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28-Homobrassinolide Induced Temperature Stress Tolerance in Mustard (*Brassica juncea* L.) Seedlings

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A B S T R A C T

Brassinosteroids (BRs) belongs to the new class of phytohormones holds promising potential in amplification of growth and development in plants under normal and stress conditions by mitigating the reactive oxygen species (ROS) in order to maintain cellular homeostasis. The current study was performed to examine the role of exogenously applied 28-Homobrassinolide (HBL) with different concentrations (0, 1 µM, 1nM, 1pM) in *Brassica juncea* seedlings subjected to cold stress (5°C) under laboratory conditions. Low temperature stress reduced the growth parameters but HBL treatment significantly augmented these morphological parameters. Further under cold stress condition, increase in H_2O_2 and MDA content was found, but their toxic effect was alleviated with exogenous appliance of HBL. Afterwards, amelioration in the content of non-enzymatic antioxidants such as carotenoids and vitamins were observed with the application of HBL which confirmed to be favourable in mitigating the harmful effects of low temperature stress. Overall, the seed treatment with HBL increased plant's potential to beat toxic effects imposed by low temperature stress by amplifying the ability of antioxidants to higher horizons thus paving the approach towards the use of eco-friendly perspectives in improving stress tolerance in plants.

***Key words*:** Low temperature stress, Growth, Reactive oxygen species, Antioxidants, 28-Homobrassinolide

Indian mustard belonging to the brassicaceae family has been consumed all over the world for centuries. Its roots, shoots and leaves are known as vegetables, whereas its seed are used as a condiment or a source of oil. In Ancient Greece it was used in cases of snake or scorpion bites, while in India and China mustard seeds have been devoured in traditional remedy since time immemorial for plentiful diseases and disorders. As an oilseed crop, after soybean and palm, rapeseed-mustard is the third most prominent crop [1]. Identification and development of abiotic stress tolerant cultivars are central economic goals for our globe. Thus, agronomical and morphological study of *Brassica* sp. performing under environmental extremes could direct the research and expansion of new stress-tolerant cultivars [2]. Due to their economic significance, as a source of good quality cooking oil, protein rich meal and multiple other value-added products, *B. juncea* is the focus of important research attention throughout the world.

Growth and development of plants depends effectively on the plant growth hormones that regulate wide array of physiological processes. Among the phytohormones, Brassinosteroids (BRs) are polyhydroxylated plant steroid hormones that can stimulate plant tolerance to variety of abiotic stress conditions including low and high temperature, drought and salinity [3-5]. BRs play protecting role in plants all the way through their developmental processes by regulating the different metabolites [6].

Every plant needs a particular temperature range to flourish well and thus temperature is the principal environmental factor that plays key role in plant growth and development. However, any kind of instability in the favourable temperature condition would put a close down on the function of physiological reactions as being sessile organisms; the plants cannot escape from these unparalleled changes. So, to tolerate dynamic temperature regime, plants must be capable to adjust their physiology actively in order to thrive well even under harmful environment [7]. Unfavourable conditions cause more production of reactive oxygen species (ROS), in so doing upsetting the cellular homeostasis. These ROS are accountable for peroxidation of lipids that escort to the irreparable metabolic, structural dysfunction and ultimately end in cell death [8]. In order to handle these ROS and sustain redox homeostasis, antioxidant defense system of plants acts as border line to protect them. High efficiency of these antioxidants can ease the oxidative damage under abiotic stress conditions [9].

Injuries are produced by stressful environments can be mitigated by synchronized regulation of different enzymatic reactions with both endogenous chemicals as well as with external signals and this synchrony is sturdily linked with phytohormones [10]. Therefore, BRs could be a vital guide for adaptive machinery in the unpleasant situations. In last few

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years, more attention has been given to the carotenoid group of pigments to understanding their purpose, mainly as antioxidants. The "core" structural part of carotenoids is a polyene backbone containing a sequence of conjugated C=C bonds. This feature is mainly accountable for the skill of these compounds to intermingle with free radicals and thus play role as effectual antioxidant [11]. Vitamins such as vitamin A (retinol), vitamin C (ascorbic acid) and vitamin E (tocopherol) acts as good antioxidants. Vitamin C is a key substrate for the detoxification of reactive oxygen entities [12]. Vitamin E is capablye to quench the singlet oxygen, scavenge different radicals, particularly the lipid peroxy radicals, and thus eventually cease the lipid peroxidation chain reactions [13]. Moreover, these vitamins are not only acknowledged for defending plant cells from free radicals but also for supplying the indispensable nutrients for plant growth [14].

