https://doi.org/10.17221/130/2021-PPS

Evaluation of genetic and induced resistance phenomena in cucumbers against the root-knot nematode (*Meloidogyne incognita*)

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Citation: Gulzar R.M.A., Rehman A.U., Umar U.U.D., Shahid M., Khan M.F. (2022): Evaluation of genetic and induced resistance phenomena in cucumbers against the root-knot nematode (*Meloidogyne incognita*). Plant Protect. Sci., 58: 338–350.

Abstract: *Meloidogyne* spp. is an important pest of cucurbits in tunnel farming of vegetables in Pakistan. A cucumber germplasm was evaluated for resistance against the root-knot nematode (RKN, *Meloidogyne incognita*) based on the number of galls and egg masses recorded under glass house conditions. All the cucumber accessions showed varying responses towards the RKN inoculation. Out of the fifteen cucumber lines, two were found to be moderately susceptible to *M. incognita*, ten were susceptible while the other three were highly susceptible. For the management of the nematodes, resistance was induced in a highly susceptible cucumber accession (28294) by the application (both drench and foliar) of elicitors, i.e., salicylic acid (SA) and benzothiadiazole (BTH). The application of SA and BTH significantly enhanced the defence mechanism of the cucumber plants when compared to the control. Increased enzymatic activities in the cucumber plants as the result of the elicitor applications were determined through spectrophotometer to correlate the induced resistance. There was a significant increase in the enzymatic activities when compared to the control in the cucumber plants, which ultimately enhanced the resistance as there was a decrease in the number of galls and egg masses per plant. The enzymatic antioxidant activity was also found to increase in response to the nematode infection. Both SA and BTH were reported to play pivotal roles in inducing resistance in cucumber plants against *M. incognita*.

Keywords: *Cucumis sativus*; *M. incognita*; SAR; benzothiadiazole; salicylic acid; enzymatic activities

Root-knot nematode (RKN) disease infection dynamics is a serious problem in vegetable and fruit production (Qi et al. 2018) and significantly hampers the yield. The most important pathogens present in the root zone area of plants are nematodes that minimise the plant growth and production. *Meloidogyne* is the most common genus among the nematodes of greenhouse vegetable crops (Tileubayeva et al. 2021). Nematodes are devastating pests that infect most cultivated plant species causing considerable agricultural losses throughout the world (Eder et al. 2018). *Meloidogyne incognita* attacks the most economically important fruits and vegetable crops, including *Citrullus lanatus* (Bello et al. 2021), *Solanum lycopersicum* (Eder et al. 2021) and *Cucumis sativus* (Kayani et al. 2017) and causes enormous losses around the world (Jones et al. 2013). The intense economic importance of root-knot nematodes (*Meloidogyne* spp.) in agriculture has elicited the interest of a large number of researchers and institutions to study them. The total yield losses due to this nematode are about

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22–30% or above (Janati et al. 2018). In Western Anatolia of Turkey, *Meloidogyne* spp. caused yield losses of up to 80% in processing tomato-growing areas (Kaskavalci 2007). As a result, the *Meloidogyne* species are among the major emerging plant parasitic nematodes (Jones et al. 2013). They have been well reported from all the hemispheres, all the continents and almost every country in the world, in tropic or temperate climates, with a diverse climate, in mountains and deserts. The number of *Meloidogyne* spp. hosts has increased year on year and about 3 000 plants belonging to all the important families have been recorded as hosts of these nematodes (Abad et al. 2003).

A few of the common control methods for nematodes include, but are not limited to, the use of chemicals, cultural methods and planting resistant cultivars (Sasanelli et al. 2021). As far as chemicals are concerned, it has been observed that ten thousand tonnes of nematicides are required by EU farmers for the control of all genera of nematodes (Wright 1981). For this purpose, several nematicides have been commonly used by farmers throughout the world. Some of the commonly used nematicides are aldicarb, ethoprophos, oxamyl, fenamiphos and dazomet. Aldicarb being a nematicide belonging to the carbamate group is linked with several hazardous effects on the environment and human health which has led to the restriction of its use in the USA and other countries in the world. The growing health and environmental concerns associated with the use of chemical nematicides have led scientists to focus more on other ecologically sound alternatives, such as the use of resistant sources, which is considered a less expensive and more environmentally friendly approach. As scientists have tried to find new resistant sources against plant pathogens, plant hormones were found to be the most important role in initiating plant defence. Hormones, such as salicylic acid (SA), jasmonic acid, ethylene, brassinosteroids, and abscisic acid, have been reported to play major roles in plant defence against nematode infections (Nahar et al. 2011; Kammerhofer et al. 2015; Kyndt et al. 2017; Song et al. 2017). SA and benzothiadiazole (BTH) have been found to act as arbitrators for systemic acquired resistance (Molinary 2008). Usually, after the establishment of a primary infection, systemic acquired resistance (SAR) develops throughout the plant body which is considered as providing long-lasting and increased resistance in host plants against secondary infections caused by the same or different pathogens. Zinovieva (2013) found that SA applications may induce systemic resistance in cucumber plants against the root-knot nematode and may also enhance the activity of phenylalanine ammonia lyase (PAL). Furthermore, BTH has been noted to act as a priming agent in plant defence leading to a reduction in the penetration and development of the root-knot nematode *M. incognita* in susceptible tomato roots (Pasqua et al. 2018). These studies clearly show that SA and BTH have the potential to act as elicitors for induced resistance in cucumbers. The current study deals with evaluating the cucumber's resistance germplasm and the role of SA and BTH in the activation of defence mechanisms in cucumber plants. The research contributes to the knowledge regarding the behaviour of the root-knot nematode (RKN) in response to SAR mechanisms in plants.

MATERIAL AND METHODS

Evaluation of genetic resistance in cucumbers against the root-knot nematode

Source of planting materials and soil sterilisation procedure. The seeds of fifteen cucumber accessions: 28295, 32029, 32028, 32027, 28523, 32534, 32805, 29435, 28522, 28293, 29643, 32030, 28294, 32149 and 32031 were collected from the National Agricultural Research Centre, Islamabad, Pakistan. A fine mixture of dry fertile soil and dry sand was made in a ratio of 40% and 60%, respectively. Formalin was used for the sterilisation of the "sandy loam soil". The formalin, in a diluted form (1 : 320), was mixed into a small heap of soil. The soil was covered afterwards for seven days under a polythene sheet. Then, the soil was mixed thoroughly after removing the plastic sheet, left open for two days and then the pots were filled. Three seeds of fifteen cucumber accessions per earthen pot (containing 2 kg of soil) were sown separately. At the 5–6 leaf stage, the plants were thinned. All the unnecessary plants were removed and only one healthy/normal plant was kept in the centre of the pot. The root-knot nematode species were confirmed by examining the perineal pattern (Taylor & Nestscher 1974). The highly susceptible tomato variety named "Money Maker" was used for the mass rearing of the root-knot nematode (*M. incognita*) to obtain the maximum inoculum

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for the experiment. For this purpose, the seedlings of tomato plants (one month old) were transplanted into small earthen pots (3 kg of soil) containing the sandy loam soil sterilised with formalin and the temperature in the glasshouse was kept at 25 ± 2 °C. The effect of the induced resistance against the root-knot nematodes was evaluated in the highly susceptible cucumber plants of accession No. 28294. For this experiment, three cucumber seeds were sown per earthen pot containing 2 kg of soil. The pots were placed in a glasshouse in a completely randomized design at a temperature of 27 ± 2 °C. At the 5–6 leaf stage, the plants were thinned, keeping a single healthy plant in the centre of the pot.

