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ORIGINAL ARTICLE

Brassinosteroids Denigrate the Seasonal Stress through Antioxidant Defense System in Seedlings of *Brassica juncea* L.

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The present work has been undertaken to study the effect of exogenously application of 24-epiBL and 28-homoBL on soluble protein, proline contents and antioxidant defense system of *Brassica juncea* L. RLM 619 under the influence of seasonal stress. It was observed that 24-epiBL and 28-homoBL treatment enhance the soluble protein, dry weight and shoot length of *B. juncea* seedlings under seasonal stress. If seeds treated with the different concentrations (10^{-6}, 10^{-8} and 10^{-10} M) of 24-epiBL and 28-homoBL revealed batter growth, protein and proline contents as compare to untreated seedlings. Similarly the activities of antioxidant enzymes SOD, CAT, APOX, DHAR, PPO and Auxinases were enhanced by the application of different concentration of both brassinosteroids, whereas MDA content was decrease with both brassinosteroids treatments. Then we have concluded that both brassinolides have the seasonal stress ameliorative properties in *B. juncea* seedlings grown under the influence of seasonal stress. This study culminates to the role of brassinolides as an anti-stress property for protection of plant from various types of stresses.

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Key words: Antioxidant enzymes, Brassica juncea, Brassinosteroids, ROS, Seasonal stress

Abbreviations: 24-epiBL - 24 Epibrassinolide, 28-homoBL – 28 Homobrassinolide, B. juncea – Brassica juncea, SOD – Superoxide dismutase, CAT – Catalase, APOX – Ascorbate peroxidase, DHAR – Dehydroascorbate Reductase, PPO – Polyphenol Oxidase, IAA – Indole Acetic Acid, MDA- malondialdehyde

Plants are confined to the place where they grow. They have a limited capacity to avoid unpredictable unfavorable changes in their environment confrontation with extreme of temperature, water shortage insufficient or excessive light or mineral nutrients attack by pathogenic bacteria, fungi viruses and viroids. They have developed ingenious molecular strategies to define themselves against such biotic and abiotic stresses, most often combined with an alteration of

growth and developmental patterns. Seasonal stress intimately associated with the external condition that adversely affects growth and development or productivity of the plants. It is now well known that seasonal stress limits the productivity of plants due to the production of reactive oxygen species (ROS) such as superoxide anion ($O^{\cdot}_2$), hydroxyl ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) radicals, which cause oxidative damage in plants (McCord 2000). To scavenge these toxic species, plants have antioxidant enzymes, such as superoxide dismutase (SOD EC 1.15.1.), peroxidase (POX EC 1.11.1.7), ascorbate peroxidase (APOX EC 1.11.1.11), catalase (CAT EC 1.11.1.6), dehydroascorbate reductase (DHAR EC 1.8.5.1) and glutathione reductase (GR EC 1.6.4.2). Since their activities and transcripts are altered when plants are subjected to stress, changes in the levels of antioxidant enzymes have been used to assess the effect of different stressors (Hernandez *et al.,* 2000). Several plant hormones are implicated in modulating the responses to various stresses, including ethylene (Vahala *et al.,* 2003), abscisic acid (Kovtun *et al.,* 2000), salicylic acid (Metwally *et al.,* 2003) and brassinosteroids (Ozdemir *et al.,* 2004). Brassinosteroids are now considered as the 6th group of phytohormones with significant growth promoting influence (Clouse and Sasse, 1998; Rao *et al.,* 2002). The ability of brassinosteroids to confer resistance to plants against abiotic stresses is gaining much attention. Brassinosteroids increased tolerance to high temperature in wheat leaves (Kulaeva *et al.,* 1991) and brome grass (Wilen *et al.,* 1995) and increased tolerance to drought stress in sugar beet (Schilling *et al.,* 1991). The ability of 28-homobrassinolide to confer resistance to moisture stress in wheat and I L. has also established (Sairam 1994, kumar 2012). We were investigating the exogenous application of 24-epiBL and 28-homoBL have any protective role on dry weight, soluble protein content and antioxidant defense system along with proline content PPO and IAA in seedlings of *B. juncea* under seasonal stress.