On the basis of several benefits of BRs that it proposes to the plants, this present research was carried out to look at its functions under cold stress. This study was conducted to evaluate the protective role of Homobrassinolide (HBL) in *Brassica juncea* exposed to low temperature by taking following objectives (i) action of HBL on growth parameters (ii) role of HBL against H_2O_2 and MDA accumulation (ii) effect of EBL on non-enzymatic antioxidants.

MATERIALS AND METHODS

Plant material and growth treatments

For the present research, *B. juncea* (L.) cv. RLC-3 seeds were procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. Seeds were sterilized with sodium hypochlorite (0.1%) washing for 15 minutes, followed by rinsing in double distilled water (DDW) for 4-5 times. Then, sterilized seeds were soaked in different concentrations (0, 1 µM, 1nM, 1pM) of HBL for 8 hours. Afterwards, the seeds were allowed to germinate on Whatman filter paper No. 1 lined in sterile petri-plates for three days in seed germinator under controlled conditions as 25°C, 16/8 hours light/dark periods, uniform light fall of 200 PAR (Photosynthetically Active Radiation) $m^{-2}s^{-1}$ and 70% humidity. These germinated seeds were further transferred to brown germination paper. 8 days old seedlings were exposed to 5°C temperature shock up to three consecutive days for 5 hours daily. All these treatments and their duration were chosen on the basis of preliminary studies those were formerly conducted in the laboratory.

Growth measurement

Ten seedlings were selected with random sampling from each treatment on 13th day for shoot length and root length, measured with the ruler (cm). Fresh weight and dry weight of random ten seedlings were measured with an electronic laboratory weighing machine.

Estimation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content

The procedure of [15] was followed to quantify the H_2O_2 content. 500 mg of fresh sample was crushed in 0.1% trichloroacetic acid followed by centrifugation. Supernatant was collected and dissolved in 0.5 ml of 10 mM potassium phosphate buffer, 1 ml of potassium iodide and then absorbance was recorded at 390 nm. Known concentrations of H_2O_2 were used for preparing standard curve.

MDA content was estimated according to [16]. 1 ml of tissue extract was added to reaction solution of 2 mL consisting of 20% (v/v) trichloroacetic acid and 0.5% (v/v) thiobarbituric acid. Then the same was set aside in water bath at 95°C for 30 minutes. Later kept in ice bath for rapid cooling and followed by centrifugation at 10,000 × g for 10 minutes. Absorbance was taken at 450 nm, 532 nm and 600 nm.

Detection of carotenoids content

The carotenoid content was determined by following the method of [17]. 200 mg fresh sample tissue was crushed in 80% acetone. Supernatant was collected by centrifugation at 3000 × g. Optical density was recorded at 470 nm, 645 nm and 663 nm.

Determination of Vitamin A, C and E content

Vitamin A was estimated according to [18] method. Fresh tissue was homogenized in 2N KOH and heated gently at 60°C for 20 minutes. 20 ml of DDW was added to it and mixed properly. By using the separating funnel extraction was done with Petroleum Ether. Further, sodium sulphate was added to eradicate moisture of the sample. Dried residues were dissolved in 1 ml of Chloroform and 2 ml of TCA. Absorbance was taken at 620 nm.

Vitamin C was detected according to the method of [19]. Fresh sample tissue was used to prepare 2 ml of extract. 8 ml of 2, 6-dichlorophenol indophenols dye was added to the extract and absorbance was recorded at 530 nm.

Estimation of Vitamin E was done with [20] method. Fresh tissue of sample was mixed steadily with 0.1 N sulphuric acid and incubated at room temperature for overnight. To the tissue extract, xylene was added in 1:1 ratio and centrifuged. 1 ml of xylene was taken out and then mixed with 1 ml of 2, 2-dipyridyl. Absorbance was noted at 460 nm. Then 0.33 ml of $FeCl_3$ was added to the reaction mixture and after 15 min, absorbance was read at 520 nm.

