

**BRASSINOSTEROID-MEDIATED CHANGES IN ROOT-KNOT NEMATODE SUSCEPTIBLE AND RESISTANT TOMATO CULTIVARS****RAVINDERJIT KAUR¹, PUJA OHRI^{*1} AND RENU BHARDWAJ²**¹Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab-143005, India^{*1}Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab-143005, India²Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab-143005, India**ABSTRACT**

Plant-parasitic nematodes are one of the major pests, causing damage to almost all cultivated crops throughout the world. Present nematode control measures pose a threat to the environment; therefore, use of natural compounds is being emphasized to control these deterrents. 28-Homobrassinolide (HBI) is one such naturally synthesized compound that is ubiquitously distributed in plant kingdom. In the present investigation, uniformly sized seeds of susceptible (Pusa Ruby) and resistant (PNR-7) tomato cultivars were treated with different concentrations of HBI. After germination, under controlled conditions seedlings were inoculated with infective juveniles of *Meloidogyne incognita*. Morphological and biochemical parameters (antioxidative enzymes/antioxidants) were assessed 72h post-nematode inoculation. Results asserted alterations in plant growth as well as in biochemical parameters post nematode penetration. But supplementing the plants with HBI improved the plant growth and ameliorated the activities of defensive enzymes and antioxidants.

KEYWORDS: Tomato cultivars, *Meloidogyne incognita*, 28-Homobrassinolide, Morphological parameters, Antioxidative enzymes, Non-enzymatic antioxidants

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INTRODUCTION

Plant parasitic nematodes (PPNs) are among the most widespread pests that either cause direct damage to their host or act as virus vectors¹⁻³. Although, over 4,100 species of plant-parasitic nematodes have been identified⁴, new species are continually being described while others, previously viewed as benign or non-damaging, are becoming major with a change in cropping patterns⁵. Methods have been developed that facilitate in curbing these deterrents, including physical, chemical and biological control⁶. But, these age-old practices are either less effective or harmful to the environment. Hence, a safer way to control the nematode pests is the use of natural products which are present in plants. These naturally synthesized compounds are eco-friendly, thereby, posing no hazardous impact on the surroundings. Of the varied natural compounds, brassinosteroids (BRs) are one of the key growth hormones found throughout the plant kingdom that regulates many aspects of growth and development, including stem elongation, pollen tube growth, leaf bending and epinasty, fruit development, ethylene biosynthesis, proton pump activity, xylem differentiation, photosynthesis, gene expression etc.⁷⁻¹². In addition to its critical role in plant growth regulation and photomorphogenesis, BRs induces plant resistance to a variety of abiotic stresses such as high and low temperature, drought, salinity and heavy metal¹³⁻¹⁵. However, scanty literature is available regarding increased resistance in plants against biotic agents such as viruses, bacteria, fungi and nematodes¹⁶⁻¹⁹. Therefore, keeping in view the previous investigations, the concurrent investigation was conducted assessing the 28-Homobrassinolide mediated defence response in root-knot nematode infected susceptible and resistant tomato cultivars.

MATERIALS AND METHODS

i. HBI treatment

28-Homobrassinolide was procured from Sigma Aldrich, Bangalore, India. Stock solution was prepared using methanol (HPLC grade; purchased from Sigma Aldrich, Bangalore, India). Uniform sized seeds of two varieties of tomato (*Lycopersicon esculentum* Mill.) viz.

Pusa Ruby (susceptible) and certified PNR-7 (resistant) were surface sterilized with 1% sodium hypochlorite for 15min followed by repeated rinsing in double-distilled water. Sterilized seeds were treated with different concentrations of HBI (10^{-11} , 10^{-9} and 10^{-7} M) for 4hrs. HBI treated seeds were then germinated in sterilized petri-plates lined with moistened Whatman sheet. For morphological and biochemical assays, thirty and sixty seeds were germinated per petri respectively. Three replicates were made for each concentration and two different controls -CI (untreated, uninoculated) and CII (untreated, nematode inoculated). The petri-plates were placed in B.O.D incubator at $24 \pm 2^\circ\text{C}$, relative humidity between 60-75% and photoperiod of 14hrs. Six-seven days old seedlings were inoculated with infective second stage juveniles of *M. incognita* (@5J₂/seedling) and estimations were carried out 72hrs post inoculation.