Extraction of nematode eggs for inoculation. To extract or isolate the eggs of *M. incognita*, the roots of the tomato plants were cut into small pieces (2–3 cm) and then shaken well manually in a one litre flask with a lid containing 1% NaOCl solution (Hussey & Barker 1973). To separate the root fragments, this suspension was passed instantly through a 200-mesh sieve, while a 400-mesh sieve was used to collect the freed eggs. To allow the eggs to become free from the NaOCl, the collected eggs were rinsed with tap water for several minutes. Finally, the suspension containing eggs was kept in a large beaker. After a three to four hour wait, the excess water was removed from the top of the beaker when the eggs had settled at the bottom. Though this process was laborious, there was a minimum chance of losing any eggs. After this, 400-mesh sieves were used to collect the eggs. By repeating this process three times, the maximum numbers of eggs were collected. The egg suspension was poured onto an extraction tray at 25 ± 2 °C in an incubator and the juveniles were collected (Whitehead & Hemming 1965). The freshly hatched second stage juveniles (J2s) were standardised and concentrated.

Inoculation of the cucumber plant. The freshly hatched J2s of *M. incognita* were introduced in the pots of the individual plants at 2 000 J2/pot in the root zone area by making holes and then covering the holes with soil. The plants of each cultivar, which were not inoculated with J2s, served as the control treatment. The experiment was conducted twice in a completely randomised design in glasshouse conditions with six replicates. During the growth period, the cucumber plants were kept at 27 ± 2 °C. The pots were regularly watered with tap water and the inoculated cucumber plants were reared for eight weeks.

Data collection. After eight weeks of inoculation, the cucumber plants were gently uprooted from the earthen pots and washed in water. For drying and weighing purposes, the washed roots were blotted onto paper. The roots were washed carefully to be free from soil. For the purpose of counting the egg masses, a phloxine B (0.14–0.15 g/L of tap water) solution was used. The roots were stained with this solution by placing the dried roots into the phloxine B solution for 15 min (Holbrook et al. 1983) and the egg masses attained a pink-red colour. The data of the nematode parameters, like the number of galls/plant root, number of egg masses/root system, and the plant parameters of the fresh root weight and shoot weight of the host plants were recorded. The disease rating and resistance or susceptible response of the cucumber accessions to the nematode were evaluated by the modified 0–6 disease rating scale (Mukhtar et al. 2013).

Evaluation of the induced resistance against root-knot nematodes

To evaluate the effect of the induced resistance against the root-knot nematodes, the cucumber plants were thinned at the 5–6 leaf stage and a single healthy plant was kept in the centre of the pot. The J2s of *M. incognita* were inoculated in pots at 2 000 J2s/pot in the root zone area of each plant by making holes and backfilling with soil. One week after inoculation, the plants were treated with T1 (50 mg/L BTH, foliar); BTH drench (50 mg/L of water), T2; BTH foliar (50 mg/L), T3; BTH drench (100 mg/L), T4; BTH foliar (100 mg/L), T5; SA drench (10 mg/L), T6; SA foliar (10 mg/L), T7; SA foliar (1 mg/L), T8; SA drench (1 mg/L), T9; Control 1, untreated, but inoculated with nematodes and T10 (healthy control); Control 2, untreated, uninoculated plants. The experiment was conducted twice in a completely randomised design with six replicates. During the growth period, the cucumber plants, temperature was kept at 27 ± 2 °C. The plants were regularly watered and allowed to grow for eight weeks. The enzymatic activities of the superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), PAL and total phenolic contents in the cucumber leaves were assayed before and after the application treatments.

Preparation of enzyme extract. The leaf samples were collected two weeks after the application of the treatments. From each treated plant, 0.5 g of leaf tissues was crushed with a pre-chilled pestle

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and mortar in 1 mL of a chilled sodium-phosphate buffer (pH 7) for the POD, SOD and CAT assays. A chilled borate buffer (pH 8.7) was used to extract the enzymes to determine the PAL activity. Whereas, 80% ethyl alcohol was used for the extraction to determine the total phenolic contents. To determine the total protein contents, the leaves were crushed in a phosphate buffer (pH 7.2). The homogenate obtained from each extract was centrifuged at 12 000 rpm for 15 min at 4 °C. The supernatant was used as the enzyme source.

Assay for the POD activity (EC 1.11.1.7). The POD enzymatic activity was measured by the method given by Chance and Maehly (1955). Briefly, the final concentration of the reaction mixture was prepared by adding 33 mM of a potassium phosphate buffer (pH 6.1), 16 mM of guaiacol, 2 mM of H_2O_2 and 200 µL of the enzyme extract in a cuvette. The change in the absorbance of the reaction mixture was recorded at 470 nm every 30 s up to 3 minutes. The POD activity was calculated using the extinction coefficient 26.6/mM/cm. One unit of POD was defined as the amount of enzyme which consumes 1 µmol of H_2O_2/min at 25 °C and the activity was given in IU/mg of protein.

Assay for the SOD activity (EC 1.15.1.1). The efficiency in preventing the nitro blue tetrazole (NBT) photoreduction was used to determine the SOD activity. Three millilitres of reaction mixture were prepared with a final concentration of 0.1 mM EDTA, 0.02 mM of riboflavin, 130 mM of methionine, 100 µL of the enzyme extract, 0.75 mM of NBT, and 50 mM of the phosphate buffer having a pH of 7.8. The reaction mixture was exposed to a fluorescent lamp with 40-Watt power for 10 minutes. The reaction mixture was observed at 560 nm with a spectrophotometer (Biorad UV Vis 3000; Bio-Rad, USA). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate, and the activity was expressed in IU/mg of protein (Giannopolitis & Ries 1997).

Assay for the CAT activity (EC 1.11.1.6). The rate of decomposition of H_2O_2 was used to measure the CAT activity (Aebi 1984). Total volume of 2 mL of the reaction solution was prepared by adding 50 mM of potassium phosphate buffer (pH 7.0), 12 mM of H_2O_2 in a final concentration and 50 µL of the enzyme extract. The rate of decrease in the absorbance at 240 nm was observed for 3 min with a spectrophotometer (Biorad UV Vis 3000; Bio-Rad, USA). An extinction coefficient of 43.6/M/cm was used to calculate the CAT activity in µmol/min/mg protein or IU/mg of protein. One unit of CAT is defined as the amount of enzyme which decomposes 1 µmol of H_2O_2/min at a pH of 7.0 at 25 °C.