Data analysis

One-way analysis of variance (ANOVA) was done with comparison of mean differences by Tukey's test using Prism Software 7. Data taken for calculations were the mean of three replicates (n = 3) and comparisons of p values\0.05 were considered significant and different from control.

RESULTS AND DISCUSSION

Growth parameters

In seedlings exposed to low temperature stress, there was significant decline in almost all growth parameters such as seed germination (Table 1), shoot and root length and in fresh and dry weight as shown in (Fig 1). Pre-sowing soaking treatment to seeds with various aforementioned concentrations of HBL improved the seed germination extensively under both room temperature and low temperature stress condition in comparison to analogous control. 1 nM HBL ameliorate the seed germination by 20% under normal conditions and 55% in the seedlings treated with 5°C temperature shock as compared to corresponding controls. Seedlings treated with HBL overcame the stress condition and supported the seedling growth.

Homobrassinolide (HBL) supplementations augmented both shoot and root length at its all concentrations. Best results were observed at 1 pM HBL for shoot length where 13% improvement was recorded and root length was ameliorated up to maximum with same concentration appliance by 22% over control. Further, shoot length and root length got affected under low temperature stress and reduced by 10% and 7% over their controls respectively. But pre-sowing soaking treatment of HBL to seedlings exposed to cold stress condition improved the shoot as well root length. Where, 1 nM Homobrassinolide

(HBL) demonstrated significant improvement by 31% in shoot length and 1 pM Homobrassinolide (HBL) concentration amplified root length by 24% as compared to respective controls.

