematodes. It can be inferred that despite pretreatment of plants with BABA, plant defense responses will not increase, while these defense responses will increase following infection by the nematodes. In conclusion, additional burden were not imposed on plant after BABA treatment. These results were consistent with results of Zimmerli *et al.* (2000). The results of BABA effects on reduction of nematode populations showed positive effect of this synthetic chemical on damage reduction of *M. incognita* on tomato through increased plant defense responses. Increased *PR1* gene expression due to pretreatment of plants with BABA shows that BABA-IR is dependent on SA signaling pathways. The relationship between BABA and SA-dependent signaling pathways to protect plants was proved by Ton *et al.* (2005). The plant hormones SA and ABA play pivotal roles in biotic and abiotic stresses, respectively. Several studies have shown that they individually or antagonistically act together in plant (Park *et al.*, 2007; Flors *et al.*, 2008; Yasuda *et al.*, 2008). Therefore, an accurate hormonal balance is essential for the survival of plants under stress conditions. Seo and Park (2010) showed positive interactions between ABA and SA signaling pathways. They showed that the *MYB96* transcription factor, which is ABA-dependent signaling pathways, induces plant resistance via an induction of SA biosynthesis and increase in pathogenesis-related proteins. Hence, *MYB96* transcription factor is an intermediate molecule of ABA-SA crosstalk. Another study has shown that external treatment of Arabidopsis with SA and ABA increased some plant metabolites more than treatment with these hormones individually. These results also showed ABA-SA crosstalk (Okamoto *et al.*, 2009). Present study revealed that level of *PR1* expression increased significantly at 48 and 72 hours after ABA-pretreatment of non-inoculated plants compared to 24 hours after ABA-pretreatment. These results were consistent with results of Seo and Park (2010). Pretreatment of plants with ABA has lower effect on induction of *PR1*-dependent defense responses than inoculated untreated plants. Probably, this is due to an antagonistic interaction between ABA and SA. This antagonistic interaction was consistent with results of Park *et al.* (2007), Flors *et al.* (2008) and Yasuda *et al.* (2008) studies. Of course, reduction of nematode populations by pretreatment of plants with ABA showed ABA had increased plant defense via another ways. ABA is activated as a result of response to stress in plants. Some studies also have shown ABA content increases during biotic stresses (Asselbergh *et al.*, 2008; Kyndt *et al.*, 2017). ABA is a key regulator of defense response in plants (Adie *et al.*, 2007). Levels of ABA increased rapidly during pathogen attack. (de Torres-Zabala *et al.*, 2007). On the other hand, it was noted that increase of SA biosynthesis due to increase of ABA and, then, enhancing of pathogenesis-related proteins leads to increased plant resistance (Seo and Park, 2010). Increased plant resistance limits nematode activity. Hence, probably, nematode decreases *NCED* expression 48 h after inoculation by effector proteins, which were secreted into feeding sites to suppress the plant defense responses.

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On the other hand, giant cell formation by nematode will cause water stress in plants. Therefore, plant tries to minimize nematode damage by enhancing the ABA level. Infection of plant with nematode increase genes involved in ABA biosynthesis such as *NCED*.

Results of Sakhabutdinova *et al.* (2003) showed that pretreatment of plants with SA increased ABA concentration in plants under drought stress and then ABA concentration decreased gradually over time. Since *NCED* gene is required for ABA biosynthesis, the results of this research is confirmed.

The results showed that pretreatment of plants with BABA led to increase in *NCED* expression in leaf tissue and, probably, ABA accumulation in plant. Different studies revealed that two Arabidopsis mutants impaired in either ABA biosynthesis (aba1) or ABA signaling (abi4) lost their ability to react to BABA treatment (Jakab *et al.*, 2005). Therefore, various studies and the present study demonstrate that BABA-IR is dependent on both ABA and SA signaling pathways (Ji *et al.*, 2015).

Overexpression of *NCED* gene increases ABA levels (Thompson *et al.*, 2000; Iuchi *et al.*, 2001). These results showed that ABA increase under stressed conditions, and no elevation of the essential precursor involved in ABA synthesis was observed under unstressed conditions, even in ABA-pretreated plants. These results were consistent with results of Fan *et al.* (2009).

In conclusion, population of *M. incognita* decreased following application of the inducers SA, BABA and ABA in nematode-inoculated tomato plants, and the pre-treated plants showed enhanced expression of *PR1* and *NCED* genes. This study confirmed that BABA-IR in the *Meloidogyne*-infected tomato depends on SA and ABA signaling pathways.

ACKNOWLEDGEMENTS

The research was supported by Shiraz University, Shiraz, Iran, and the authors thank the responsible authorities for their valuable assistance.

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القاء مقاومت سیستمیک علیه نماتد ریشه‌گرهی در گوجه‌فرنگی با استفاده از القاگرهای شیمیایی

ح. چاره‌گانی، ا. کارگر، م. جواهری و ع. نیازی

چکیده

مقاومت سیستمیک اکتسابی (SAR) به عنوان یک استراتژی مقاومتی علیه نماتدهای انگل گیاهی، حالتی از مقاومت است که بعد از یک آلودگی اولیه گیاه با بیمارگرهای گیاهی افزایش پیدا می‌کند. القاء SAR با افزایش موضعی و سیستمیک سالیسیلیک اسید (SA) همراه است. هم‌زمان با افزایش سالیسیلیک اسید در گیاه، بیان ژن *PR1* اتفاق می‌افتد. در این آزمایش اثر سه القاگر شیمیایی شامل SA، آبسیزیک اسید (ABA) و بتاآمینوبوتیریک اسید (BABA) روی گوجه‌فرنگی آلوده به نماتد ریشه‌گرهی *Meloidogyne incognita* بررسی شد. بیان ژن‌های *PR1* و *NCED* به ترتیب به‌عنوان ژن نشانگر SAR و ژن فعال کننده وابسته به ABA در شرایط اتاقک رشد مورد ارزیابی قرار گرفت. نتایج نشان داد که تمام القاگرها باعث کاهش جمعیت نماتد در مقایسه با شاهد شدند. طول، وزن تر و خشک شاخساره و وزن تر ریشه در گیاهان گوجه‌فرنگی تیمار شده با BABA در مقایسه با شاهد به ترتیب ۲۲، ۲۷، ۲۶ و ۱۴ درصد افزایش و تعداد تخم، گال، کیسه‌تخم و فاکتور تولیدمثل به ترتیب ۳۴، ۲۳، ۳۲ و ۳۴ درصد کاهش یافت. همه القاگرها باعث افزایش بیان ژن *PR1* و *NCED* در گوجه‌فرنگی‌های آلوده به نماتد شدند. این نتایج نشان داد که SA، ABA و BABA باعث فعال شدن مقاومت مشابهی در گیاهان گوجه‌فرنگی می‌شوند و SA و ABA تا حدودی با یکدیگر ارتباط دارند. پیش تیمار گوجه‌فرنگی آلوده به *M. incognita* با SA، ABA و BABA موجب فعال شدن واکنش SAR شده که باعث کنترل نماتد در شرایط کنترل شده می‌گردد.

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