Assay for the PAL activity (E.C. 4.3.1.24). The rate of conversion of L-phenylalanine to trans-cinnamic acid was used to determine the PAL activity (Dickerson et al. 1984). The reaction mixture contained 400 µL of the enzyme extract, 500 µL of a 0.1 M borate buffer (pH 8.8), 5 µL of 12 mM L-phenylalanine. After incubation for 30 min at 30 °C, the absorbance of the solution was taken by the spectrophotometer at 290 nm. An extinction coefficient of 9 630/M/cm was used to calculate the amount of trans-cinnamic acid synthesised. The enzymatic activity was expressed as nmol trans-cinnamic acid/min/mg protein or IU/mg of protein.

Assay for the total phenolic contents. The total phenolic contents were measured by the method described by Zieslin and Ben Zaken (1993). The reaction solution was prepared by adding 1 mL of methanolic extract with 5 mL of distilled water and 250 µL of 1 N Folin-Ciocalteau reagent and kept at 25 °C. The absorbance of the developed blue colour was measured by the spectrophotometer at 725 nm. Gallic acid (GA) was used as the standard and the total phenolic contents were expressed as µg GA/mg protein.

Assay for the total soluble proteins. The Bradford method (Bradford 1976) was used to determine the total soluble proteins. Bovine serum albumin was used as the standard and the absorbance of the samples was recorded at 595 nm and the protein concentration was expressed in mg/g.

Statistical analysis

All the mean values of the enzymatic and nematode data were subjected to an analysis of variance (ANOVA) and compared by the least significant difference (LSD) test at a 0.05 level of significance using the SAS program (version 8.1).

RESULTS

Screening of cucumber accessions against the root-knot nematodes

Galling index. The fifteen screened cucumber cultivars varied widely in their susceptibility to *M. incognita.* Out of the fifteen, two accessions,

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i.e., 32028 and 32534, showed a moderately susceptible response with a maximum galling index (MGI) of 4. Three accessions, 28294, 32149 and 32805, were rated highly susceptible each having the MGI of 6 and a significantly high mean number of galls (156.33, 133.33 and 115.00, respectively), while ten accessions (28295, 32029, 32027, 28523, 32031, 29435, 28522, 28293, 29643 and 32030) were rated as being susceptible each recording a galling index of 5 which was not statistically ($P > 0.05$) different from each other.

No. of egg masses. The No. of egg masses produced varied significantly among the tested accessions. A higher No. of egg masses were formed on the highly susceptible accessions, i.e., 28294, 32805 and 32149, with an average No. of egg masses of 44.33, 41.66 and 39.66, respectively. On the susceptible accessions, the No. of egg masses were 32.66 and 30.66 on accession 32027 and 28293, respectively. Whereas on the moderately susceptible accessions, 32028 and 32534, the average No. of egg masses were found to be 15 and 15.33, respectively.

Root weight. The root weight varied significantly among all the tested accessions. Among the fifteen accessions, the accessions, i.e., 32028, 32534 and 29435, gained the minimum root weight with an MGI of 4 and were considered moderately susceptible to *M. incognita* and they were found to be statistically dissimilar ($P < 0.05$) from the others. Two accessions, viz. 28294 and 32149, gained the maximum root weight with an MGI of 6 and were considered highly susceptible to *M. incognita* and they were found at par statistically ($P < 0.05$) (Table 1).

Shoot weight. The maximum shoot weight was observed in accession 32028 followed by accessions 32030, 32534 and 28522 with a MGI of 4 and 5 and were considered moderately susceptible and susceptible, respectively, and were different statistically ($P < 0.05$). While accession, viz. 32149, had a minimum shoot weight with a MGI of 6 and was considered highly susceptible (Table 1).

Table 1. Modified rating scale for the assessment of level of resistance or susceptibility based on number of galls

Number of galls	Galling index	Resistance rating
0	0	immune
1–2	1	highly resistant
3–10	2	resistant
11–30	3	moderately resistant
31–70	4	moderately susceptible
71–100	5	susceptible
> 100	6	highly susceptible

Evaluation of the induced resistance in the cucumber crop against the root-knot nematode

The present study revealed that when the 20-day-old cucumber seedlings were infected with the J2s of *M. incognita* and the SA or BTH applications were given at different concentrations, the defence mechanism was activated which ultimately enhanced the SOD, POD, CAT, and PAL antioxidant enzymatic activities and also the total protein and phenol contents when compared with the non-treated plants. In response cucumber plant showed varied degree of induced resistance which was assessed by decreased MGI as compared to the control.

SOD activity. When the SA and BTH were applied to the cucumber plants in two different concentrations (50 mg/L and 100 mg/L), they notably induced changes in the SOD activity when compared to all the control plants. The SOD activity was at a maximum (219.35 IU/mg) after the nematode infestation when the BTH was applied as a drench treatment at 50 mg/L and the minimum SOD activity (142.57 IU/mg) was recorded when the BTH was applied as a drench treatment at 100 mg/L (Figure 1).

POD activity. The cucumber plants showed variable response in the case of the POD activity when treated with SA when compared to the control plants. The POD activity was found to be the maximum (1 095.21 IU/mg) when SA was applied as a foliar treatment at 10 mg/L. The minimum POD activity (191.16 IU/mg) was recorded when the SA was applied as a foliar treatment at 1 mg/L (Figure 2).

CAT activity. When the cucumber plants were treated with SA and BTH, the catalase activity varied considerably when compared to the control plants. The catalase activity was found to be the maximum (2 564.24 IU/mg) when the BTH was applied as a foliar treatment at 100 mg/L and found to be the minimum (876.49 IU/mg) when the SA was applied as a foliar treatment at 10 mg/L (Figure 3).

PAL activity. SA had significant effects on the PAL activity in the cucumber plants when compared to the control plants. The PAL activity was at a maximum (15.92 IU/mg) when the BTH was applied as a drench treatment at 50 mg/L after in-

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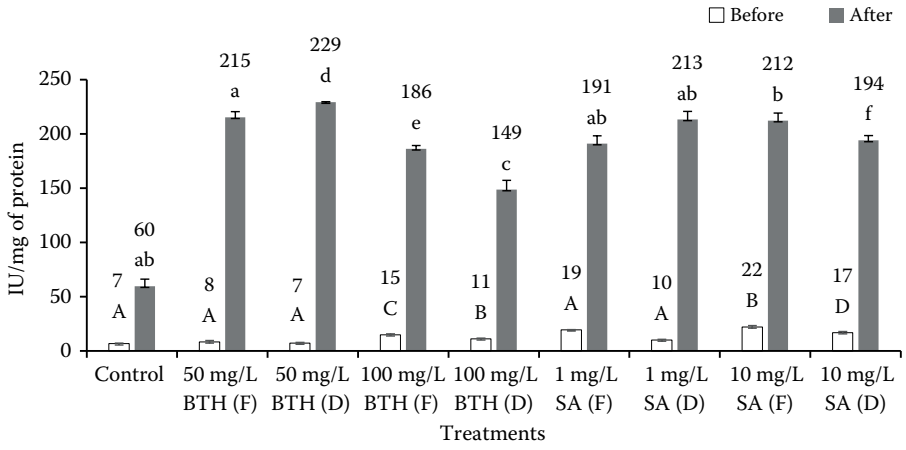