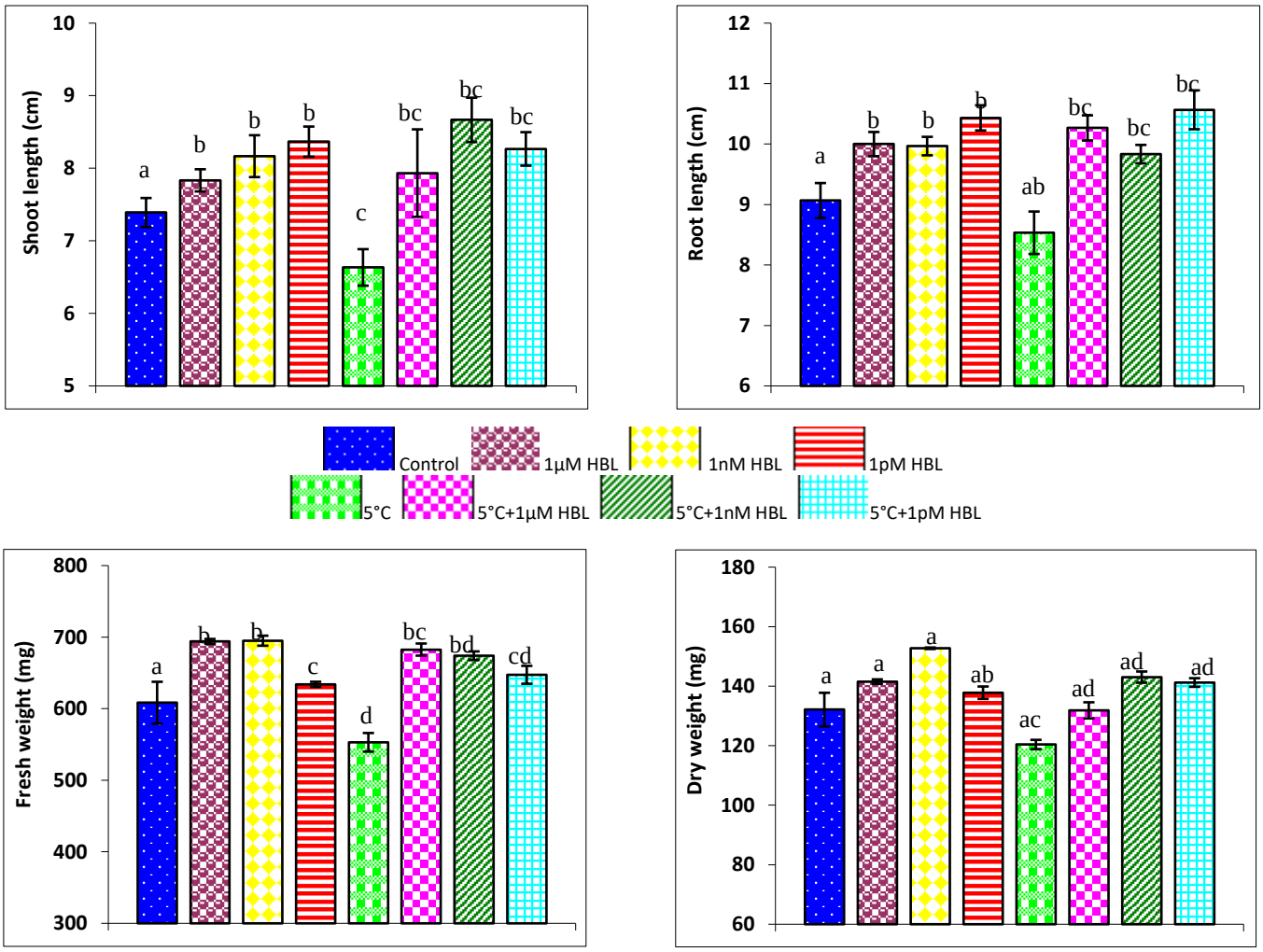

Fig 1 Effect of 28-Homobrassinolide on growth parameters of *B. juncea* seedlings under control and cold stress conditions

Measurement (A) Shoot Length, (B) Root Length (C) Fresh Weight and (D) Dry Weight. On 13th day, 10 random seedlings were collected to evaluate these parameters.

Data represent the mean of three replicates. Error bars represent standard deviation (SD) and different letters above the bars represent a significant difference as determined by one-way ANOVA.

Seedlings those were exposed to low temperature stress had diminution in fresh and dry weight. Exogenous application of Homobrassinolide (HBL) (1µM, 1nm, 1pM) exposed to low temperature stress enhanced the fresh weight evidently and maximum augmentation was shown by 23% under 1 µM Homobrassinolide (HBL) treatment in comparison to control. Seedlings also showed amelioration in dry weight with HBL supplementation with respect to control whether there was control or stressful condition. At all Homobrassinolide (HBL) concentrations mentioned above, increase in dry weight was reported and maximum amplification was observed under 1 nM EBL by 16% under normal conditions and by 19% under low temperature condition, with respect to analogous controls, respectively (Fig 1).

Table 1 28-Homobrassinolide improved the seed germination and limited the oxidative damage by diminishing H_2O_2 and MDA content in *B. juncea* seedlings under room temperature and low temperature stress condition

Treatments	Seed germination (%)	H_2O_2 content ($\mu g g^{-1}$FW)	MDA content ($\mu mol g^{-1}$FW)
Control	76.66±6.67^{a}	0.63±0.0^{a}	1.33±0.04^{a}
1 µM HBL	91.11±1.92^{b}	0.47±0.03^{b}	1.27±0.03^{a}
1 nM HBL	92.22±5.09^{b}	0.52±0.02^{c}	1.14±0.02^{a}
1 pM HBL	86.66±0.00^{a}	0.53±0.03^{c}	1.04±0.06^{a}
5°C	56.66±3.34^{c}	0.73±0.01^{d}	3.26±0.16^{b}
5°C +1 µM HBL	75.55±1.92^{ab}	0.56±0.01^{cd}	1.30±0.01^{ab}
5°C +1 nM HBL	87.78±3.85^{bc}	0.53±0.01^{cd}	1.17±0.01^{ab}
5°C +1 pM HBL	78.89±1.93^{ac}	0.62±0.00^{ab}	1.57±0.02^{c}
F ratio $_{(7.16)}$	30.34	67.02	331.9
	(A)	(B)	(C)

(A) After pre-sowing soaking treatment, the seed germination was recorded on 4th day; (B) H_2O_2 content was quantified on 13th day by following potassium iodide (KI) oxidation (C) Lipid peroxidation was measured as malondialdehyde (MDA) levels on 13th day. Fresh tissue of sample was collected, homogenized and used for TBA-based lipid.