MATERIALS AND METHODS

Study Material:

The seeds of *B. juncea* L. cv. RLM 619 were procured from Department of Plant Breeding, Punjab Agriculture University, Ludhiana, India. Seeds were surface sterilized with 0.01% $HgCl_2$ and rinsed 5-6 times with double distilled water. The sterilized seeds were soaked for 5 h in different concentrations of 24-epiBL and 28- homoBL (0, 10^{-6}, 10^{-8} and 10^{-10} M). The treated seeds were sown in three replications in soil under field conditions. Morphological data in terms of seedling growth (shoot length) and dry weight were measured at regular interval of 15 days until maturity. The dry weight was calculated by using formula Dry weight = dry weight/ fresh weight X 100. The samples were harvested after regular interval of 15 days after sowing for the various biochemical analyses.

Enzymatic Analysis:

Estimation of various antioxidant enzyme activities and total protein content were done regular interval of 15th days to maturity by homogenizing 1g fresh plant material in 3 ml of 100 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 4°C for 20 min at 15000x g. The supernatant was used for estimation of total protein content and antioxidant enzyme activities (SOD, CAT, APOX, and activities of PPO (EC 1.14.18.1) and IAA. For proline content the tissue was grind with sulphosalysilic acid and for MDA content tissue was homogenized with TCA solution. Activity of SOD was estimated by studying the

increase in absorbance of superoxide nitro blue tetrazolium at 540 nm using the method of Kono (1978). CAT activity was measured according to Aebi (1983). 3ml reaction mixture containing 1.5 ml of 100 mM phosphate buffer (pH 7.0), 0.05 ml of 75 mM H_2O_2, 0.05 ml enzyme extract and distilled water was taken. The reaction was started by addition of H_2O_2 and CAT activity was measured as decrease in absorbance at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed per minute. Activity of APOX was measured following the method of Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm. DHAR activity was measured according to the method of Dalton *et al.* (1986). DHAR activity was assayed by following the increase in absorbance at 265nm. Enzyme activity was determined using the extinction coefficient of 6.2 mM^{-1} cm^{-1}. The reaction mixture contained 100 mM phosphate buffer (pH 7.0), 5 mM Ascorbate, 5 mM H_2O_2 and the enzyme extract. PPO activity was measured using the method of Bastin and Unleur (1972). Lipid peroxidation was determined as the content of malondialdehyde (MDA) using the thiobarbituric acid (TBA) reaction as described by Heath and Peacker (1968). The reaction mixture contained 60 mM phosphate buffer (pH 7.0) and 0.01 M chlorogenic acid. Reaction mixture was incubated at 30°C ± 2°C for one hour. OD was measured at 430 nm. IAA activity was measured according to method of Gordon and Weber (1951). Reaction mixture contained salkowski reagent and 0.01 % IAA solution. Enzyme extract was incubated at 40°C for 20 min in dark. Carbohydrate content was estimated by the method of Dubois *et al.* (1956). Reaction mixture contains phenol reagents and H_2SO_4 then sample incubated at room temperature for 30 minutes and absorbance was taken at 485 nm. Proline accumulation was determined using the method of Betes *et al.* (1973). Fresh sample was homogenized with 3% sulphosalicylic acid and 72h that proline was released the homogenate was centrifuged at 3000g for 20 min the supernatant was treated with treated with acetic and ninhydrin boiled for 1h and then absorbance at 520nm was determined by Uv-visible spectrophotometer. Contents of proline were expressed as mg g^{-1} FW. Protein estimation was estimated by following the method of Lowry *et al.* (1951).

Statistical analysis:

All analyses were done on a completely randomized design. All data obtained was subjected to one-way analysis of variance (ANOVA) and the mean differences were compared by honestly standard deviation (HSD) test. Each data was the mean of three replicates (n =3) except for shoot lengths of *B. juncea* seedlings (n =10) and comparisons with P-values <0.05 were considered significantly different.