ii. Morphological assay

Percentage germination, total plant height and total plant biomass were recorded in untreated and treated plants of both the cultivars.

iii. Biochemical assay

For biochemical studies, antioxidative enzymes and non-enzymatic antioxidants were assayed using standard protocols. For antioxidative enzymes, roots and shoots were separately weighed and homogenized in potassium phosphate buffer (PPB). Homogenate was then centrifuged using Heraeus Biofuge Stratos at 10,000 rpm for 30min at 4°C . Supernatant was used for assays while the pellet was discarded. Specific activities of enzymes were measured spectrophotometrically [Thermo Scientific Genesys (G10S UV-VIS) spectrophotometer] using standard protocols. Specific activity of catalase (CAT) was determined as decrease in absorbance of H_2O_2 at 240 nm for a min²⁰. The activities of ascorbate peroxidase (APOX), guaiacol peroxidase (GPOX), glutathione reductase (GR) and glutathione peroxidase (GPOD) were determined using standard protocols²¹⁻²⁴. Superoxide dismutase (SOD) was determined by monitoring its ability to inhibit photochemical reduction of nitrobluetetrazolium (NBT) at 540 nm²⁵. Protein

concentration was also determined²⁶. For estimating non-enzymatic antioxidants, roots and shoots were separated, weighed and then crushed in pre-chilled pestle and mortar using ice-cold 80% methanol. Extracts were collected and centrifuged at 10,000 rpm for 20min at 4°C. Supernatant was used for estimations while the pellet was discarded. Total phenolic content (TPC) was estimated with slight modifications²⁷, while Total flavonoid content (TFC) was determined using AlCl₃ method²⁸. For ascorbic acid content (AsC), roots and shoots were weighed and crushed separately using chilled 2% Meta-phosphoric acid in chilled pestle-mortar and centrifuged at low speed (2500rpm) for 15min at 4°C. The residues were discarded and the supernatants were used for estimations²⁹. Estimation for total glutathione content (GSH) was carried out by homogenising fresh plant tissue (both root and shoot) in pestle and mortar under ice-cold conditions in 0.02 M disodium salt of Ethylenediaminetetracetic acid. The homogenate was centrifuged at 3000g for 15 min at 4°C. The pellet was discarded and supernatants were kept ice-cold until used³⁰.

iv. Statistical Analysis

Statistical comparisons of the data obtained were performed using Assistat (7.6) beta software (Federal University of Campina-Grande City, Campina Grande, Brazil). For each assay, data were subjected to one-way analysis of variance (ANOVA). Comparisons between means of treatment combinations were compared by Tukey's multiple range test ($P < 0.05$).

RESULTS

1. Morphological parameters

In the susceptible cultivar, highly significant effect of 28-Homobrassinolide was observed on percentage germination ($F_{0.01} = 18.492$; $p < 0.01$). Maximum germination was found in 10^{-9} M followed by 10^{-7} and 10^{-11} M. Similarly, in resistant plants, HBI pre-sowing treatment significantly ($F_{0.01} = 84.480$; $p < 0.01$) enhanced the percentage germination. Total plant height in Pusa Ruby was slightly suppressed in Control II when compared with the normal plants. However, with HBI treatment, the total plant height got enhanced in a linear trend. Total plant height analyzed in the resistant

cultivar illustrated a slight increase post-nematode inoculation as compared to non-inoculated plants. Further, it enhanced linearly with HBI concentrations ($F_{0.01} = 36.964$; $p < 0.01$). However, total plant biomass increased in both the cultivars after nematode inoculation and further with brassinosteroid treatment (Table1).