Figure 1. Effect of the salicylic acid (SA) and benzothiadiazole (BTH) applications on the superoxide dismutase enzymatic activity in the cucumber plants

D = drench; F = foliar; $^{A–D}$differences among treatments before application; $^{a–f}$differences among treatments after application

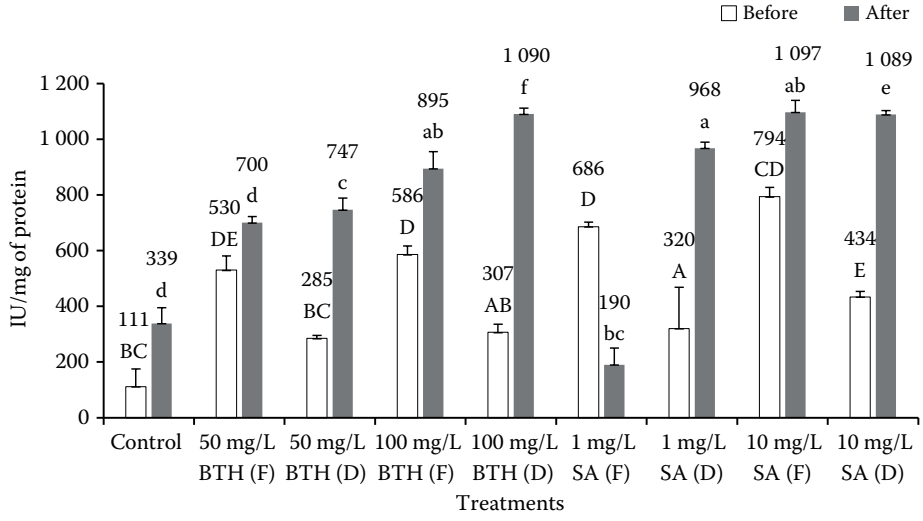

Figure 2. Effect of the salicylic acid (SA) and benzothiadiazole (BTH) applications on the peroxidase enzymatic activity in the cucumber plants

D = drench; F = foliar; $^{A–E}$differences among treatments before application; $^{a–f}$differences among treatments after application

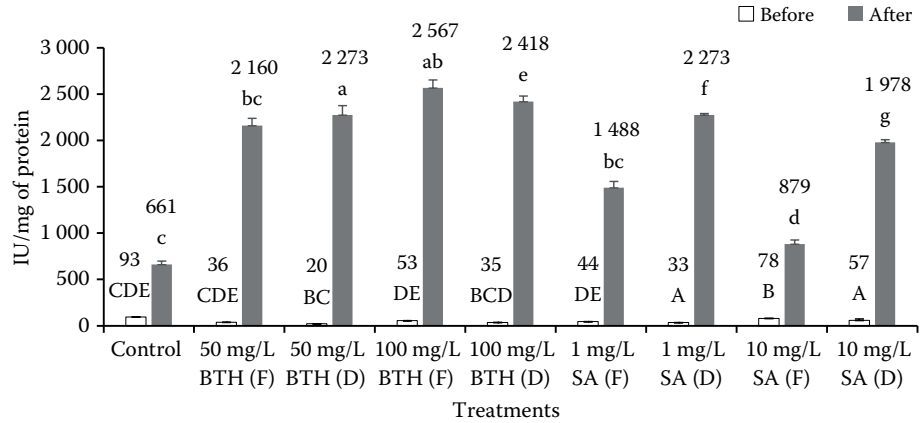

Figure 3. Effect of the salicylic acid (SA) and benzothiadiazole (BTH) applications on the catalase enzymatic activity in the cucumber plants

D = drench; F = foliar; $^{A–E}$differences among treatments before application; $^{a–g}$differences among treatments after application

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oculation with the root-knot nematode. The minimum PAL activity (12.46 IU/mg) was recorded when the SA was applied as a drench treatment at 1 mg/L (Figure 4).

Total phenolic content. The present study revealed that the phenolic content increased significantly in the cucumber plants treated with SA when compared to the control plants. The maximum phenolic compounds (315.62 µg GA/mg) were produced when SA was applied as a drench treatment (10 mg/L) after inoculation with *Meloidogyne incognita*. On the contrary, the minimum phenolic compounds (178.24 µg GA/mg) were produced when BTH was applied as a foliar treatment at 100 mg/L (Figure 5).

Effect of SA and BTH on the cucumber resistance against *M. incognita*

Highly susceptible cucumber cultivars of accession number 028294 were grown under glass house conditions. Ten treatments (10 different treatments along with control were given to plants within each replication) were applied to the plants. The maximum reduction in the number of galls and egg masses were seen in the treatment where BTH was applied as a drench treatment at 100 mg/L. Whereas the minimum reduction was recorded when SA was applied at 1 mg/L as a foliar treatment (Table 2). The number of galls and egg masses were significantly reduced in the soil drenching treatment when compared to the exogenous applications of BTH and

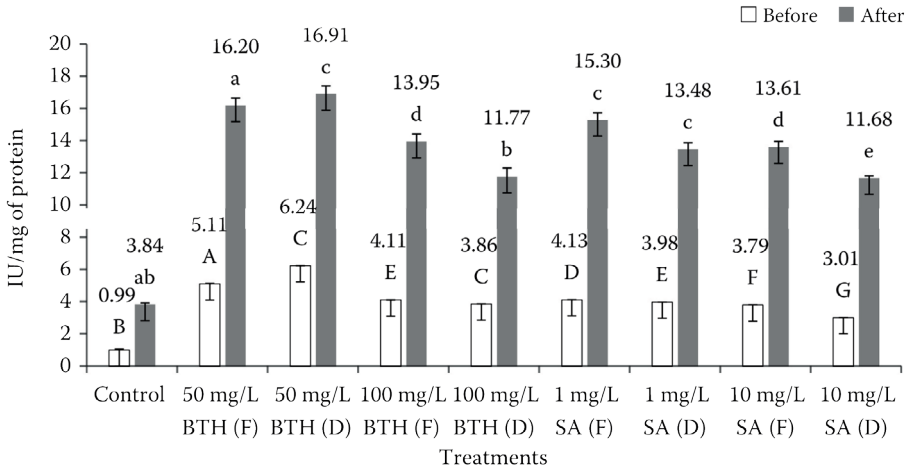

Figure 4. Effect of the salicylic acid (SA) and benzothiadiazole (BTH) applications on the phenylalanine ammonia-lyase enzymatic activity in the cucumber plants