RESULTS

24-epoBL and 28-homoBL treated plants exhibiting a significant increase in growth and developments. The maximum seed emergence of mustard seedlings were found in 10^{-8} M conc of 28-homoBL as compared to control and other concentration of both brassinolides (Fig. 1). Moreover, maximum increase in shoot length was observed in 10^{-8} M 28-homoBL treated plants 19.59 ± 0.689 cm as compared to control plants 13.73 ± 1.50 cm at 15 days old seedlings. The shoot length were exhibiting increasing trend with time as shown in Fig 1. In case of moisture content the maximum moisture content was observed in 10^{-10} M conc of 24-epiBL as compared to control plants. The moisture content was decrease with time, the

maximum moisture content was found at 15 days and minimum at maturity time Fig. 1. Maximum dry weight was found in 10^{-10} M 24-epiBL as compared to control plants. The dry weight was increased with time and maximum dry weight was observed at maturity time. (Fig 1.)

The protein content was increase with the treatments of both brassinolides. But maximum protein content was observed in 10^{-8} M conc of 24-epiBL. The protein content was exhibiting the increasing trend with time but during maturity, the protein content was decrease as shown in Fig. 1. The activities of antioxidant enzymes were increase with the treatment of both brassinolides SOD activity was maximum in 10^{-10} M conc of 28-homoBL as compared to control plants. The activity of SOD was found to be increase with the time. The maximum activity of SOD was observed at the time of 60 days i.e. at the reproductive phase of the plants. The activity of SOD was decrease at time of maturity (Fig.3). Similarly the activity of cat was found maximum in 10^{-10} M conc of 28-homoBL treated plants as compared to controls. CAT activity was also maximum at reproductive phase and minimum at the time of maturity (Fig. 3). APOX and POD activities were also exhibiting increasing trend with both brassinolides as compared to control. The activities of both enzymes were increase with time. Maximum activities were found at reproductive phase and minimum at maturity phase as shown in Fig. 2 and 3. The activities of DHAR and MDHAR were also increase with the treatments of both brassinolides as compared to control and maximum activities of both enzymes were maximum at reproductive phase Fig. 8 and 9. Whereas the MDA content was decrease with the treatment of both brassinosteroids as compared to control plants. The MDA content was increase with time and maximum MDA content was observed at maturity phase, which indicate the more accumulation of ROS at maturity. The activities of PPO and IAA also enhanced with the treatment of both brassinosteroids treatments as compared to control. The activities of both enzymes were increased with time and maximum activities were observed at reproductive phase. The proline content was exhibiting similar trend with brassinolides.

DISCUSSION

The plants acclimatize the negative effects of seasonal stress and show specific and nonspecific response, which leads to tolerance to the abiotic stress. The general stress response reactions involving plant growth regulators are of prime importance in stress protection and tolerance mechanisms. Brassinolides play an essential role in plant growth, development and are involved in the modulation of stress responses (Sirhindi *et al.,* 2009; Cao *et al.,* 2005; Hayat *et al.,* 2007). Enhanced resistance of brassinosteroids treated plants to extreme temperature, salt, pathogen attack and environmental stresses have been reported by Krishna (2003). The present study revealed the effect of 24-epiBL and 28-homoBL in reducing the toxic effect of seasonal stress by improving the antioxidant defense system. In present study the total soluble protein contents were enhanced in seedlings of *B. juncea* after the pre-soaking treatments of both brassinolides as compare to control seedlings. Seasonal stresses often cause membrane damage, decrease hydrolytic enzyme activity and increased lipid peroxidation in cells. It may stimulate formation of ROS, Such as H_2O_2, $O^{\cdot 2-}$ and $OH^{\cdot}$ radicals.