2. Antioxidative enzymes

Specific activity of CAT in Pusa Ruby roots showed enhanced activity after nematode inoculation as compared to Control I. Further, when the seeds were treated with HBI, CAT activity was found to increase significantly ($F_{0.05} = 4.484$; $p < 0.05$) with an increase in concentration. Alternately, in case of APOX and GPOX, specific activities were found suppressed in nematode inoculated plants in comparison to normal plants. But here also, specific activities of enzymes increased with increase in HBI concentrations though non-significantly. Observations recorded for GR showed similar trend as that of CAT where specific activity of GR got enhanced significantly ($F_{0.05} = 3.940$; $p < 0.05$) after nematode infection and brassinosteroid treatment. In case of GPOD, enzyme activity was found to decrease with nematode inoculation but it increased with steroid treatment. On the contrary, specific activity of SOD increased post-nematode inoculation and it decreased significantly ($F_{0.05} = 5.563$; $p < 0.05$) with increase in concentration although it was found to be higher than the control plants. In shoots, the specific activities of CAT and APOX were enhanced after nematode infection and further with steroid application. Also, the specific activity of GPOX which got suppressed in Control II also got enhanced in HBI treated plants. The specific activities of GR and GPOD were found to increase both post-nematode inoculation and HBI treatment significantly ($F_{0.05} = 3.642$; 3.641 ; $p < 0.05$ respectively). In case of SOD, specific activity of enzyme got enhanced in Control II but it decreased with increase in HBI concentration (Table2). In the roots of resistant cultivar (PNR-7), specific activities of all antioxidative enzymes were found to increase in Control II when compared to Control I. Further, when HBI seed treatment was applied, specific activities of these enzymes further increased significantly especially in APOX, GPOX and GPOD ($F_{0.05} =$

4.000; 19.657; 65.111; $p < 0.01$; 0.01 respectively) while specific activities of CAT and SOD increased non-significantly. On the other hand in shoots, specific activity of CAT got suppressed in Control II when compared to Control I but got increased with increase in HBI concentrations. Enzyme activity recorded for APOX and GPOX increased post-nematode inoculation in comparison to Control I and as the HBI concentrations increased, the enzyme activities also increased significantly ($F_{0.05} = 4.110$; 4.238; $p < 0.05$ respectively). On the other hand, GR activity was found to decrease after nematode infection but the activity level again regained with brassinosteroid treatment. Specific activities of GPOD and SOD also demonstrated similar results. In both the cases, enzyme activities increased post-nematode inoculation and HBI treatment significantly ($F_{0.05} = 4.834$; 5.917; $p < 0.05$ respectively) (Table3).

3. Non-Enzymatic Antioxidants

Estimations of non-enzymatic antioxidants in the roots of susceptible plants demonstrated decreased activities of TPC, TFC and GSH after nematode inoculation. However, brassinosteroid application resulted in an

overall increase in TPC, TFC and GSH. In contrast, AsC increased slightly in Control II but was found suppressed significantly ($F_{0.05} = 4.275$; $p < 0.05$) after HBI treatment. In the shoots, TPC, TFC and AsC were enhanced after nematode infection. Further, with HBI application, the overall content of TPC and TFC increased and that of AsC decreased. GSH was also found suppressed in Control II but an overall increase was observed in HBI treated plants (Table4). In roots of PNR-7, increase in TPC, AsC, GSH and decrease in TFC were observed in Control II as compared to Control I. Further, in HBI treated seedlings, the overall content of these antioxidants was found enhanced. In shoots, TPC, AsC and GSH were enhanced while TFC was found suppressed in nematode inoculated seedlings. But, the overall content of TPC, AsC and GSH was found enhanced (significantly in TPC and GSH ($F_{0.01} = 22.082$; 25.114; $p < 0.01$ respectively) and that of TFC was found significantly reduced ($F_{0.01} = 107.253$; $p < 0.01$) (Table5). In addition to these, when the two cultivars were compared with each other, overall higher values were seen in the resistant plants as compared to the susceptible check for all the parameters taken into account.