Data is represented here with standard deviation (SD) and different letters after SD represent a significant difference as determined by one-way ANOVA and F ratio is simply signifying the ratio of two variances.

EBL decreased H_2O_2 and MDA content

Hydrogen peroxide and malondialdehyde (MDA) content was enhanced under stress which spots the onset of oxidative stress. Exogenous application of HBL declined the H_2O_2 content under stress or without stress condition. All HBL concentrations notably diminished the H_2O_2 content on 13th day of growth but it was 1 nM concentration that showed best results by 28% decrease in its content as compared to control under cold stress. Moreover, the HBL supplementation also decreased the MDA content significantly in the seedlings treated with stress as well as in seedlings those were raised under room temperature. 1 pM HBL appliance reduced its content up to minimum by 22% over that of control under room temperature and 1 nM HBL application reduced the MDA content by 64% in comparison to control treated with stress alone (Table 1).

Carotenoids content

Exposure of seedlings to low temperature stress resulted in enhancement in content if carotenoids up to 8% with respect to control on 13th day after sowing. Whereas HBL application ameliorated its content significantly under stress or normal conditions and maximum expansion was observed with the treatment of 1 pM HBL by 27% as compared to control under normal condition while 16% enhancement was recorded in the seedlings subjected to cold stress, in comparison to corresponding controls, respectively (Fig 2).