D = drench; F = foliar; $^{A–G}$differences among treatments before application; $^{a–e}$differences among treatments after application

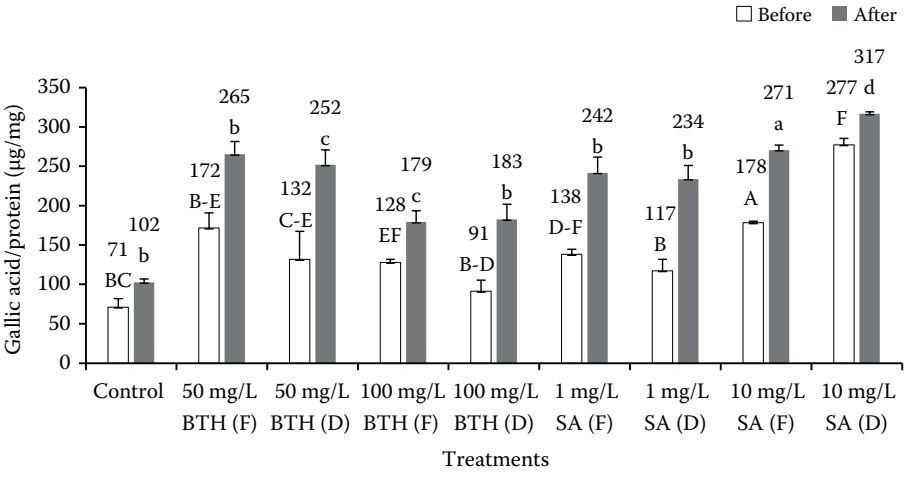

Figure 5. Effect of the salicylic acid (SA) and benzothiadiazole (BTH) applications on the total phenolic content in the cucumber plants

D = drench; F = foliar; $^{A–F}$differences among treatments before application; $^{a–d}$differences among treatments after application

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Table 2. Response of cucumber accessions against nematode (*Meloidogyne incognita*)

Sr. No.	Accession No.	No. of galls	No. of egg masses	Fresh root weight (g)	Shoot weight (g)	Mean galling index	Response
1	28295	92.33^{de}	27.67^{cd}	6.47^{edf}	17.79^{b}	5	S
2	32029	82.33^{f}	28.33^{cd}	7.08^{bed}	16.62^{d}	5	S
3	32028	43.33^{g}	15.00^{f}	5.44^{g}	19.28^{a}	4	MS
4	32027	92.67^{de}	32.66^{bcd}	6.88^{fg}	16.73^{cd}	5	S
5	28523	93.33^{de}	25.67^{de}	6.63^{g}	16.82^{cd}	5	S
6	32534	47.67^{g}	15.33^{f}	5.99^{efg}	18.87^{a}	4	MS
7	32031	94.33^{dc}	33.66^{bc}	6.62^{de}	17.76^{b}	5	S
8	29435	86.66^{ef}	19.00^{ef}	6.12^{def}	17.36^{bc}	5	S
9	28522	85.00^{ef}	28.66^{cd}	7.04^{bcd}	17.94^{b}	5	S
10	28293	94.67^{de}	30.66^{cd}	7.61^{ab}	16.72^{cd}	5	S
11	29643	92.00^{def}	25.66^{de}	7.42^{abc}	16.87^{cd}	5	S
12	32030	99.33^{d}	29.33^{cd}	7.29^{abc}	19.06^{a}	5	S
13	28294	156.33^{a}	44.33^{a}	7.96^{a}	15.16^{e}	6	HS
14	32149	133.33^{b}	39.66^{ab}	7.81^{a}	14.78^{e}	6	HS
15	32805	115.00^{c}	41.66^{a}	6.78^{cd}	14.92^{e}	6	HS
LSD	–	9.98	7.80	0.67	0.72	–	–

HS = highly susceptible; LSD = least significant difference; MS = moderately susceptible; S = susceptible

$^{a–g}$Means followed by same letter in each column are not statistically different at $P \leq 0.05$

SA. The mean galling index (MGI) was significantly lower in the treatments where BTH and SA were applied in the highest doses as a soil drench treatment. The data generated from this study showed that the exogenously applied SA and BTH treatments, when applied to the cucumber seedlings before inoculation of the juvenile *M. incognita,* minimised the reproduction of the pathogen (Table 3).

DISCUSSION

Plant parasitic nematodes play an important role in minimising the production of agricultural products throughout the world. Among them, *Meloidogyne* spp. are considered the most damaging pests having a large number of host crops (Wendimu 2021) especially in the cucurbitaceous

Table 3. Effect of the different concentrations of salicylic acid (SA) and benzothiadiazole (BTH) on the highly susceptible (HS) cucumber cultivar (28294)

Treatment	No. of galls	No. of egg masses	Fresh root weight (g)	Shoot weight(g)	Mean galling index
T1 (50 mg/L BTH, foliar)	76.50 ± 4.97^{ab}	71.17 ± 4.66^{a}	6.65 ± 0.22^{c}	16.18 ± 0.15^{bc}	4.66 ± 0.21^{ab}
T2 (50 mg/L BTH, drench)	69.33 ± 4.11^{ab}	58.50 ± 3.50^{c}	5.43 ± 0.18^{d}	12.56 ± 0.26^{e}	4.50 ± 0.22^{ab}
T3 (100 mg/L BTH, foliar)	74.17 ± 5.24^{ab}	69.17 ± 4.58^{ab}	6.94 ± 0.08^{bc}	16.89 ± 0.22^{b}	4.50 ± 0.22^{ab}
T4 (100 mg/L BTH, drench)	66.33 ± 4.05^{b}	57.50 ± 3.30^{c}	7.17 ± 0.13^{abc}	15.44 ± 0.18^{cd}	4.33 ± 0.21^{b}
T5 (1 mg/L SA, foliar)	77.17 ± 4.37^{ab}	72.17 ± 3.24^{a}	7.95 ± 0.23^{a}	16.33 ± 0.36^{b}	4.66 ± 0.21^{ab}
T6 (1 mg/L SA, drench)	71.67 ± 3.37^{ab}	58.83 ± 4.09^{bc}	7.95 ± 0.53^{a}	17.89 ± 0.26^{a}	4.50 ± 0.21^{ab}
T7 (10 mg/L SA, foliar)	76.50 ± 4.16^{ab}	70.17 ± 3.66^{a}	6.38 ± 0.27^{c}	14.80 ± 0.32^{d}	4.66 ± 0.22^{ab}
T8 (10 mg/L SA, drench)	68.33 ± 5.42^{ab}	56.67 ± 4.63^{c}	7.78 ± 0.26^{ab}	17.83 ± 0.11^{a}	4.33 ± 0.21^{b}
T9 (infested control, only nematodes)	78.33 ± 2.89^{a}	72.33 ± 3.45^{a}	6.72 ± 0.48^{c}	16.95 ± 0.28^{b}	5.00 ± 0.00^{a}
T10 (healthy control)	–	–	5.51 ± 0.38^{d}	12.88 ± 0.78^{e}	–
Least significant difference	11.20	10.49	0.92	0.79	0.55

