EVALUATION OF BRASSINOLIDE EFFECT ON GROWTH, PROTEINS AND ANTIOXIDATIVE ENZYME ACTIVITIES IN *BRASSICA JUNCEA* L.

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The present study investigated the linkages between exogenous application of two commercially available brassinolides (24-epiBL and 28-homoBL) on total protein, carbohydrates and antioxidant enzyme system in *Brassica juncea* to elucidate the antioxidative protective mechanism. Seeds treated with different concentrations of 24-epiBL and 28-homoBL (10^{-6}M, 10^{-8}M, and 10^{-10} M) exhibited better growth as compared to control seedlings. This improvement was observed more by 24-epiBL as compared to 28-homoBL in 20 days old seedlings. Enzymes involved in antioxidant defense system of the plant showed increasing trend with both brassinolides treatments but concentration dependent manner. Superoxide dismutase (SOD 1.15.1.1), ascorbate peroxidase (APOX EC: 1.11.1.11) and dehydroascorbate reductase (DHAR EC: 1.8.5.1) exhibited amelioration with 24-epiBL while catalase (CAT 1.11.1.6), polyphenol oxidase (PPO 1.14.18.1) and indole acetic acid oxidase (IAAO) showed increased activity level with 28-homoBL. However, treatment of seeds with different concentrations of both 24-epiBL and 28-homoBL helped in enhancing the growth, protein and various enzymatic activities involved in defense system operated in *B. juncea.* These results suggested that application of brassinolides may cause brassinosteroid induced elevation in defense system that enable the plant to behave better than control plants.

Key Words: Antioxidant enzymes, *Brassica juncea,* Brassinosteroids, Reactive oxygen species

Plants are exposed to various external and internal stimuli are which act as stress factor and limit the productivity of plants. Under the influence of these sources plants produce excess ROS as O_2^-, OH^-, and H_2O_2 etc. which damage the plants by disturbing metabolic machinery of the system. To protect from such reactive species plants have well developed antioxidant defense system (ADS) which includes both enzymatic and non-enzymatic contents such as SOD, CAT, APOX, DHAR, PPO and IAAO etc. Antioxidant defense system also operates during normal course of growth and development of plant, exhibiting increased activity at seed or seedling stage enabling the plant to acclimatize with stress condition. Brassinosteroids are relatively new class of plant steroid hormones discovered by (Mitchell *et al.* 1970). Their action is accompanied by stimulation of growth process and can also protect plants against many stresses, such as heavy metals, salt, heat shock and cold (Krishna 2003, Janeczko *et al.* 2005). However, the mechanism of brassinosteroid action in plants is still not fully explained and extremely little is known about the interaction of different active forms of brassinolides at particular stage of growth and development. Keeping this in view an attempt was made to study the effect of 24-epiBL on activities of growth and biochemical changes in 20 day old seedlings of *B. juncea* L. growing under field conditions.

MATERIAL AND METHODS

The seeds of *Brassica juncea* L. c.v. RCM 619 were procured from Department of Plant Breeding, Punjab Agriculture University, Ludhiana India. Seeds were surface sterilized

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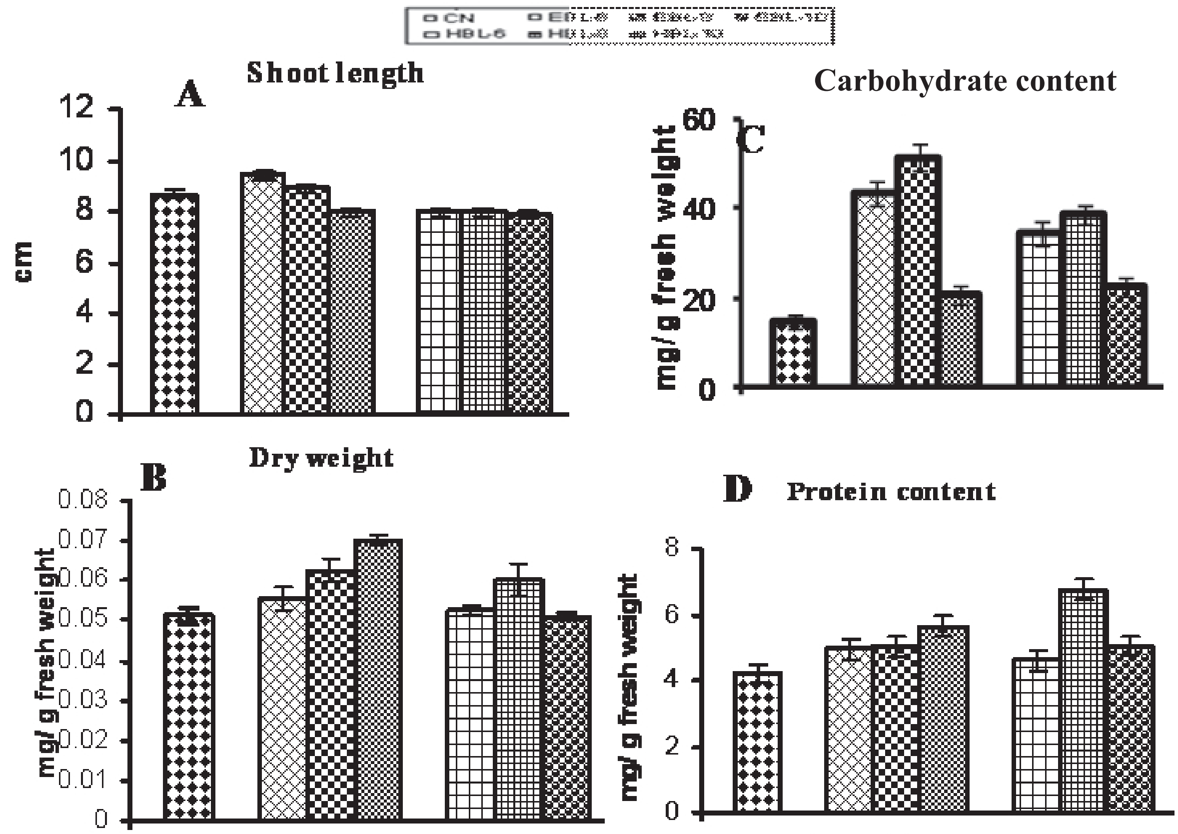