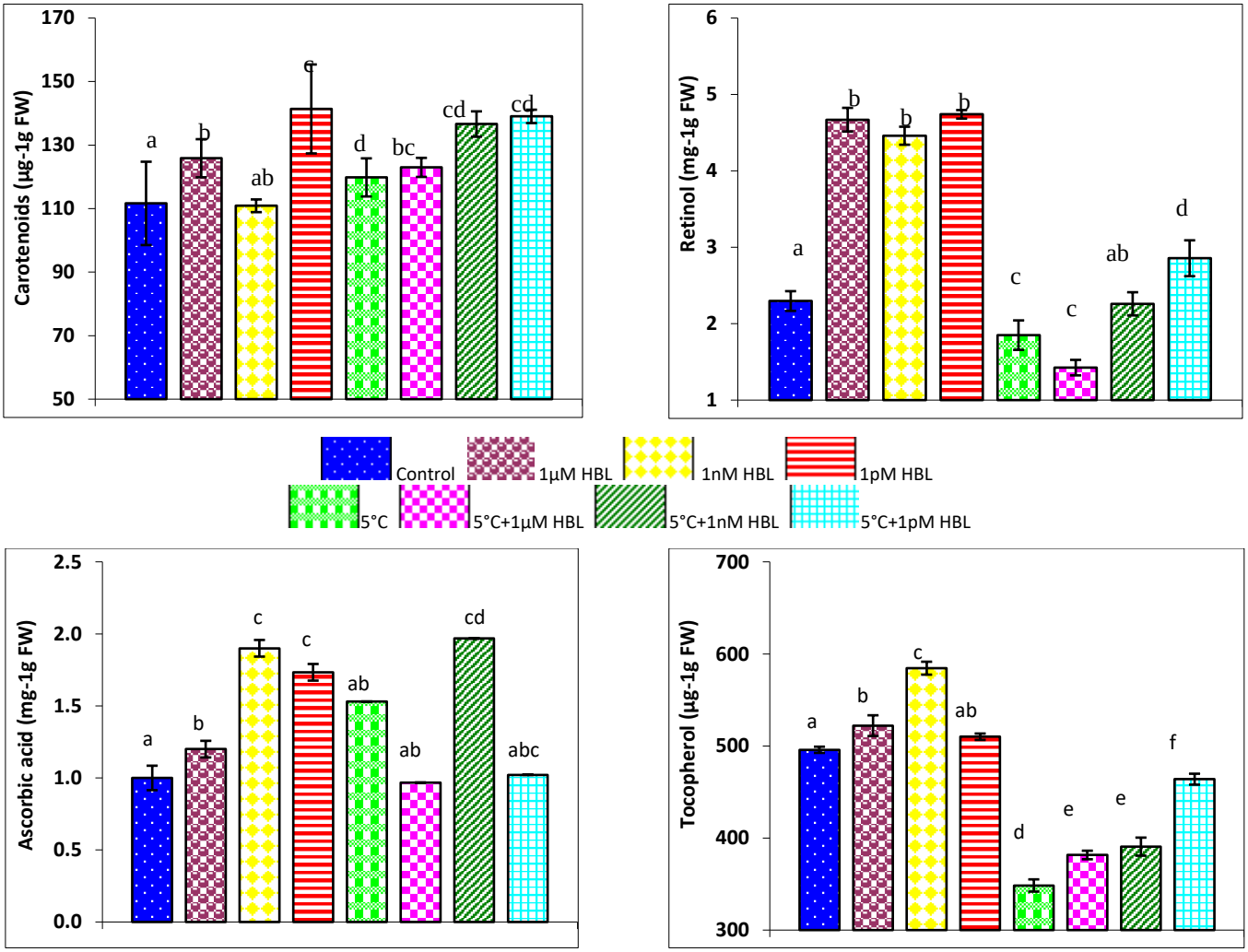

Fig 2 28-Homobrassinolide influenced the antioxidant levels in *B. juncea* seedlings under room temperature and cold stress conditions

Fresh sample tissue was collected on 13th day, homogenized and then centrifuged. Resulting supernatant was used for determining the content of non-enzymatic antioxidants as (A) Carotenoids, (B) Retinol, (C) Ascorbic acid and (D) Tocopherol.

Error bars represent standard deviation (SD) and different letters above the bars represent a significant difference as determined by one-way ANOVA.

Vitamins

Under the exposure to low temperature, Vitamin A and E content was decreased by 20% and 30% in comparison to their respective controls whereas vitamin C content was increased under stress condition by 53% over its control (Fig 2). Further, the significant enhancement was observed in vitamin A, C and E content in the seedlings treated with different concentrations of HBL under normal and stressful environment. However, for vitamins mentioned above, HBL behaved in a dose dependent manner. For vitamin A, 1 pM HBL treatment ameliorated the content by 106% under normal condition and

by 54% under cold stress condition in comparison to analogous control. Further, 1 nM EBL augmented the vitamin C content by 90% under room temperature and by 29% under temperature stress condition as compared to corresponding controls. The content of vitamin E was ameliorated up to maximum by 1 nM EBL under normal conditions by 18% and by 1 pM HBL under low temperature by 33% over their respective controls.

Temperature is a fundamental abiotic factor as it regulates several developmental processes from germination up to senescence in plants. These processes of growth and development engross a large number of biochemical reactions which necessitate optimum temperature for efficient operation [21]. But due to fluctuations in the environmental circumstances there always arise deviations in the temperature beyond the critical threshold that is sufficient to cause irreversible injuries to plant growth [22]. Plant growth hormones play vital role in making plants tolerant to the changing environmental conditions, by arbitrating growth and development. BRs have been found to exert anti stress effects on plants.

Present study was conducted to explore the defensive role of HBL in protecting the mustard seedlings against low temperature stress. In our findings, exogenous supplementation of HBL improved the seedling growth by avoiding the harmful effects of stress environment. These findings are in accordance with the reports of [23] where stress effected the growth but application of HBL significantly reduced the damaging effects. H_2O_2 plays twofold role in plants as at lower concentrations it behaves as a signaling molecule but escorts to oxidative damage at higher concentration [24]. Further we found that under cold stress, the levels of H_2O_2 rise rapidly marking the beginning of oxidative burst which further augmented the MDA content due to peroxidation of lipids. The increase in content of H_2O_2 under stress conditions is in accordance with the study of [25] on *Brassica juncea*. Oxidative burst leads to limited or complete dysfunction of cell membranes that ultimately causes lipid peroxidation and finally MDA as the end product [26]. In our findings, HBL lessened the H_2O_2 production and harmonize with the study of [27] and this result is providing confirmation for the fact that HBL amplifies the ROS scavenging capability of antioxidants in *B. juncea* seedlings that eventually reduce lipid peroxidation. Similar pattern of results was observed in *Zea mays* where BR protested the cell by inhibiting the formation of MDA [28].