$^{a–e}$Means followed by the same letter in each column are not statistically different at $P \leq 0.05$

https://doi.org/10.17221/130/2021-PPS

family. Using resistant crop cultivars is considered the most effective strategy to manage the population of RKNs. In the current study, the reactions of the cucumber accessions to *M. incognita* were assessed based on the galling and egg masses produced on the roots. The tested plants showed different responses against *M. incognita*. No accession was found to be resistant or highly resistant against *M. incognita*. All the accessions were found to be highly susceptible, susceptible or moderately susceptible. Resistance within a plant species is often due to specific genes that can segregate within the species. By contrast, for non-host species or resistant cultivars, the nematode cannot reproduce on that species or group of plants due to a broader absence of host traits required for parasitism. To reproduce, the infective second-stage juveniles must be attracted to the host roots, penetrate the epidermis and migrate through the root cortex to establish a feeding site in the vascular parenchyma that provides sufficient nutrition for the development and egg production (Punithaveni et al. 2015). In our research, we especially focused on the resistance of the cucumber crop which is highly vulnerable to the infection dynamics of the RKN in all the vegetable growing areas of Pakistan. We collected germplasm samples and went through resistance evaluation procedures via screening under glasshouse conditions. The experiment was conducted with standard procedures and, hence, we found that the cucumber accessions varied widely in their susceptibility to *M. incognita* in terms of the total number of galls, total number of egg masses, fresh root weight and shoot weight. Our research depicted that out of fifteen accessions, ten were found to be susceptible to RKN while two were found to be moderately susceptible and three were found to be highly susceptible. None of the accessions were shown to have a moderately resistant, resistant or highly resistant response. Out of the fifteen accessions, three accessions had the maximum number of galls, similarly three cultivars produced the highest number of egg masses, while two accessions gained the maximum root weight and the maximum shoot weight was observed in one accession. In the highly susceptible cultivars, the production of the maximum egg masses on the roots explain that a maximum number of juveniles were successful in completing their life cycles after entering the host. Whereas, in contrast, in the resistant cultivars, only a few juveniles were able to infect the roots and become mature, which is reflected by their reproductive factors and the number of egg masses (Khan et al. 2019). Our results are in parallel with Moussa (1981) who conducted a study on the cucumber and indicated the occurrence of four *Meloidogyne* spp. in descending order of frequency *M. incognita*, *M. javanica*, *M. arenaria* and *M. thamesi* with a varying response in the resistance in all the evaluated varieties. Similarly, Kassi and Hussain (1987) showed the response of cucumber varieties towards RKN infections, where Energia VFN, Novia and F-187 possessed susceptibility to *M. javanica*. C32 VFN was the most susceptible as the highest number of galls occurred on the roots, the lowest occurred in Novia and no galls developed on Energia VFN. Our results varied from Walter's experiments (Walter et al. 1993), who studied the response of 24 cultigens of the horned cucumber (*C. metuliferus* Naud.) and 884 cucumber cultivars (*Cucumis sativus*) to check their level of resistance against RKNs. Out of the 24 cultigens, only two cultigens showed a highly susceptible response against all the tested nematode species, while all the other horned cucumber cultigens showed a resistant response to *M. incognita*, *M. arenaria* and *M. hapla.* All 884 cultigens showed a resistant response against *M. hapla* and very few showed a resistant response against *M. incognita*. Whereas, 50 cultigens were considered somewhat resistant to *M. arenaria* and *M. incognita*. Our findings are similar to previous research about the highly susceptible response of cucumber germplasms against RKNs. The germplasm screening is a continuous process because sexual reproduction in RKNs possesses the capability to break any type of resistance by adjusting themselves against the subjected crop species.

As we found a scarcity of resistance in the cucumber germplasms against RKNs, we evaluated the mechanism of the induced resistance in cucumber plants as the result of elicitor applications. Plants of a susceptible cucumber cultivar were treated with the varying concentrations of SA and BTH which are considered signalling molecules that induce resistance. We also explored the plant enzymatic activity in response to the infection dynamics of RKNs. Antioxidants like SOD, POD, CAT and PAL were determined as biochemical markers of resistance and all the antioxidants were found to increase in response to the RKN infection followed by the application of BTH and

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SA (Molinari 2001). Similarly, the CAT activity was at a maximum when SA and BTH were applied as foliar treatments before inoculation of *M. incognita* at 10 mg/L and 50 mg/L, respectively. Likewise, the PAL activity was at a maximum when SA was applied as a foliar treatment after inoculation of *M. incognita* at 1 mg/L. Whereas PAL was recorded at a maximum when BTH was applied as a foliar treatment before inoculation of *M. incognita* at 50 mg/L. In the same way, the SOD and POD activity was at a maximum when SA and BTH were applied as foliar treatments before inoculation of *M. incognita* at 10 mg/L and 100 mg/L, respectively. Our results are in accordance with Molinari (2002), who reported the effect on nematode reproduction as the result of the exogenous application of SA on susceptible tomatoes before inoculation with RKNs. Moreover, it was already reported that SA sprayed onto leaves of the tomato could induce some resistance to *M. incognita* and improved the plant growth (Nandi et al. 2000). SOD is a first line of defence in dissimulating superoxide into non-toxic forms, which is foremostly activated by microbes against the ROS generated in cytosol during respiration and photosynthesis (Clarke et al. 2017). The present study suggests that the activity of the antioxidative enzymes (SOD and CAT) in the cucumber leaves increased as the relative galling formation decreased in the cucumber roots. The activities of SOD and POD increased after the inoculation of *M. incognita*, due to defence signalling during the attack tend to up regulate the activities of specific antioxidants (Singh et al. 2019). Our results are confirmatory with Nguyen et al. (2011) in which they described that the application of any resistance inducing product could induce the SOD-specific activity in cucumber leaves as a defensive response against RKN infections. Molinari (2001) also studied the inhibition of CAT activity in roots of resistant tomatoes as a result of a nematode infestation or SA application, whereas in our experiment, the CAT activity was significantly increased in both the elicitor treated plants and the RKN infested cucumber susceptible plants. In this study, the POD activity was increased after the *M. incognita* inoculation and reached a maximum after the establishment of nematodes in the roots. The enhanced POD activity is relative to the resistance mechanisms developed by the plants (Bajestani et al. 2019), and directly linked to its involvement in providing structural rigidity to plant tissues through enhanced lignin synthesis in the cell walls in order to prevent the nematode penetration in plants (Moghbeli et al. 2017). Suppression of the POD enzymatic activity was indicated by the weakening of the host defence mechanisms in *M. incognita* inoculated cucumber host plants. This eventually helped in the further spread of RKNs and more knots developed on the roots. The number of eggs per gram of root fresh weight was reduced where SA and BTH were applied in drench treated plants, whereas the degree of infestation in terms of the galling index was apparently unaffected. Comparably, the number of egg masses per root system did not substantially change in tomato seedlings treated by a root dip in 1 mM SA before inoculation with RKNs (Molinari 2008). It was found that the phenolic and protein contents increased significantly in cucumber plants infested with RKNs followed by the foliar and drench application of SA and BTH when compared to the control plants. The application of SA and BTH resulted in an increase in the phenolic content, which ultimately induced the resistance (Molinari 2001).