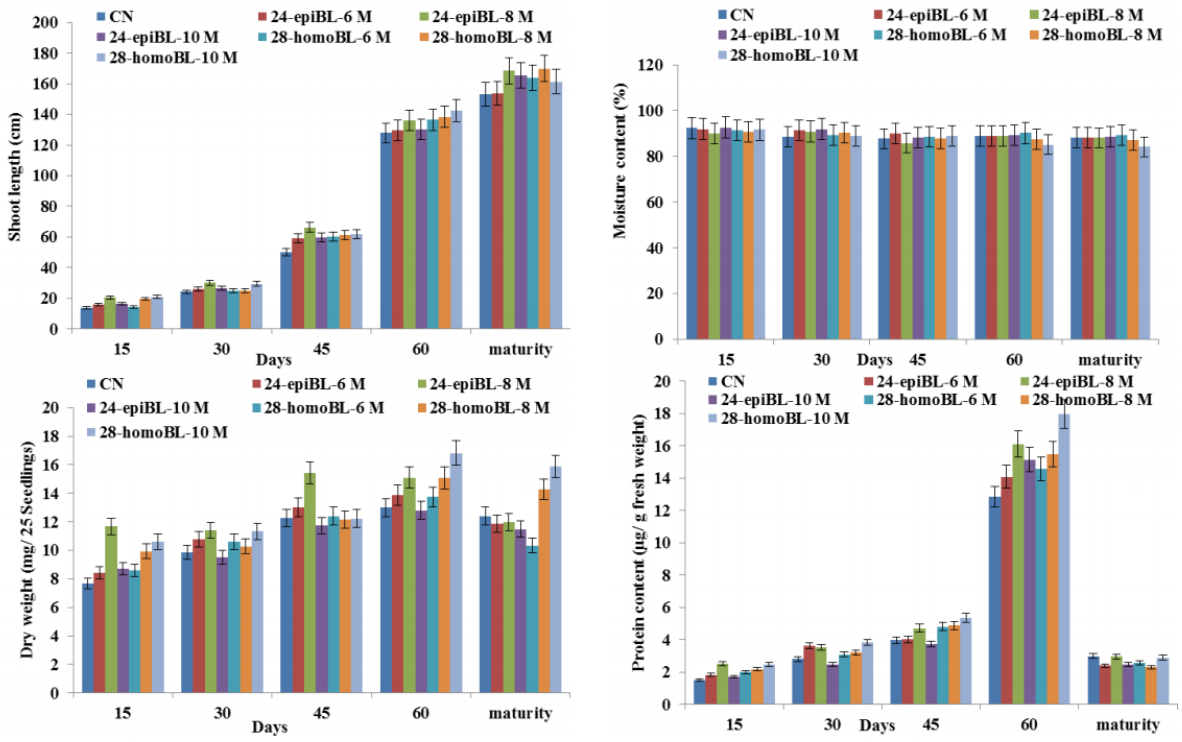

Figure 1. Effects of seasonal stress and different concentration (10^{-6}, 10^{-8}, 10^{-10} M) of 24-epiBL and 28-homoBL on Shoot length (A), Percentage moisture content (B), Dry weight (C), and Protein contents (D) Data are mean of three replicate and are significant difference from control at P<0.05.