Till date, a number of non-enzymatic antioxidants are known those play an imperative role in the detoxification of ROS. Among them, carotenoids are proficient antioxidants defending plants against oxidative harm [29]. Lycopene acts as an intermediate in biosynthetic pathway of carotenoids and also behaves as scavenger of ROS. In current study, amelioration in carotenoid content revealed that HBL played main role here to augment the content. Ascorbic acid also plays role in shielding organelles and cells from ROS [30]. It controls the cell division and cell expansion by acting as a cofactor of many enzymes, by modulating plant sense and further by implicating in hormone biosynthesis and antioxidant regeneration [31]. Moreover, the detoxification of H_2O_2 is done by ascorbate peroxidase where ascorbate acts as an electron donor [32]. In this study, elevation in content of ascorbic acid was reported which supported the survival of seedlings under stress conditions. Vitamin E plays an important role in the protection of thylakoid membrane from photo-oxidative damage [33]. Both tocopherol and ascorbate have metal ion chelating movement that reduces the formation of ROS. HBL showed enhancement in the content of these antioxidants under normal or stress conditions.

CONCLUSION

In conclusion, examination of the role of Homobrassinolide (HBL) was found to improve the antioxidant defense system of *Brassica juncea* (L.) to combat the ROS imbalance under cold stress. This investigation further combined the fact that HBL seed priming diminished the extent of damage proficiently at harsh low temperature stress by reducing the effect of stress on growth and permitting the adaptation to unfavourable changes of environment. Thus, due to its ameliorative potential in making crops/plants tolerant to harsh environment; Homobrassinolide (HBL) may prove to be an excellent candidate for *Brassica juncea* (L.) to look after from various stress conditions.

LITERATURE CITED

1. Shahi SK, Kumar A, Kumar S, Vaishya NK. 2022. Effect of sulphur and zinc nutrition on growth and yield performance of mustard (*Brassica juncea* L.) and soil properties. *Res. Jr. Od. Agril. Sciences* 13(1): 081-085.
2. Mohan N, Kumari N, Jattan M, Avtar R, Rani B. 2020. Response of antioxidative system of *Brassica juncea* (L.) Czern. to terminal heat stress. *Bangladesh Journal of Botany* 49(4): 1185-1188.
3. Harpreet K, Geetika S, Renu B, Poonam S, Mir M. 2014. 28-homobrassinolide modulate antenna complexes and carbon skeleton of *Brassica juncea* L. under temperature stress. *Journal of Stress Physiology and Biochemistry* 10(3): 186-196.
4. Serna M, Coll Y, Zapata PJ, Botella MÁ, Pretel MT, Amorós A. 2015. A brassinosteroid analogue prevented the effect of salt stress on ethylene synthesis and polyamines in lettuce plants. *Scientia Horticulturae* 185: 105-112.
5. Sharma I, Kaur N, Pati PK. 2017. Brassinosteroids: A promising option in deciphering remedial strategies for abiotic stress tolerance in rice. *Frontiers in Plant Science* 8: 2151.
6. Sirhindi G, Kaur H, Bhardwaj R, Nirmal KS, Sharma P. 2014. Thermo-protective role of 28-homobrassinolide in *Brassica juncea* plants. *American Journal of Plant Sciences* 5(15): 2431.
7. Kaur H, Sirhindi G, Bhardwaj R. 2017. Influence of 28-homobrassinolide on photochemical efficiency in *Brassica juncea* under dual stress of extreme temperatures and salt. *Canadian Journal of Pure and Applied Sciences* 11: 4205-4213.
8. Yin YL, Zhou Y, Zhou YH, Shi K, Zhou J, Yu Y, Yu JQ, Xia XJ. 2016. Interplay between mitogen-activated protein kinase and nitric oxide in brassinosteroid-induced pesticide metabolism in *Solanum lycopersicum*. *Jr. Hazard Mater.* 316: 221-231.
9. Ahmad P. 2018. Upregulation of antioxidant and glyoxalase systems mitigates NaCl stress in *Brassica juncea* by supplementation of zinc and calcium. *Journal of Plant Interactions* 13: 151-162.
10. Pacifici E, Polverari L, Sabatini S. 2015. Plant hormone cross-talk: the pivot of root growth. *Journal of Experimental Botany* 66(4): 1113-1121.
11. Young AJ, Lowe GL. 2018. Carotenoids-antioxidant properties. *Antioxidants* 7(2): 28.
12. Qian HF, Peng XF, Han X, Ren J, Zhan KY, Zhu M. 2014. The stress factor, exogenous ascorbic acid, affects plant growth and the antioxidant system in *Arabidopsis thaliana*. *Russian Journal of Plant Physiology* 61(4): 467-475.
13. Schneider C. 2005. Chemistry and biology of vitamin E. *Molecular nutrition & food research* 49(1): 7-30.