Figure 1. Effect of different concentration (10^{-6}, 10^{-8}, 10^{-10}) of 24-eqi BL and 28-homo BL on Shoot length (A), Dry weight (B), Carbohydrate (C), and Protein contents (D) of B. juncea seedling. Data are means of three replicates and are significant at $P<0.05$.

with 0.01% $HgCl_2$ and rinsed 5-6 times with double distilled water. The sterilized seeds were soaked for 8 h in different concentration of 24-epiBL and 28-homoBL (0, 10^{-6}, 10^{-8}, 10^{-10} M). The treated seeds were sown as three replicates in soil under field condition. Morphological data in terms of shoot length and dry weight was measured 20 days after sowing (DAS). Seedlings were harvested for analysis of various enzymes.

Estimation of various antioxidant enzyme activities and total protein content was done in by homogenizing 1g fresh plant material in 3 ml of 100 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 4 ºC for 20 min at 15000 g. The supernatant was used for estimation of total protein content and enzyme activities (SOD, CAT, APOX, PPO (EC 1.14.18.1) and IAAO. 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). 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) at 265nm. PPO activity was measured using the method of Bastin and Unluer (1972). IAAO activity was measured according to method of Gordon and Weber (1951). Carbohydrate content was estimated by the method of Dubois *et. al. (*1956). Total protein content was estimated as per method of Lowry *et al.* (1951). 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 lowest standard deviations

(LSD) test. Each data point was the mean of three replicates (n=3) except for shoot and root lengths of *B. juncea* seedlings (n=10) and comparisons with P-values <0.05 were considered significantly different. In all the figures, the spread of values is shown as error bars representing standard errors of the means.

RESULTS AND DISCUSSION

Effect of two active forms of brassinolide (24-epiBL and 28-homoBL) on growth shoot length, dry weight, total carbohydrate and total protein content has been in shown fig (1). Among two active forms of brassinolide, 24-epiBL has more significant effect on shoot length as compared to 28-homoBL and control untreated seedlings as reported in earlier study by Sirhindi *et. al.* (2009) in 10 DAS in *B. juncea* seedlings. 24-epiBL proved to be more effective in increasing shoot length. 10^{-10} M 24-epiBL (9.65 ± 0.94) was most effective concentration among both the brassinolides as compared to control seedlings (8.63 ± 0.97). There are number of reports available in literature which show that brassinosteroids play an essential role in plant growth and development (Li Kai-rang *et al.* 2005, Bhardwaj *et al.* 2007 and Karytal *et al.* 2009). Present study reveals the effect of both brassinolides in improving seedling growth in dose and type dependent manner. The carbohydrate content in the seedlings treated with brassinolide was enhanced as compared to control seedlings. 10^{-8} M concentration of both brassinolides exhibited the best response as compared to control seedlings. Both dry weight and protein content exhibited best response in