Table I
Effect of 28-Homobrassinolide on morphological parameters of tomato cultivars 72hrs after nematode inoculation

Parameters Studied		Mean \pm S.E.M.					F value (df=4)
		Control I	Control II	10^{-11} M	10^{-9} M	10^{-7} M	
Percentage Germination	Pusa Ruby	54.22 \pm 1.47 ^b	55.66 \pm 4.20 ^b	60.11 \pm 1.44 ^b	75.33 \pm 1.45 ^a	73.00 \pm 1.53 ^a	18.492 ^{**}
	PNR-7	60.61 \pm 0.31 ^c	62.66 \pm 0.71 ^c	71.97 \pm 0.98 ^b	82.52 \pm 1.60 ^a	78.77 \pm 1.16 ^a	84.480 ^{**}
Total Plant Height (cm)	Pusa Ruby	6.74 \pm 1.16	6.65 \pm 1.22	8.51 \pm 0.86	8.55 \pm 0.081	8.68 \pm 0.91	1.217 ^{ns}
	PNR-7	5.54 \pm 0.31 ^d	5.88 \pm 0.24 ^{cd}	7.36 \pm 0.73 ^{bc}	8.85 \pm 0.059 ^b	11.00 \pm 0.062 ^a	36.964 ^{**}
Total Plant Biomass (gm)	Pusa Ruby	0.438 \pm 0.027 ^a	0.476 \pm 0.003 ^a	0.501 \pm 0.001 ^a	0.578 \pm 0.01 ^a	0.550 \pm 0.001 ^a	1.338 ^{ns}
	PNR-7	0.367 \pm 0.01 ^c	0.431 \pm 0.018 ^{bc}	0.470 \pm 0.001 ^{bc}	0.518 \pm 0.004 ^{ab}	0.597 \pm 0.021 ^a	15.3410 ^{**}

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

Table II
Effect of 28-Homobrassinolide on specific activities of antioxidative enzymes of Pusa Ruby after 72 hours of nematode inoculation

Enzyme studied		Mean \pm S.E.M. (Units/mg protein)					F value (df=4)
		Control I	Control II	10 ⁻¹¹ M	10 ⁻⁹ M	10 ⁻⁷ M	
Catalase	Root	15.02 \pm 3.99 ^b	39.96 \pm 14.5 ^{ab}	53.40 \pm 9.51 ^{ab}	68.20 \pm 10.3 ^{ab}	78.23 \pm 16.2 ^a	4.484*
	Shoot	30.88 \pm 3.49 ^b	31.02 \pm 6.01 ^b	72.00 \pm 13.6 ^a	58.62 \pm 8.00 ^{ab}	57.56 \pm 3.04 ^{ab}	5.288*
Ascorbate peroxidase	Root	0.097 \pm 0.035 ^a	0.073 \pm 0.022 ^a	0.117 \pm 0.016 ^a	0.148 \pm 0.008 ^a	0.228 \pm 0.114 ^a	1.192^{ns}
	Shoot	0.027 \pm 0.012 ^a	0.077 \pm 0.005 ^a	0.100 \pm 0.020 ^a	0.087 \pm 0.021 ^a	0.118 \pm 0.038 ^a	2.435^{ns}
Guaiacol peroxidase	Root	0.054 \pm 0.025 ^a	0.023 \pm 0.008 ^a	0.045 \pm 0.011 ^a	0.095 \pm 0.044 ^a	0.078 \pm 0.015 ^a	1.342^{ns}
	Shoot	0.051 \pm 0.002 ^a	0.031 \pm 0.009 ^a	0.043 \pm 0.009 ^a	0.062 \pm 0.020 ^a	0.077 \pm 0.016 ^a	1.905^{ns}
Glutathione reductase	Root	0.036 \pm 0.005 ^b	0.080 \pm 0.050 ^{ab}	0.113 \pm 0.069 ^{ab}	0.203 \pm 0.055 ^{ab}	0.373 \pm 0.111 ^a	3.940*
	Shoot	0.003 \pm 0.001 ^b	0.010 \pm 0.001 ^{ab}	0.016 \pm 0.005 ^{ab}	0.022 \pm 0.007 ^a	0.019 \pm 0.002 ^{ab}	3.642*
Glutathione peroxidase	Root	0.058 \pm 0.029 ^a	0.050 \pm 0.007 ^a	0.143 \pm 0.039 ^a	0.233 \pm 0.038 ^a	0.312 \pm 0.164 ^a	2.073^{ns}
	Shoot	0.004 \pm 0.001 ^b	0.011 \pm 0.003 ^{ab}	0.029 \pm 0.004 ^{ab}	0.029 \pm 0.004 ^{ab}	0.036 \pm 0.006 ^{ab}	3.641*
Superoxide dismutase	Root	15.57 \pm 2.54 ^b	28.85 \pm 3.91 ^{ab}	60.95 \pm 8.86 ^a	48.26 \pm 10.2 ^{ab}	35.02 \pm 8.50 ^{ab}	5.563*
	Shoot	8.01 \pm 2.77 ^a	10.53 \pm 2.74 ^a	7.69 \pm 1.28 ^a	7.08 \pm 0.965 ^a	7.85 \pm 1.41 ^a	0.449^{ns}