14. Blesseena A, Deotale RD, Raut DA, Pise S. 2020. Efficiency of foliar fertilization of tocopherol and micronutrients on chemical, biochemical parameters, yield and yield attributing factors in chickpea. *Journal Soils and Crops* 30(1): 74-80.
15. Velikova V, Yordanov I, Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. *Plant Science* 151: 59-66.
16. Heath RL, Packer L. 1968. Photoperoxidation in isolated chloroplasts. *Archives of Biochemistry and Biophysics* 125: 189-198.
17. Lichtenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* 148: 350-382.
18. Bayfield RF, Cole ER. 1980. Colorimetric estimation of vitamin A with trichloroacetic acid. *Methods in Enzymology* 67: 189-195.
19. Chinoy JJ, Singh YD, Gurumurti K. 1976. Colorimetric determination of ascorbic acid turnover in plants. *Indian Journal of Plant Physiology* 19(2): 121-130.
20. Rosenberg HR. 1992. *Chemistry and Physiology of Vitamins*. Inter Science Publishers Inc New York. pp 452-453.
21. Źróbek-Sokolnik A. 2012. Temperature stress and responses of plants. *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate*. Springer, New York, NY. pp 113-134.
22. Wahid A, Gelani S, Ashraf M, Foolad MR. 2007. Heat tolerance in plants: an overview. *Environmental and Experimental Botany* 61(3): 199-223
23. Fariduddin Q, Yusuf M, Chalkoo S, Hayat S, Ahmad A. 2011. 28-homobrassinolide improves growth and photosynthesis in *Cucumis sativus* L. through an enhanced antioxidant system in the presence of chilling stress. *Photosynthetica* 49(1): 55-64.
24. Quan LJ, Zhang B, Shi WW, Li HY. 2008. Hydrogen peroxide in plants: A versatile molecule of the reactive oxygen species network. *Journal of Integrative Plant Biology* 50: 2-18.
25. Sharma N, Hundal GS, Sharma I, Bhardwaj R. 2014. 28-Homobrassinolide alters protein content and activities of glutathione-Stransferase and polyphenol oxidase in *Raphanus sativus* L. plants under heavy metal stress. *Toxico. Int.* 21: 44.
26. Ahmad P, Hashem A, Abd-Allah EF, Alqarawi AA, John R, Egamberdieva D, Gucel S. 2015. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. *Frontiers in Plant Science* 6: 868.
27. Ahmed AHH, Darwish E, Alobaidy MG. 2017. Impact of putrescine and 24-epibrassinolide on growth, yield and chemical constituents of cotton (*Gossypium barbadense* L.) plant grown under drought stress conditions. *Asian Jr. Plant Science* 16: 9-23.
28. Arora N, Bhardwaj R, Sharma P, Arora HK. 2008. 28-Homobrassinolide alleviates oxidative stress in salt-treated maize (*Zea mays* L.) plants. *Brazilian Journal of Plant Physiology* 20: 153-157.
29. Stahl W, Sies H. 2003. Antioxidant activity of carotenoids. *Molecular Aspects of Medicine* 24(6): 345-351.
30. Al-Mukhtar N, Mohmmed A, Mahdi H. 2016. The effects of potassium dichromate on testes, accessory sex glands, liver and kidney in adult male rats treated with ascorbic acid. *Australian Journal of Basic and Applied Sciences* 10(4): 99-108.
31. Gallie DR. 2012. The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. *Journal of Experimental Botany* 64(2): 433-443
32. Das K. 2020. Ascorbate and tocopherols in mitigating oxidative stress. protective chemical agents in the amelioration of plant abiotic stress: *Biochemical and Molecular Perspectives*. pp 102-121
33. Havaux M, Eymery F, Porfirova S, Rey P, Dörmann P. 2005. Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. *The Plant Cell* 17(12): 3451-3469.