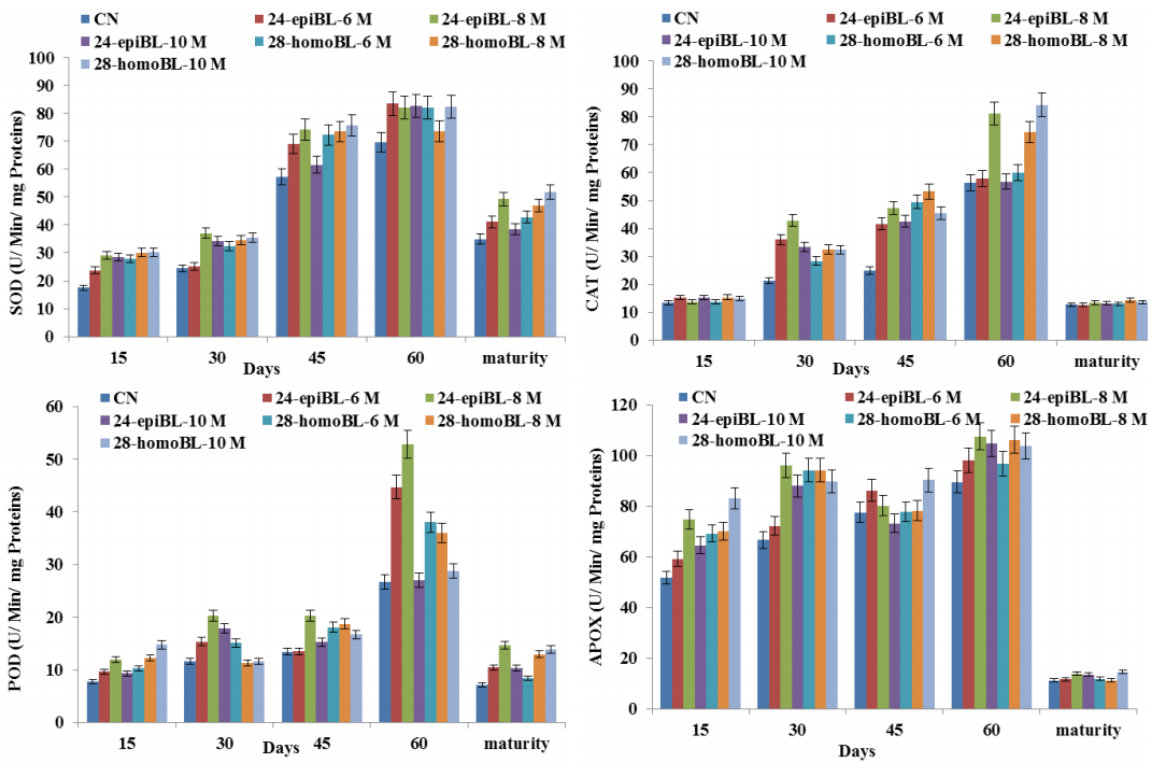

Figure 2. Effects of seasonal stress and different concentration (10^{-6}, 10^{-8}, 10^{-10} M) of 24-epiBL and 28-homoBL on Superoxide dismutase (A), Catalase (B) (C), Guaiacol peroxidase and Ascorbate peroxidase (D) Data are means of three replicate and are significant difference from control at P<0.05.

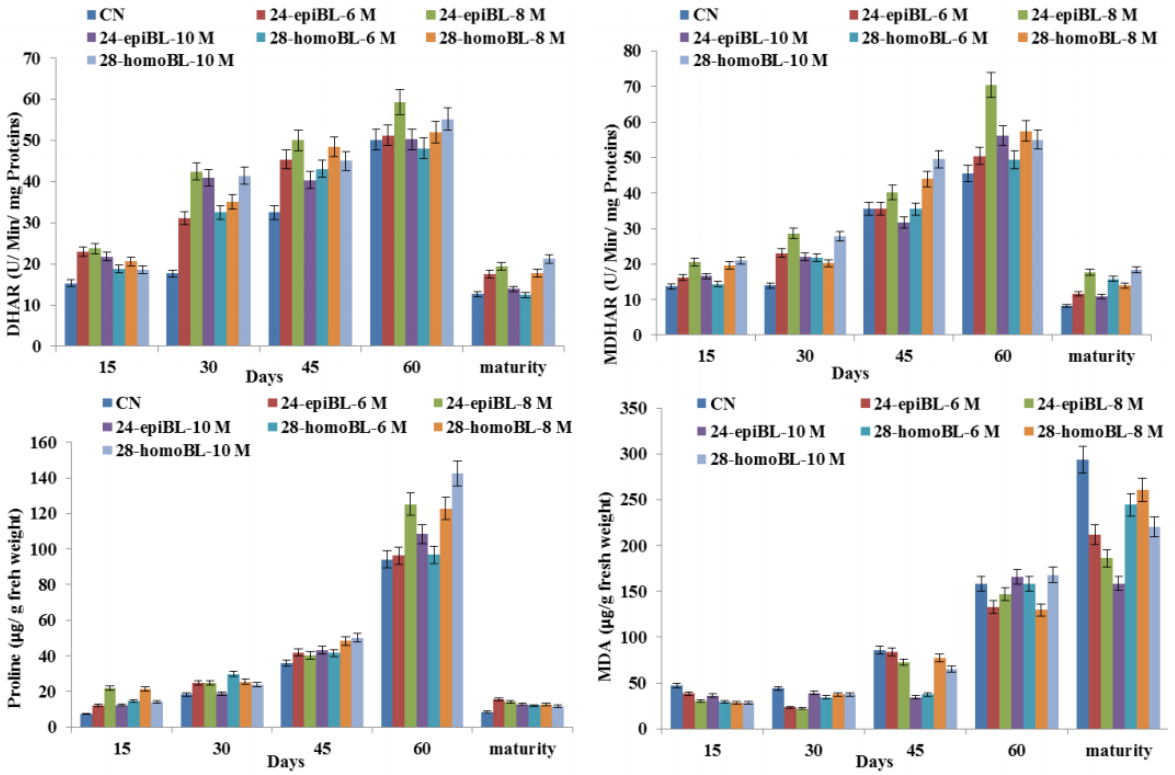

Figure 3. Effect of seasonal stress and different concentration (10^{-6}, 10^{-8}, 10^{-10} M) of 24-epiBL and 28-homoBL on Dehydroascorbate peroxidase (A), Monodehydroascorbate peroxidase (B), Proline content (C) lipid peroxidation (D). Data are means of three replicate and are significant difference from control at P<0.05.