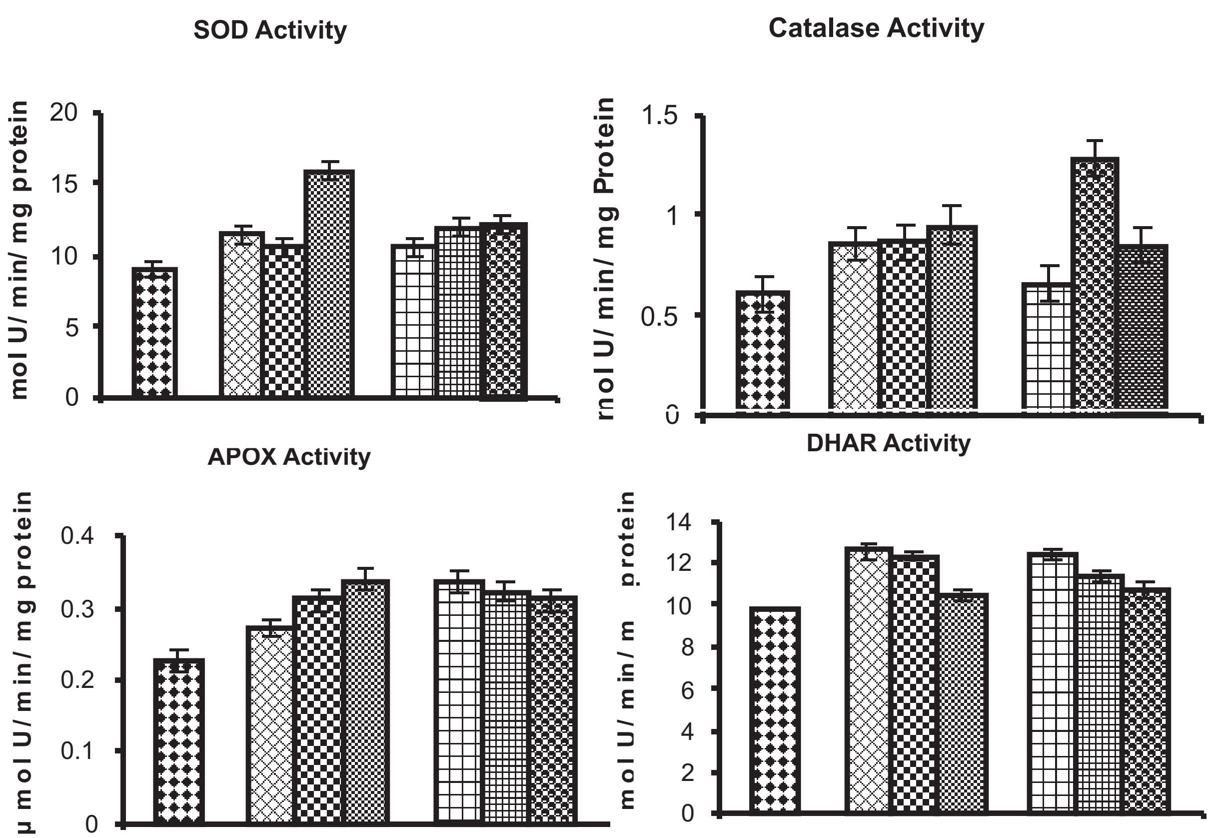

Figure 2. Effect of different concentration (10-6, 10-8, 10-10 M) of 24-epiBL and 28-homoBL on Superoxide dismutase (A), Catalase (B), Ascorbate Peroxidase (C), and Dehydroascorbate Reductase (D) of *B. juncea* seedlings. Data are means of three replicates and are significant at P<0.05.

