BIOLOGIA PLANTARUM 52 (4): 767-770, 2008

BRIEF COMMUNICATION

Effects of 28-homobrassinolide on nickel uptake, protein content and antioxidative defence system in *Brassica juncea*

P. SHARMA[1], R. BHARDWAJ[1]*, N. ARORA[1], H.K. ARORA[1] and A. KUMAR[2]

Department of Botanical and Environmental Sciences Guru Nanak Dev University, Amritsar-143005, Punjab, India[1]
Serum Institute of India Ltd., 212/2, Hadapsar, Pune-411028, India[2]

Abstract

The effects of 28-homobrassinolide (HBL) on nickel uptake, protein content and activities of antioxidative enzymes were determined in the seedlings of *Brassica juncea* L. The seeds were treated with different concentrations (0, 0.01, 1 and 100 nM) of HBL for 8 h and then sown in the Petri plates containing various concentrations (0, 25, 50 and 100 mg dm^{-3}) of nickel. After 7 d, observations were made on shoot and root length, Ni uptake, protein content and activities of antioxidative enzymes (guaiacol peroxidase, catalase, glutathione reductase, ascorbate peroxidase and superoxide dismutase). The growth of seedlings was inhibited by Ni, however, less after HBL pre-treatment. The protein content and antioxidative enzyme activities were also increased by HBL treatment.

Additional key words: antioxidative enzymes, brassinosteroids, heavy metal toxicity, Indian mustard.

The soil in which plants grow may contain phytotoxic levels of metals including Zn, Cr, Cu, Hg and Ni, *etc*. Though heavy metals are essential as micronutrients, their higher concentrations are toxic for plants (Panda *et al*. 2003). Out of these metals, nickel (Ni) in increased concentration is a potential inhibitor of photosynthesis and decrease the chlorophyll content and stomatal conductance. Excess of Ni also affects root anatomy and induced the accumulation of proline. Further Ni-induced peroxidation of membrane lipids is associated with extensive degradation of intracellular membranes and organelles, particularly chloroplasts (Meharg 1993, Lin and Kao 2007, Maksimović *et al.* 2007). It ultimately results in oxidative stress in plants. Plants possess strong antioxidative defence system to scavenge reactive oxygen species (ROS) in order to protect themselves from the oxidative stresses (Cao *et al*. 2004). Several plant hormones are implicated in modulating the plant responses to oxidative stresses like ethylene (Vahala *et al*. 2003), abscisic acid (Kovtun *et al*. 2000) and salicylic acid (SA) (Metwally *et al*. 2003).

Brassinosteroids (BRs) are a family of more than 60 naturally occurring plant steroid hormones found in a wide variety of plant species (Bhardwaj *et al.* 2006). BRs are implicated in many physiological responses like cell elongation, cell division, vascular differentiation, seed germination and photomorphogenesis (Mandava 1988, Clouse and Sasse 1998). The stress-protective properties of BRs have been recently studied in plants under cold (Dhaubhadel *et al*. 1999), heat (Dhaubhadel *et al.* 2002), salinity (Ozdemir *et al*. 2004) and heavy metal stress (Bajguz 2000a). With their stress-protective properties, it becomes important to study their role in oxidative defence system of plants. The present study was designed to observe the effect of 28-homobrassinolide on growth, Ni uptake, protein content and antioxidative enzyme activities in *B. juncea* seedlings.

The certified seeds of Indian mustard (*Brassica juncea* L. cv. PBR 91) used in the present investigation were procured from the Department of Plant Breeding, Punjab Agriculture University, Ludhiana. The experiment was conducted in Petri dishes at temperature of 25 ± 0.5 ºC, 16-h photoperiod, irradiance of 110 μmol m^{-2} s^{-1} (supplied by cool white fluorescent tubes) and relative humidity

Received 4 August 2007, *accepted* 8 June 2008.
Abbreviations: APX - ascorbate peroxidase; BCF - bioconcentration factor; BRs - brassinosteroids; CAT - catalase; GR - glutathione reductase; HBL - 28-homobrassinolide; POD - guaiacol peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.
Acknowledgements: The financial assistance from UGC New Delhi is gratefully acknowledged.
* Corresponding author; fax: (+91) 183 2258819, e-mail: renubhardwaj82@gmail.com

85 - 90 %. The seeds were surface sterilized with 0.01 % $HgCl_2$ for 5 min and thoroughly washed with double distilled water 3 - 4 times. The sterilized seeds were soaked for 8 h in different concentrations (0, 0.01, 1 and 100 nM) of HBL (*Sigma Aldrich*, St. Louis, USA). HBL treated seeds were then transferred to sheets of sterile *Whatman* No. #1 filter paper moistened with different concentrations (0, 25, 50 and 100 mg dm^{-3}) of nickel ($NiSO_4 \cdot 7 H_2O$).