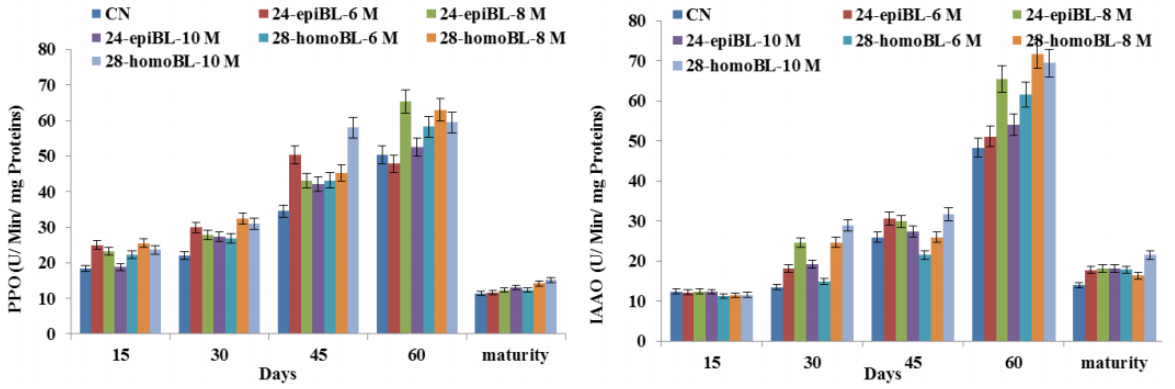

Figure 4. Effects of seasonal stress and different concentration (10^{-6}, 10^{-8}, 10^{-10} M) of 24-epiBL and 28-homoBL on Polyphenol oxidase (A), Indole acetic acid oxidase (B). Data are means of three replicate and are significant difference from control at P<0.05.

To neutralize the toxicity of ROS, plants have enzymatic (SOD, CAT, APOX, DHAR and GR) and nonenzymatic (e.g. ascorbate, glutathione, tocopherols and proline) defense system (Mittler 2002; Schutzendubel and Polle 2002; Arora *et al.,* 2007). Among ROS, Superoxide radical ($O_2^{\cdot}$) is dismutated by SOD into H_2O_2 and this H_2O_2 further scavenged by CAT and various peroxidases. APOX and GR also play a key role by reducing H_2O_2 into water through the ascorbate- glutathione cycle (Nocter and Foyer 1998). Brassinosteroids protect the plants from toxic action of ROS either by

directly acting on them or indirectly by regulating the enzymatic and nonenzymatic system of plants. The observations in the present study revealed that both brassinolides applied to *B. juncea* seedlings resulted in increased activities of antioxidative enzymes (SOD, CAT, APOX, DHAR), which are the part of defense system of cell. Mazorra *et al.,* (2002) found enhanced CAT activity in tomato under different temperature treated with BRs. Hayat *et al.* (2007) also reported that the activities of antioxidative enzymes (CAT, SOD and APOX) were increased in roots and aerial parts of *B. juncea* plants when 28-homoBL foliar treatments were given. Similar reports were found by Nunez *et al.* (2003), who revealed that the application of BRs caused the activation of antioxidative enzymes under water and salt stresses. Polyphenol oxidase is an enzyme that catalyzes the hydroxylation of monophenols to *o*-diphenols and their oxidation to *o*-diquinones. This enzyme is widely distributed in higher plant (Steffens *et al.,* 1994; Hind *et al.,* 1995 and Trebst and Depka 1995). MDA content was decrease with the treatment of both brassinolides, suggested that brassinosteroids detoxify the ROS by enhancing the activities of antioxidative enzymes. PPO plays an important role to control oxidative processes, although it is not considered a component of antioxidant defense system, but the activity of PPO was increased under stressful condition (Rachkovskaya *et al.,* 1980; Voskresenskaya *et al.,* 2006; Zhukova *et al.,* 1996). In present study PPO activity was enhanced by the application of 24-epiBL and 28-homoBL. The activity of IAA was increased under water stress in pea plant reported by Darbyshire (1971). In Present study the activity of IAA was increased under the influence of both brassinolides as compare to control seedlings under the seasonal stress.