Several studies support our evidence that SA and BTH both have inducing effects in vegetables (Achuo et al. 2002). The SA application induces resistance against *Ralstonia solanacearum* by the accumulation of phenolics and increased lignin deposition in the cell walls of roots and also increases the enzymatic activities (Mandal 2010). SA induces the accumulation of phenolics and increases the PAL enzyme activity in okra that leads to resistance against *Erysiphe cichoracearum* (Vimla & Suriachandraselvan 2009). BTH and SA induce defence related enzymes, i.e., POD, PAL and SOD in mustard which results in the prevention of an *Alternaria brassicae* invasion (Sharma & Sohal 2010). BTH induces the synthesis of chitinase and β-1,3-glucanase in sugar beets that provide resistance against the *tobacco necrosis virus* (TNV) (Burketová et al. 1999). In a nutshell, in the absence of genetic resistance against RKNs in cucumbers, induced resistance through elicitors like SA and BTH may provide an alternate strategy to mitigate RKN infections. Furthermore, the incorporation of drench applications of SA and BTH in integrated management RKNs may be effective.

Cucurbits are highly vulnerable to RKNs in Pakistan. The screening of cucurbits, especially the cucumber, against RKNs may lead to cheaper, envi-

https://doi.org/10.17221/130/2021-PPS

ronmentally friendly and feasible control measures for the management of the nematode. The present research may guide plant breeders to ponder upon the breeding of cucumbers for resistant sources against RKNs. Although the elicitors are not able to control the disease, they may play an important role by triggering the defence mechanism of the plant against the nematode and for future direction, the genes or proteins involved in the defence mechanism and their interaction with RKNs should be established. The application of elicitors may become an integral part of integrated nematode management strategies for protected cucumber production.

Acknowledgement: The authors gratefully acknowledge the scientific and technical support from Plant Pathological Research Institute, Ayub Agricultural Research Institute, Faisalabad. Further the authors also acknowledge National Agriculture Research Centre, Pakistan Agriculture Research Council, Islamabad for providing cucumber germplasm.

REFERENCES

Abad P., Favery B., Rosso M.N., Castagnone-Sereno P. (2003): Root-knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. Molecular Plant Pathology, 4: 217–224.

Achuo A.E., Audenaert K., Meziane H., Höfte M. (2002): The SA-dependent defense pathway is active against different pathogens in tomato and tobacco. Mededelingen (Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen), 67: 149–157.

Aebi H. (1984): Catalase *in vitro*. In: Jura N., Murphy J.M. (eds). Methods in Enzymology. Waltham, Elsevier Academic Press: 121–126.

Anwar S.A., Zia A., Hussain M., Kamran M. (2007): Host susceptibility of selected plants to *Meloidogyne incognita* in the Punjab, Pakistan. International Journal of Nematology, 17: 144–150.

Bajestani M.S., Moghadam E.M., Aghnoum R., Rohani H. (2019): Genotypic and biochemical variation in the response of barley to the Root-knot nematode (*Meloidogyne javanica*) at seedling stage. Pakistan Journal of Phytopathology, 31: 7–17.

Bello T.T., Coyne D.L., Fourie H. (2021): Reproduction potential of Nigerian *Meloidogyne* spp. and the response of six commercial watermelon cultivars to predominant species. Journal of Plant Diseases and Protection, 128: 831–842.

Bradford M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Analytical Biochemistry, 72: 248–254.

Burketová L., Šindelářová M., Šindelář L. (1999): Benzothiadiazole as an inducer of β-1,3-glucanase and chitinase isozymes in sugar beet. Biologia Plantarum, 42: 279–287.

Chance B., Maehly A.C. (1955): Assay of catalase and peroxidase. Methods in Enzymology, 2: 764–775.

Clarke C.R., Vinatzer B.A. (2017): Characterizing the immune-eliciting activity of putative microbe-associated molecular patterns in tomato. In: Shan L., He P. (eds). Plant Pattern Recognition Receptors. Methods and Protocols. New York, Springer: 249–261.

Dickerson D.P., Pascholati S.F., Hagerman A.E., Butler L.G., Nicholson R.L. (1984): Phenylalanine ammonia-lyase and hydroxycinnamate CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiology Plant Pathology, 25: 111–123.

Eder A.B., Mauro F.B., Carlos B.J., Gilbert E.J., Thales R., Janice D.A.E. (2018): Imaging mass spectrometry of endogenous polypeptides and secondary metabolites from galls induced by root knot nematodes in tomato roots. Journal of Molecular Plant Microbe Interactions, 31: 1048–1059.

Eder R., Consoli E., Krauss J., Dahlin P. (2021): Polysulfides applied as formulated garlic extract to protect tomato plants against the root-knot nematode *Meloidogyne incognita*. Plants, 10: 394. doi: 10.3390/plants10020394

Elekcioglu I.H., Ohnesorge B., Lung G., Uygun N. (1994): Plant parasitic nematodes in the Mediterranean region of Turkey. Nematol Mediterranian, 22: 59–63.

Giannopolitis C.N., Ries S.K. (1997): Superoxide dismutase I. Occurrence in higher plants. Plant Physiology, 59: 309–314.

Holbrook C.C., Knauft D.A., Dickson D.W. (1983): A technique for screening peanut for resistance to *Meloidogyne arenaria*. Plant Disease, 57: 957–958.

Janati S., Houari A., Wifaya A., Essarioui A., Mimouni A., Hormatallah A., Sbaghi M., Dababat A.A., Mokrini F. (2018): Occurrence of the root-knot nematode species in vegetable crops in Souss region of Morocco. The Plant Pathology Journal, 34: 308–315.

Jones J.T., Haegeman A., Danchin E.G., Gaur H.S., Helder J., Jones M.G. (2013): Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology, 14: 946–961.

Kammerhofer N., Radakovic Z., Regis J.M., Dobrev P., Vankova R., Grundler F.M. (2015): Role of stress-related hormones in plant defence during early infection of the

https://doi.org/10.17221/130/2021-PPS

cyst nematode *Heterodera schachtii* in *Arabidopsis*. New Phytology, 207: 778–789.

Kaskavalci G. (2007): Effect of soil solarization and organic amendments treatment for controlling *Meloidogyne incognita* in tomato cultivars in Western Anatolia. Turkish Journal of Agriculture and Forestry, 31: 159–167.

