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Effect of 28-Homobrassinolide on tomato seedlings during nematode pathogenesis

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Abstract : 28-Homobrassinolide was evaluated for its influence on susceptible (Pusa Ruby) and resistant (PNR-7) cultivars of tomato inoculated with second stage juveniles of root-knot nematode, *Meloidogyne incognita*. Morphological and biochemical parameters were investigated in roots 168 hrs after nematode inoculation. In susceptible cultivar, nematode invasion reduced the plant growth and development but HBl treatment improved the growth as observed from the amelioration of total antioxidant contents. In case of resistant plants, nematodes were not able to invade the roots and here also, pre-sowing treatment of seeds further enhanced the growth of plants. Moreover, nematode inoculation activated the level of antioxidants which further got enhanced with HBl treatment. Also, when the two varieties were compared for morphological and biochemical parameters, overall higher values were found in the resistant cultivar than in the susceptible one

Key Words: ***Meloidogyne incognita*, 28-Homobrassinolide, Tomato cultivars, Morphological parameters, Antioxidants.**

INTRODUCTION

Brassinosteroids (BRs) are hydroxylated derivatives of cholestane, which play an essential role in plant growth and development by influencing various physiological processes including seed germination, stem elongation, cell division and expansion and xylem differentiation[1]. In addition to that, BRs have protective role in response to various stresses including temperature, drought, salinity, organic pollutants and heavy metals[1,2,3,4]. The potential role of BRs in pathogen defence has also been the topic of recent studies. Potato plants sprayed with BRs had a lower incidence of infection by *Phytophthora infestans*[5]. Similarly, BR-induced disease resistance has also been noted in barley, potato-tubers, tobacco, rice and cucumber plants[6,7,8].

Since root-knot nematodes are closely associated with plants and cause heavy stress to the plants, the present study was formulated to evaluate the effect of 28-Homobrassinolide on susceptible and resistant cultivars of tomato plants during nematode pathogenesis.

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MATERIALS AND METHODS

Uniformly sized surface sterilized seeds of tomato (*Lycopersicon esculentum* Mill.) cultivars Pusa Ruby (susceptible) and PNR-7 (resistant) were soaked in different concentrations of HBl (10^{-11}, 10^{-9} and 10^{-7} M) and kept for germination in 80 mm autoclaved petri-plates lined with moistened Whatman Sheet No-1. Thirty seeds were germinated per petri for morphological and biochemical estimations. A total of five sets were made including two controls (CI; untreated, uninoculated and CII; untreated, inoculated) with three replicates each. The petri-plates were placed in B.O.D incubator at a temperature of 24±2ºC and photoperiod of 14hr. Seven days old seedlings were inoculated with second stage juveniles @$5J_2$/seedling. Morphological and biochemical estimations were carried out in both cultivars 168 hrs post nematode inoculation in treated and untreated plants.

Morphological observations were made on root length, root weight and number of galls.

For biochemical estimations, roots of each cultivar were separated, weighed and crushed in pre-chilled pestle and mortar using ice-cold 80% methanol. The extract was collected and centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used for estimations while the pellet was discarded. Total phenolic content (TPC) was estimated with slight modifications[9] while total flavonoid content (TFC) was estimated using $AlCl_3$ method[10]. For ascorbic acid content (AsC), roots were crushed in pestle-mortar in chilled 2%Meta phosphoric acid and centrifuged at low speed (2500 rpm) for 15 minutes. The residues were discarded and the supernatants were used for estimations[11]. For glutathione content (GSH) extract was prepared by homogenizing fresh roots in pestle and mortar under ice-cold conditions in 0.02 M disodium salt of Ethylenediaminetetracetic acid. The homogenate was centrifuged at 3000 g for 15 min at 4°C. The pellet was discarded while supernatants were kept ice cold until used for the assay[12].

Statistical Analysis: For each assay, data was subjected to one-way analysis of variance (ANOVA) using Assistat (7.6) beta software. Comparisons between means of treatment combinations were compared by Tukey's multiple range test ($p<0.05$).

RESULTS AND DISCUSSION

Observations made on morphological parameters indicated that pre-sowing treatment of tomato seeds with HBl enhanced the overall growth of susceptible cultivar and decreased the nematode invasion. In resistant plants, much effect was not observed after nematode inoculation but here also, HBl treatment enhanced the overall growth of plants and reduced the nematode invasion to plants (Table1).

These observations are consistent with the previous studies[13] where it was found that *M. javanica* significantly affected the growth of susceptible mung bean plants. Reports have also documented the effects of *M. incognita* on growth and yield of resistant and susceptible tomato varieties under controlled growth chamber conditions. The nematode invasion in roots 7 days of inoculation was drastically reduced in resistant cultivars (Hisar Lalit and PBNR-7) as compared to susceptible (Pusa Ruby) one[14]. Similarly, in the present study also, it was found that nematode invasion in roots 7 days of inoculation was significantly reduced in resistant cultivar as compared to susceptible one. In addition to that, pre-sowing of tomato varieties with HBl improved the seed vigor which hampered the root knot nematode penetration thus improving the growth of both the varieties. These results are in accordance with the previous studies conducted in *Raphanus sativus* where it was reported that root length decreases with nematode invasion but increases with brassinolide treatment[15].

In susceptible cultivar, TPC, TFC, AsC increased in Control II but GSH content decreased. However, HBl treatment resulted in significant increase in the content of antioxidants (Table2). Total antioxidant content in resistant cultivar showed increase in TPC, AsC and GSH but a slight decrease in TFC post nematode inoculation (Control II) as compared to Control I. But here also, application of HBl further enhanced total antioxidant contents significantly (Table3).