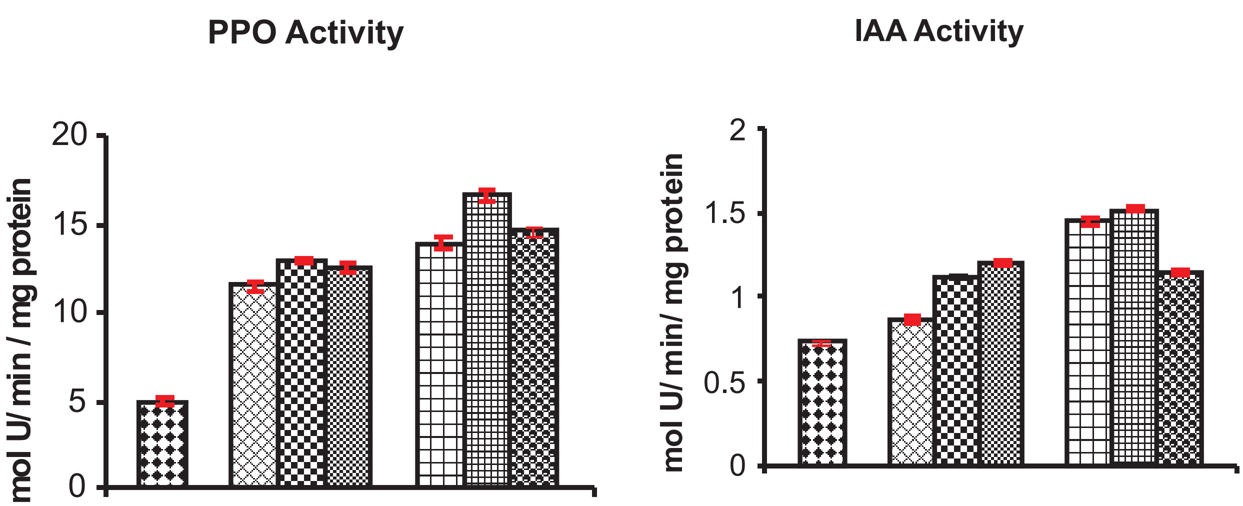

Figure 3. Effect of different concentration (10-6, 10-8, 10-10 M) of 24-epiBL and 28-homoBL on Polyphenol Oxidase activity (A), Indole Acetic Acid Oxidase activity (B) of *B. juncea* seedlings. Data are means of three replicates and are significant at P<0.05.

10^{-10} M 24-epiBL & 10^{-8} M 28-homoBL. 10^{-10} M of 24-epiBL (0.07 ± 0.001) generated the best response as compared to control seedlings (0.51 ± 0.002) in dry weight and Protein content also increased with treatment of both brassinolides. But maximum protein content was observed in 10^{-10} M concentration of 24-epiBL 5.64 ± 1.5 mg/g FW^{-1} as compared to control untreated seedlings (4.21 ± 1.03 mg/g Fw^{-1}) (Figure 2).

Activities of antioxidative enzymes (Fig. 2) were found to increase with treatment of both brassinolides. Maximum increase in SOD activity was observed in seedlings treated with 10^{-10} M 24-epiBL (15.0 ± 1.25 mol U/ min/ mg protein) as compared to control seedlings (9.00 ± 1.30). SOD is an important antioxidative enzyme which plays essential role in detoxification of free radicals into stable H_2O_2 (vital signaling molecule). Free radicals lead to membrane disruption, lipid peroxidation and decrease in hydrolse enzyme activity (Buchanan-wallaston *et al.* 1997). CAT is another important enzyme that plays crucial role in breakdown of H_2O_2 in H_2O and O_2. Maximum enhancement in CAT activity was observed in treatment of 10^{-10} M conc. of 28-homoBL (1.28 ± 0.20 mol U/ min/ mg protein) as compared to control seedlings (0.60 ± 0.09 mol U/ min/ mg protein). APOX further reduces H_2O_2 to water through ascorbate-glutathione cycle (Noctor and Foyer, 1998).

APOX showed maximum activity in seedling treated with 10^{-10} M conc. of 24-epiBL (0.34 ± 0.01 mol U/ min/ mg protein) as compared to control seedlings (0.23 ± 0.0105 mol U/ min/ mg protein). DHAR is another enzyme of ADS used for regeneration of Ascorbic acid, produced via non-enzymatic reaction from oxiglutathione (GSSG) in Asada-Halliwell pathway (Sairam and Tyagi 2004). Interestingly DHAR activity increased to significant level in seedlings treated with 10^{-6}M epiBL as compared to control.

PPO and Auxinases play an important role in growth and development, although these are not considered as component of antioxidant defense system. Present study clearly indicates that brassinolides protect plants from toxic effects of ROS either by directly acting on them or indirectly by regulating the enzymatic and nonenzymatic system of plants. Similarly, Hayat *et al.* (2007) observed that the activities of antioxidant enzymes increased with foliar treatment of 28-homoBL in roots and aerial parts of *B. juncea* L. seedlings. Activities of

PPO and auxinase (Fig. 3) were found to increase with treatments of both brassinolide as compare to control seedlings. 10^{-8}M homoBL was the best treatment to increase activities of these enzymes in *B. juncea* at 10 DAS. However these enzymes showed increase in their activity in seedlings treated with different concentrations of both brassinolides.6

In general, all antioxidative enzyme activities increased in brassinolides treatments, especially 10^{-10}M 24-epiBL exhibited highest SOD and APOX activity, and 10^{-8} M 28-homoBL catalase activity and 10^{-6} M of both 24-epiBL and 28-homoBL exhibited highest DHAR activity. Increasing 28-homoBL conc. exhibits decline trend in APOX and DHAR activities whereas increasing 24-epiBL showed decline in DHAR activity only through it remained higher than control in all cases.

CONCLUSION

The present study clearly indicates that brassinolides play a crucial role in growth and development of plants but in very much collaborative manner in a means that some activities ameliorated by 24-epiBL and some 28-homoBL in very much dose dependent manner. But further studies are needed to explore signaling pathway and reasons for this behavior of different brassinolides on *B. juncea* plants.

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