Kassi A.H., Hussain S.I. (1987): Screening of some tomato cultivars for their resistance to *Meloidogyne javanica* under Iraqi conditions. International Nematology Network Newsletter, 4: 27–29.

Kayani M.Z., Mukhtar T., Hussain M.A. (2017): Effects of southern root knot nematode population densities and plant age on growth and yield parameters of cucumber. Crop Protection, 92: 207–212.

Khan M.T., Mukhtar T., Saeed M. (2019): Resistance or susceptibility of eight aubergine cultivars to *Meloidogyne javanica*. Pakistan Journal of Zoology, 51: 2187–2192.

Kyndt T., Nahar K., Haeck A., Verbeek R., Demeestere K., Gheysen G. (2017): Interplay between carotenoids, abscisic acid and jasmonate guides the compatible rice-*Meloidogyne graminicola* interaction. Frontiers in Plant Science, 8: 951. doi: 10.3389/fpls.2017.00951

Mandal S. (2010): Induction of phenolics, lignin and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors. African Journal of Biotechnology, 9: 8038–8047.

Moghbeli E., Nemati S.H., Aroiee H., Olfati J.A. (2017): Evaluation of resistance, enzymatic response, and phenolic compounds in roots of F1 cucumber hybrids to *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. Journal of Horticulture Research, 25: 117–124.

Molinari S. (2001): Inhibition of H_2O_2-degrading enzymes in the response of *Mi*-bearing tomato to root-knot nematodes and salicylic acid treatment. Nematologia Mediterranea, 29: 235–239.

Molinari S. (2008): Salicylic acid as an elicitor of resistance to root-knot nematodes in tomato. Acta Horticulture, 789: 119–126.

Moussa F.F., Elgindi D.M., Kheir A.M., Koraiem A.M. (1981): Reaction of certain cucurbit cultivars to nine populations of *Meloidogyne incognita*. Bulletin of Zoological Society Egypt, 31: 93.

Mukhtar T., Kayani M.Z., Hussain M.A. (2013): Response of selected cucumber cultivars to *Meloidogyne incognita*. Crop Protection, 44: 13–17.

Nahar K., Kyndt T., De Vleesschauwer D., Hofte M., Gheysen G. (2011): The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. Plant Physiology, 157: 305–316.

Nandi B., Nandy R.K., Mukhopadhyay S., Nair G.B., Shimada T., Ghose A.C. (2000): Rapid method for species-specific identification of *Vibrio cholerae* using primers targeted to the gene of outer membrane protein OmpW. Journal of Clinical Microbiology, 38: 4145–4151.

Nguyen D.M.C., Seo D.J., Park R.D. (2011): Changes in antioxidative enzyme activities in cucumber plants with regard to biological control of root-knot nematode, *Meloidogyne incognita* with *Cinnamomum cassia* crude extracts. Journal of Korean Society and Applied Biological Chemistry, 54: 507–514.

Pasqua V., Costantino P., Federico P., Silvana D.L., Alba G.U., Maria T.M. (2018): Changes in lignin biosynthesis and monomer composition in response to benzothiadiazole and root-knot nematode *Meloidogyne incognita* infection in tomato. Journal of Plant Physiology, 230: 40–50.

Punithaveni V., Jansirani P., Sivakumar M. (2015): Screening of cucurbitaceous rootstocks and cucumber scions for root knot nematode resistance (*Meloidogyne incognita* Kofoid and White). Electronic Journal of Plant Breeding, 6: 486–492.

Qi W., Ke C., Haiyan L., Gengrui Z., Weichao F., Changwen C., Xinwei W., Lirong W. (2018): Lignification plays an important role on resistance to root-knot nematode (*Meloidogyne incognita*) based on contrastive analysis in peach. Scientia Horticulturae, 238: 1–6.

Sasanelli N., Konrat A., Migunova V., Toderas I., Iurcu-Straistaru E., Rusu S., Bivol A., Andoni C., Veronico P. (2021): Review on control methods against plant parasitic nematodes applied in southern member states (C Zone) of the European Union. Agriculture, 11: 602. doi: 10.3390/agriculture11070602

Sasser J.N., Freckman D.W. (1987): World perspective of Nematology. The role of society. In: Veech J.A., Dickson D.W. (eds). Vistas on Nematology: A Commemoration of the Twenty-Fifth Anniversary of the Society of Nematologist. Hyattville, Society of Nematologist, Inc.: 7–14.

Sharma S., Sohal B.S. (2010): Foliar spray of benzothiadiazole and salicylic acid on *Brassica juncea* var. RLM619 to combat *Alternaria* blight in field trials. Crop Improvement, 31: 87–92.

Singh U.B., Singh S., Khan W., Malviya D., Sahu P.K., Chaurasia R., Sharma S.K., Saxena A.K. (2019): *Drechslerella dactyloides* and *Dactylaria brochopaga* mediated induction of defense related mediator molecules in tomato plants pre-challenged with *Meloidogyne incognita*. Indian Phytopathology, 72: 309–320.

Song L., Xu X.C., Wang F.N., Wang Y., Xia X.J., Shi K. (2017): Brassino steroids act as a positive regulator for resistance against root-knot nematode involving respiratory burst oxidase homolog-dependent activation of MAPKs in tomato. Plant Cell Environment, 41: 1113–1125.

Taylor D.P., Nestscher C. (1974): An improved technique for preparing perennial pattern of *Meloidogyne* spp. Nematologica, 20: 268–269.

https://doi.org/10.17221/130/2021-PPS

Vimala R., Suriachandraselvan M. (2009): Induced resistance in bhendi against powdery mildew by foliar application of salicylic acid. Journal of Biopesticides, 2: 111–114.

Walter S.A., Wehner T.C., Barkel K.R. (1993): Root-knot nematode resistance in cucumber and horned cucumber. Horticulture Science, 28: 151–154.

Whitehead A.G., Hemming J.R. (1965): A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology, 55: 25–38.

Wright D.J. (1981): Nematicides: Mode of action and new approaches to chemical control. In: Zuckerman B.M., Rohde R.A. (eds). Plant-Parasitic Nematodes. London and New York, Academic Press: 421–449.

Yigezu Wendimu G. (2021): Biology, taxonomy, and management of the root-knot nematode (*Meloidogyne incognita*) in sweet potato. Advances in Agriculture, 2021: 8820211. doi: 10.1155/2021/8820211

Yuksel H. (1974): Considerations on the state of root knot nematodes (*Meloidogyne* spp.) in Turkey and their population problems. Journal of Agricultural Faculty of Atatürk University, 5: 83–105.

Zieslin N., Ben-Zaken R. (1993): Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiology and Biochemistry, 31: 333–339.

Zinovieva S.V., Vasyukova N.I., Udalova Z.V., Gerasimova N.G. (2013): The participation of salicylic and jasmonic acids in genetic and induced resistance of tomato to *Meloidogyne incognita*. Biology Bulletin, 40: 297–303.

Received: September 27, 2021
Accepted: April 12, 2022
Published online: July 1, 2022