An increase in total content of antioxidants in plants treated with brassinosteroids has been reported earlier[8] where root and foliar applications of 24-epibrassinolide (EBL) reduced symptoms of fusarium wilt (*Fusarium oxysporum*) and influenced phenolic and flavonoid metabolism in roots of cucumber plants (*Cucumis sativus* L. cv. Jinyan No. 4). The results showed that EBL enhanced resistance to fusarium wilt by increase in antioxidant system. Recently, studies pertaining to effects of brassinosteroid analogues on total phenols, antioxidant activity, sugars, organic acids and yield of field grown endive (*Cichorium endivia* L.) have been reported[16]. Here, it was found that all treatments (4ppm, 8ppm, 12ppm) with DI-100 and DI-31 (brassinosteroid analogues) in Tomex Amin (a commercial fertilizer) significantly increased total antioxidant activity and total phenols of field grown endives.

Present study showed that HBl treatment improved plant growth and altered the level of antioxidants in both cultivars of tomato plants after nematode inoculation. Hence, it provides evidence of BR induced resistance in plants during nematode pathogenesis.

Table1. Effect of 28-Homobrassinolide on morphological parameters of tomato cultivars 168 hrs after nematode inoculation (n= 3± S.E.M)

Control/ Treated	Root length (cm)		Root weight (gm)		Number of galls	
	Pusa Ruby	PNR-7	Pusa Ruby	PNR-7	Pusa Ruby	PNR-7
Control I	3.62±0.40	4.43±0.43	0.134±0.13	0.222±0.05	0^{c}	0
Control II	2.27±0.40	4.47±0.38	0.081±0.081	0.248±0.04	6.00 ± 0.58^{a}	1.00±0.58
10^{-11}M	3.52±0.50	4.63±0.35	0.135±0.13	0.253±0.04	2.00 ± 0.58^{bc}	0
10^{-9} M	3.49±0.47	5.37±0.93	0.129±0.13	0.269±0.08	2.00 ± 0.33^{b}	0
10^{-7} M	4.17±0.18	5.93±0.30	0.137±0.14	0.278±0.04	3.00 ± 0.58^{b}	0
F-value	2.91^{ns}	1.56^{ns}	2.64^{ns}	0.63^{ns}	21.25^{**}	1.00^{ns}

** = Significant at 1%, ns = Non-Significant, Control I = (Untreated, Uninoculated), Control II = (Untreated, Inoculated); Averages followed by same letter do not differ statistically between themselves according to Tukey's Test at a level of 5% of probability

Table2. Effect of 28-Homobrassinolide on antioxidant content ($mg^{-1}g$ tissue) in roots of Pusa Ruby 168 hrs after nematode inoculation (n= 3± S.E.M)

Control/ Treated	Total Phenolic Content	Total Flavonoid Content	Ascorbic Acid Content	Total Glutathione Content
Control I	73.77 ± 2.03^{a}	1.36 ± 0.07^{b}	14.36 ± 3.04^{b}	1.81 ± 0.19^{a}
Control II	74.09 ± 6.62^{a}	1.39 ± 0.16^{b}	16.11 ± 0.83^{ab}	1.00 ± 0.073^{b}
10^{-11}M	78.33 ± 1.91^{a}	1.55 ± 0.009^{ab}	22.62 ± 0.072^{a}	1.11 ± 0.11^{ab}
10^{-9}M	88.12 ± 3.57^{a}	2.70 ± 0.19^{a}	22.62 ± 0.31^{a}	1.26 ± 0.19^{ab}
10^{-7}M	89.78 ± 1.50^{a}	1.50 ± 0.54^{ab}	19.94 ± 0.33^{ab}	1.38 ± 0.18^{ab}
F value	4.410^{*}	4.399^{*}	6.998^{**}	4.024^{*}

** = Significant at 1%, * = Significant at 5%, Control I = (Untreated, Uninoculated), Control II = (Untreated, Inoculated); Averages followed by same letter do not differ statistically between themselves according to Tukey's Test at a level of 5% of probability

Table3. Effect of 28-homobrassinolide on antioxidant content ($mg^{-1}g$ tissue) in roots of PNR-7 168hrs after nematode inoculation (n= 3± S.E.M)

Control/ Treated	Total Phenolic Content	Total Flavonoid Content	Ascorbic Acid Content	Total Glutathione Content
Control I	76.12 ± 3.99^{b}	1.60 ± 0.19^{c}	87.83 ± 12^{a}	2.29 ± 0.19^{a}
Control II	81.42 ± 2.95^{b}	1.52 ± 0.11^{c}	95.24 ± 6.04^{a}	2.48 ± 0.073^{b}
10^{-11}M	87.25 ± 0.85^{b}	3.64 ± 0.04^{b}	106.77 ± 1.58^{a}	2.43 ± 0.055^{b}
10^{-9}M	86.36 ± 2.88^{b}	3.52 ± 0.08^{b}	144.35 ± 16.4^{a}	3.22 ± 0.16^{b}
10^{-7}M	105.23 ± 3.37^{a}	5.48 ± 0.07^{a}	127.32 ± 16.6^{a}	3.04 ± 0.43^{ab}
F value	13.394^{**}	223.160^{**}	3.663^{*}	3.372^{ns}

** = Significant at 1%, * = Significant at 5%, ns = Non-Significant, Control I = (Untreated, Uninoculated), Control II = (Untreated, Inoculated); Averages followed by same letter do not differ statistically between themselves according to Tukey's Test at a level of 5% of probability

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