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

Table III
Effect of 28-Homobrassinolide on specific activities of antioxidative enzymes of PNR-7 after 72 hours of nematode inoculation

Enzyme studied		Mean \pm S.E.M. (Units/mg protein)					F value (df=4)
		Control I	Control II	10 ⁻¹¹ M	10 ⁻⁹ M	10 ⁻⁷ M	
Catalase	Root	77.83 \pm 25.00 ^a	80.05 \pm 3.49 ^a	107.56 \pm 30.9 ^a	90.31 \pm 5.90 ^a	128.69 \pm 13.6 ^a	1.252^{ns}
	Shoot	52.99 \pm 11.7 ^a	45.97 \pm 5.41 ^a	62.07 \pm 21.5 ^a	74.66 \pm 4.11 ^a	81.76 \pm 51.1 ^a	0.336^{ns}
Ascorbate peroxidase	Root	0.631 \pm 0.499 ^b	1.47 \pm 0.400 ^{ab}	2.46 \pm 1.300 ^{ab}	3.32 \pm 0.355 ^{ab}	4.40 \pm 0.739 ^a	4.000*
	Shoot	0.124 \pm 0.005 ^b	0.273 \pm 0.018 ^b	1.83 \pm 1.52 ^{ab}	4.50 \pm 1.16 ^a	2.44 \pm 0.472 ^{ab}	4.110*
Guaiacol peroxidase	Root	0.177 \pm 0.136 ^c	0.357 \pm 0.047 ^c	0.647 \pm 0.080 ^{bc}	1.318 \pm 0.058 ^{ab}	2.121 \pm 0.362 ^a	19.657**
	Shoot	0.046 \pm 0.006 ^b	0.122 \pm 0.033 ^b	0.458 \pm 0.399 ^{ab}	1.493 \pm 0.212 ^a	0.797 \pm 0.452 ^{ab}	4.238*
Glutathione reductase	Root	0.161 \pm 0.041 ^a	0.998 \pm 0.278 ^a	2.125 \pm 1.02 ^a	1.836 \pm 0.385 ^a	1.574 \pm 0.906 ^a	1.454^{ns}
	Shoot	0.024 \pm 0.007 ^a	0.011 \pm 0.001 ^a	0.610 \pm 0.524 ^a	1.096 \pm 0.872 ^a	0.904 \pm 0.567 ^a	0.914^{ns}
Glutathione peroxidase	Root	0.071 \pm 0.009 ^c	0.143 \pm 0.039 ^c	0.304 \pm 0.050 ^{bc}	0.524 \pm 0.036 ^b	1.206 \pm 0.103 ^a	65.111**
	Shoot	0.028 \pm 0.004 ^b	0.067 \pm 0.014 ^{ab}	0.096 \pm 0.024 ^{ab}	0.155 \pm 0.017 ^{ab}	0.235 \pm 0.075 ^a	4.834*
Superoxide dismutase	Root	43.58 \pm 4.52 ^a	62.46 \pm 35.0 ^a	99.44 \pm 36.9 ^a	182.28 \pm 111 ^a	139.47 \pm 65.1 ^b	0.830^{ns}
	Shoot	25.47 \pm 11.8 ^b	57.52 \pm 12.6 ^{ab}	46.64 \pm 6.42 ^b	95.70 \pm 22.7 ^{ab}	138.22 \pm 28.8 ^a	5.917*