CONCLUSION:

The present study clearly indicates seasonal stress protective properties of BRs in *B. juncea* seedlings. Stress ameliorative properties of BRs are clearly demonstrated by batter growth, dry weight, carbohydrate, protein contents and activities of antioxidative enzymes in brassinolides treated seedlings. It points to the possibility of BRs regulated stress protection in plants but extensive studies are needed on various aspects related to stress.

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REFERENCES

Aebi, H. (1983) Catalase. In: *Methods of Enzymatic Analysis*, eds Bergmeyer H.U. Verlag Chemie, Weinhan. Pp 673-684.

Bastin, Unleur, O. (1972) Effect of actinomycin–D on the formation of enzymes in *Jerusalem artichoke* tuber slices. *Planta,* **102**, 357-361.

Bates, L.S., Waldren, R.P., and Teare, I.D. (1973) Rapid determination of free proline for water-stress studies. *Plant Soil,* **39**, 205–207.

Cao, S., Xu, Q., Cao, Y., Qian, K., An, K., Zhu, Y., Binzeng, H., Zhao, H. and Kuai, B. (2005). Loss-of- function mutation in DET2 gene lead to an enhanced resistance to oxidative stress in *Arabidopsis*. *Physiol. Plant,* **123**, 57-66.

Clouse, S.D. and Sasse, J.M. (1998). Brassinosteroids: Essential regulators of plant growth and developments. *Annu. Rev. Plant. Physiol. Plant Mol. Biol.,* **49**, 427-451.

Dalton, D.A., Russell, S.A., Hanus, F.J., Pascoe, G.A. and Evans, H.J. (1986) Enzymatic reactions of

ascorbate and glutathione that prevent peroxide damage in soybean root nodules. *Proc. Natl. Acad. Sci.* USA, **83**, 3811-3815.

Darbyshire, B. (1971) The effect of water stress on Indole acetic acid oxidase in Pea plants. *Plant Physiol.,* **47**, 65-67.

Dubois, M., Gills, K. A., Hamilton, J. K., Robers, P.A. and Smith, F. (1956). Colorometric methods for the determination of sugars and related substances. *Ann. Chem.,* **28**, 350–356.

Gordon, S.A., Weber, R.P. (1951) The effect of x-radiation on indoleacetic acid and auxin levels in the plant. *Ameri. Jour. of Bot.,* **37**, 78.

Hayat, S., Ali, B., Aiman, H.S. and Ahmad, A. (2007). Brassinosteroids enhanced antioxidants under cadmium stress in *Brassica juncea. Environ. Exp. Bot.* **60**, 33-41.

Heath, R., Packer, L. (1968) Photo peroxidation in isolated chloroplasts I Kinetics and stoichiometry of fatty acid and peroxidation. *Arch. Biochem. Biophys.*, **196**, 385-395

Hernández, J.A., Jimenez, A., Mullineaux, P. and Sevilla, F. (2000) Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. *Plant Cell Environ.,* **23**, 853–862.

Hind, G., Marshak, D. and Coughalan, S. (1995) Spinach thylakoid polyphenol oxidase: cloning, characterization, and relation to a putative protein kinase. *Biochem.,* **34**, 8157-8164.

Kono, Y. (1978) Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. *Archives of Biochem. Biophys.,***186**, 189-195

Kovtun, Y., Chiu, W.L. and Tena, S.J. (2000) Functional analysis of oxidative stress – activated mitogen – activated protein kinase cascade in plants. *Proc Natl. Acad. Sci. USA*., **97**, 2940-2945.