Germination percentage was recorded and after 7 d, the seedlings were harvested and shoot and root lengths were measured. Samples of ground, dried plant material were digested in mixture of H_2SO_4:HNO_3:$HClO_4$ (1:5:1) according to Allen *et al.* (1976). After filtration the analysis of Ni uptake was done using atomic absorption spectrophotometer (*AA-6200 Shimadzu*, Kyoto, Japan). The bio-concentration factor (BCF) was calculated as the ratio of Ni concentration in plant tissues at harvest [mg kg^{-1}] and initial concentration in the external solution [mg dm^{-3}].

Shoots (1 g) were homogenized in an ice-chilled mortar with 3 dm^3 of 100 mM potassium phosphate buffer (pH 7.0) containing 1 % insoluble polyvinyl-pyrolidone at 4 °C. The homogenate was centrifuged at 15 000 *g* for 20 min and the supernatant was used for enzyme and protein analysis. Protein content was determined by the method of Lowry *et al.* (1951). Guaiacol peroxidase (POD; EC 1.11.1.7) was estimated according to the method given by Putter (1974). The rate of formation of oxidized guaiacol was followed spectrophotometrically at 436 nm. Catalase (CAT; EC 1.11.1.6) activity was determined by following the initial rate of disappearance of H_2O_2 at 240 nm (Aebi 1974). Enzyme activity was determined using the coefficient of absorbance of 6.93×10^{-3} mM^{-1} cm^{-1}. The activity of glutathione reductase (GR; EC 1.6.4.2) was measured by the method of Carlberg and Mannervik (1975). The ascorbate peroxidase (APX; EC 1.11.1.11) activity was assayed by monitoring the decrease in absorbance at 290 nm (Nakano and Asada 1981). Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by the method of Kono (1978). One unit of the SOD activity was defined as the enzyme concentration required inhibiting reduction of NBT by 50 %. The data obtained was statistically analyzed by one-way-*ANOVA* using the methodology proposed by Bailey (1995).

The seed germination and seedling growth was significantly reduced by the Ni treatment. The HBL alone enhanced the germination percentage as well as shoot and root length (Table 1). As concern Ni-treated plant in combination with HBL, the maximum germination was observed at 100 mg dm^{-3} Ni and 1 nM HBL. Maximum shoot and root length of Ni-treated seedling was at the lowest concentration 25 mg(Ni) dm^{-3} combined with 100 nM and 0.01 nM HBL, respectively (Table 1). The Ni uptake and bioconcentration factors (BCF) showed a significant reduction under the influence of HBL. Maximum reduction in Ni uptake and BCF was observed in seedlings treated with 50 and 100 mg(Ni) dm^{-3} supplemented with 100 nM of HBL (Table 1). It has been reported by Hayat and Ahmad (2003) and Ali *et al.* (2005) that the germination percentage of *Lens culinaris* and *Cicer arietinum* increased by seed soaking in 10^{-10} M or 10^{-8} M HBL. Similar results were obtained by Ozdemir *et al*. (2004) who observed that growth of rice seedling was improved by EBL treatments under salt stress. In our earlier studies, EBL decreased uptake of Cu, Zn, Mn, Ni and Co in *Brassica campestris* and *B. juncea* (Kaur and Bhardwaj 2003, Sharma and Bhardwaj 2007a,b). According to Bajguz (2000a), the amounts of Cu, Cd and

Table 1. Effect of 28-homobrassinolide on morphological parameters and Ni uptake in *Brassica juncea* seedlings under Ni stress. Means ± SE, $n = 6$, * - significantly different values from control.