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

Table IV
Effect of 28-Homobrassinolide on antioxidant content in Pusa Ruby after 72 hours of nematode inoculation

Antioxidant studied		Mean \pm S.E.M. (mg/g)					F value (df=4)
		Control I	Control II	10 ⁻¹¹ M	10 ⁻⁹ M	10 ⁻⁷ M	
Total Phenolic Content	Root	71.14 \pm 2.94	64.69 \pm 3.73	70.74 \pm 4.85	76.12 \pm 3.39	90.74 \pm 5.48	2.081^{ns}
	Shoot	97.69 \pm 0.543	109.76 \pm 24.6	127.03 \pm 7.60	105.69 \pm 5.06	116.72 \pm 8.06	2.003^{ns}
Total Flavonoid Content	Root	3.68 \pm 0.051	3.54 \pm 0.069	3.52 \pm 0.033	3.64 \pm 0.00	3.56 \pm 0.019	2.458^{ns}
	Shoot	10.13 \pm 0.12 ^b	11.06 \pm 0.00 ^a	11.40 \pm 0.12 ^a	11.80 \pm 0.11 ^a	10.36 \pm 0.30 ^b	23.076**
Ascorbic acid Content	Root	42.08 \pm 1.26 ^a	43.79 \pm 3.75 ^a	35.14 \pm 0.16 ^a	43.25 \pm 1.09 ^a	35.95 \pm 1.84 ^a	4.275*
	Shoot	38.57 \pm 3.36	45.87 \pm 1.17	48.48 \pm 1.31	42.53 \pm 3.22	42.08 \pm 2.13	2.463^{ns}
Total Glutathione Content	Root	2.23 \pm 0.27	1.97 \pm 0.25	2.28 \pm 0.25	2.28 \pm 0.23	2.15 \pm 0.20	0.306^{ns}
	Shoot	3.57 \pm 0.40	3.05 \pm 0.26	2.97 \pm 0.33	3.33 \pm 0.24	4.17 \pm 0.25	2.536^{ns}

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

Table V
Effect of 28-Homobrassinolide on antioxidant content in PNR-7 after 72 hours of nematode inoculation

Antioxidant studied		Mean \pm S.E.M. (mg/g)					F value (df=4)
		Control I	Control II	10 ⁻¹¹ M	10 ⁻⁹ M	10 ⁻⁷ M	
Total Phenolic Content	Root	99.03 \pm 2.49 ^{ab}	105.89 \pm 3.02 ^b	109.40 \pm 3.23 ^b	116.77 \pm 2.26 ^a	115.54 \pm 2.14 ^a	13.498**
	Shoot	165.07 \pm 2.64 ^b	173.65 \pm 5.86 ^c	206.14 \pm 4.32 ^b	237.13 \pm 7.40 ^a	217.76 \pm 2.48 ^{ab}	
Total Flavonoid Content	Root	10.07 \pm 0.33 ^a	6.28 \pm 0.37 ^c	7.94 \pm 0.26 ^b	8.36 \pm 0.35 ^{bc}	7.96 \pm 0.36 ^{bc}	19.429**
	Shoot	27.87 \pm 0.26 ^a	14.08 \pm 0.15 ^c	18.88 \pm 0.68 ^b	12.97 \pm 0.73 ^c	13.08 \pm 0.88 ^c	
Ascorbic acid Content	Root	310.12 \pm 64.4	312.70 \pm 11.5	656.47 \pm 149	639.74 \pm 205	519.81 \pm 58.7	1.595^{ns}
	Shoot	52.08 \pm 1.87	67.83 \pm 1.68	91.80 \pm 14.5	79.59 \pm 9.01	101.26 \pm 1.49	
Total Glutathione Content	Root	2.73 \pm 0.11 ^{ab}	3.17 \pm 0.10 ^a	1.75 \pm 0.13 ^c	2.25 \pm 0.058 ^b	2.97 \pm 0.12 ^a	29.881**
	Shoot	1.27 \pm 0.057 ^c	2.39 \pm 0.028 ^b	3.15 \pm 0.17 ^a	2.49 \pm 0.11 ^c	2.51 \pm 0.28 ^c	