Krishna, P. (2003) Brassinosteroids-mediated stress responses. *Jour. Plant Growth Regul*., **22**, 289-297

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with folin-phenol reagent. *The Jour. of Biol. Chemis.,* **193**, 265-275.

Mazorra, L.M., Nunez, M., Hechavarria, M., Coll, F. and Sanchez- Blanco, M.J. (2002) Influence of brassinosteroids on antioxidant enzymes activity in tomato under different temperature. *Biol. Plant.* **45**, 593-596.

Mc Cord, J.M. (2000) The evolution of free radicals and oxidative stress. *Am. Jour. Med.* **108**, 652-659.

Metwally, A., Finkemeier, I., Georgi, M. and Dietz, K.J. (2003). Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiol.* **132**, 272-281.

Mittler, R. (2002) Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, **7**, 405-410.

Nakano, Y. and Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. *Plant and Cell Physiol.,* **22(5)**, 867-880.

Noctor, G. and Foyer, C.H. (1998) Ascorbate and glutathione: Keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 249–279.

Nunez, M., Mazzafera, P., Mazorra, L.M., Siqueira, W.J. and Zullo, M.A.T. (2003). Influence of brassinosteroid analogue on antioxidant enzymes in rice grown in culture medium with NaCl. *Biol. Plant,* **47**, 67-70

Ozdemir, F., Bor, M., Demiral, T. and Turkan, I. (2004) Effects of 24- epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. *Plant Growth Regul.,* **42**, 203-211

Rachkovskaya, M.M., and Kim, L.O. (1980) Changes in activities of some oxidases as an index of plant adaptation to industrial pollutants, *Gazoustoichivost' rastenii* (Plant Tolerance to Gases), Nikolaevskii, V.S., Ed., Novosibirsk: Nauka.

Rao, S.S.R., Vardhini, B.V., Sujatha, E. and Anuradha, S. (2002). Brassinosteroids. A new class of phytohormones. *Curr. Sci.* 1239-1245.

Sairam, R.K. (1994) Effect of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture stress condition of two wheat varieties. *Plant Growth Regul.,* **14**, 173–181.

Schilling, G., Schiller, C. and Otto, S. (1991) Influence of brassinosteroid on organ relations and enzyme activities of sugar beet plants. In: *Brassinosteroids: Chemistry, Bioactivity and application* eds. Cutlar HG, Yokata T and Adam G. ACS Symp Ser 474. Washington, DC: *Am. Chem. Soc.,* 208-219.

Schützendübel, A. and Polle, A. (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Jour. of Exp. Bot.,* **53**, 1351-1365.

Sharma, P. and Bhardwaj, R. (2007). Effects of 24-Epibrassinolide on growth and metal uptake in *Brassica juncea* L. under copper metal stress. *Acta Physiol. Planta.,* **29**, 259-263.

Sirhindi, G., Kumar, S., Bhardwaj, R. and Kumar, M. (2009) Effect of 24-epibrassinolide and 28-homobrassinolide on antioxidant defense system in seedlings of *Brassica juncea* L. *Physiol. Molecul. Biol. Plants*, **15(4),** 35-41.

Steffens, J.C., Harel, E. and Hunt, M.D. (1994). Polyphenol oxidase. In: *Recent Advances in* Phytochemistry, genetic engineering of plant secondary metabolism*. eds.* Ellis BE, Kuroki GW, Stafford HA, Plenum Press, New York. 28 275-312.

Trebst, A. and Depika, B. (1995). Polyphenol oxidase and photosynthesis research. *Photosyn. Res.,* **46,** 41-44.

Vahala, J., Keinanen, M., Schutzendubel, A., Polle, A. and Kangasjarvi, J. (2003) Differential effects of elevated ozone on two hybrid aspen genotypes predisposed to chronic ozone fumigation role of ethylene and salicylic acid. *Plant Physiol.,* **132**, 196-205.

Voskresenskaya, O.L., Chernavina, I.A. and Aksenova, V.A. (1986). Effect of zinc surplus on iron accumulation and activity of iron-containing enzymes in oat. *Plant Physiol.,* **33**, 1056–1060.

Zhukova, L.A., Populyatsionnaya, Z. and Lugovykh, R. (1995) Population Life of Meadow Plants, *Yoshkar-Ola: Mar Gos Univ.*