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

DISCUSSION

Concurrent environmental stresses are detrimental to plants, causing severe agricultural losses³¹. Among these, nematode infection is one such that exacerbates stress on plants, since their parasitism in roots severely disrupts nutrient flow thereby resulting in reduced growth³²⁻³³. In the current investigation also, modulations in the physiological parameters post-nematode inoculation has been observed. Plant growth parameters were suppressed with nematode inoculation in terms of total plant height but, total plant biomass was increased. However, in both cases HBI application resulted in increased height and biomass. This increase align with the fact that BRs application stimulated cell elongation, cell division and differentiation which ultimately promoted growth³⁴⁻³⁶. Similarly, BRs have been found to induce resistance in tobacco plants against tobacco mosaic virus, the bacterial pathogen *Pseudomonas syringae* and the fungal pathogen *Oidium* sp. In rice too, BR induced resistance to *Magnaporthe grisea* and *Xanthomonas oryzae*¹⁷. Besides this, overall activities of ROS scavenging enzymes were attenuated by HBI pre-treatment. This increase in the activities of enzymes was suggested as a factor contributing to BR induced disease resistance³⁷. These results are also in relation with the earlier studies where EBI pre-treatment greatly increased the activities of SOD, APOX, GPOX and CAT hence, suggesting less peroxidative stress in BR treated plants³⁸. Accumulation of antioxidants

often occurs in plants to interact with its environment for adaptation and defence against herbivores and pathogens³⁹⁻⁴⁰. An increase in the content of antioxidants after brassinosteroid treatment indicated that seed pre-treatment might be the result of activation of transcription/ translation of specific genes during pathogenesis. Such as increased phenolic and flavonoid synthesis increased the activities of enzymes such as polyphenol oxidase and phenylalanine ammonium lyase after pathogen infection⁴¹⁻⁴². Further, in susceptible plants, ascorbic acid content decreased. This decline suggested an induced accumulation of free radicals, which would injure the plant and causing its death. Similar results have also been documented in Japanese black pine during pine wood nematode *Bursaphelenchus xylophilus* infection. Here also, ascorbic acid content in pine seedlings decreased gradually after nematode invasion⁴³. Also, when the two cultivars were compared with each other, higher values were recorded in the resistant plants which are in concordance with earlier studies⁴⁴⁻⁴⁶.

CONCLUSION

Nematode stress in plants enhances reactive oxygen species (ROS) accumulation, thereby, disturbing its detoxification. These over generated ROS undergo a series of oxidation/reduction reactions (known as the Halliwell-Asada pathway)⁴⁷⁻⁴⁸. ROS-

scavenging antioxidant enzymes such as SOD, CAT, APOX, GR, DHAR, MDHAR, POD etc. maintain the balance between ROS production and its detoxification⁴⁹ along with activation of some secondary metabolites including phenols, tocopherols, carotenoids etc.⁵⁰⁻⁵³. Over-production of ROS under stress might increase oxidative degradation of phospholipids, resulting in the disruption of cell membrane permeability that cannot be restored by antioxidant enzymes⁵⁴⁻⁵⁵. Conclusively, it can be affirmed that HBI treatment made treated seedlings less affected to nematode stress by improving seed vigour, plant growth along with activated defence system. Further, detailed investigations pertaining to the additive/synergistic role of

BRs along with other phytohormones under stress could play a critical role in understanding the signalling mechanisms in plants.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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