HBL [nM]	Ni [mg dm^{-3}]	Germination [%]	Shoot length [cm]	Root length [cm]	Ni uptake [mg g^{-1}(d.m.)]	BCF
0	0	92.66 ± 0.66	4.76 ± 0.28	4.80 ± 0.28	0.006 ± 0.0007	0
100	0	92.66 ± 1.45	6.00 ± 0.24*	6.16 ± 0.17*	0.004 ± 0.0008*	0
1	0	93.00 ± 1.72	5.70 ± 0.15	5.52 ± 0.36	0.003 ± 0.001*	0
0.01	0	94.22 ± 1.15*	5.20 ± 0.76	5.10 ± 0.10	0.003 ± 0.001*	0
0	25	89.3 ± 1.63	4.66 ± 0.44	4.66 ± 0.16	0.456 ± 0.022	18.26 ± 0.91
100	25	91.6 ± 0.62	5.66 ± 0.33*	4.83 ± 0.16	0.117 ± 0.005*	4.72 ± 0.21*
1	25	90.6 ± 0.55	5.06 ± 0.66*	5.06 ± 0.06	0.098 ± 0.003*	3.94 ± 0.14
0.01	25	88.8 ± 0.58	4.83 ± 0.16*	5.50 ± 0.28*	0.127 ± 0.006*	5.06 ± 0.26*
0	50	86.6 ± 0.45	4.33 ± 0.33	4.33 ± 0.15	0.564 ± 0.023	11.28 ± 0.47
100	50	91.6 ± 0.70*	5.00 ± 0.11*	4.66 ± 0.24	0.088 ± 0.010*	1.75 ± 0.03
1	50	88.6 ± 0.72	4.93 ± 0.06	4.50 ± 0.18	0.119 ± 0.008*	2.39 ± 0.18*
0.01	50	90.4 ± 0.69	4.46 ± 0.35	4.93 ± 0.06	0.110 ± 0.003*	2.20 ± 0.07
0	100	83.3 ± 1.12	2.50 ± 0.28	2.83 ± 0.17	0.768 ± 0.014	7.68 ± 0.14
100	100	92.3 ± 0.63*	4.00 ± 0.28*	5.16 ± 0.44	0.094 ± 0.041*	0.94 ± 0.04
1	100	92.5 ± 1.15*	3.33 ± 0.33*	4.76 ± 0.15	0.108 ± 0.018*	1.08 ± 0.18*
0.01	100	90.2 ± 0.40	3.00 ± 0.16*	3.83 ± 0.17	0.109 ± 0.011*	1.09 ± 0.10*

Table 2. Influence of 28-homobrassinolide on soluble protein content [mg g^{-1}(f.m.)] and specific activity of antioxidative enzymes [U mg^{-1}(protein) min^{-1}] in *Brassica juncea* seedlings under Ni stress. Means ± SE, n = 3, * - significantly different values from control.

HBL [nM]	Ni [mg dm^{-3}]	Protein content	POD	CAT	GR	APX	SOD
0	0	6.98 ± 1.01	15.45 ± 1.98	13.42 ± 1.52	12.40 ± 1.12	12.56 ± 1.26	4.98 ± 0.89
100	0	13.35 ± 1.56*	26.40 ± 2.82*	16.78 ± 1.92	26.48 ± 1.90*	22.56 ± 2.16*	16.48 ± 1.82*
1	0	12.40 ± 1.83*	27.84 ± 1.98*	23.96 ± 2.15*	34.98 ± 2.43*	24.84 ± 3.05*	15.07 ± 1.96*
0.01	0	10.75 ± 1.44*	28.96 ± 2.14*	27.54 ± 1.84*	30.14 ± 2.76*	24.13 ± 2.74*	12.92 ± 2.11*
0	25	4.06 ± 0.07	23.21 ± 2.65	11.91 ± 2.79	3.23 ± 0.30	9.25 ± 1.05	3.67 ± 0.22
100	25	6.55 ± 0.03*	33.21 ± 1.39*	13.91 ± 1.28	5.73 ± 0.85	13.17 ± 0.83*	10.26 ± 0.39*
1	25	7.38 ± 0.23	27.97 ± 1.26	13.35 ± 3.71	4.43 ± 0.39	12.31 ± 0.24	7.61 ± 1.67*
0.01	25	6.03 ± 0.16	25.38 ± 0.54	11.95 ± 2.61	3.93 ± 0.46	11.01 ± 0.24	4.76 ± 0.23
0	50	3.24 ± 0.16	14.15 ± 1.19	8.83 ± 0.79	2.95 ± 0.15	6.05 ± 0.28	4.60 ± 0.52
100	50	6.36 ± 0.22*	23.12 ± 1.23*	14.47 ± 1.28	6.23 ± 0.26*	9.57 ± 0.33	12.33 ± 0.53*
1	50	5.84 ± 0.36*	17.49 ± 2.23	13.35 ± 1.97	3.18 ± 0.13	8.53 ± 0.83*	9.02 ± 0.72*
0.01	50	5.71 ± 0.14	15.15 ± 3.26	10.65 ± 2.36	3.00 ± 0.05	7.09 ± 0.65	7.16 ± 0.83
0	100	2.84 ± 0.05	12.18 ± 1.22	5.55 ± 0.23	1.95 ± 0.02	4.55 ± 0.14	5.72 ± 0.25
100	100	5.78 ± 0.32*	20.07 ± 2.02*	8.58 ± 1.12*	6.11 ± 1.03*	9.16 ± 0.12	15.37 ± 1.35*
1	100	5.77 ± 0.16	15.94 ± 1.67	7.65 ± 0.89	3.18 ± 0.22	7.07 ± 0.78	11.31 ± 1.22*
0.01	100	5.51 ± 0.25*	13.17 ± 1.22	6.05 ± 0.34	2.98 ± 0.11	6.06 ± 1.05	8.31 ± 1.08

Zn accumulated by the cells of *Chlorella vulgaris* decreased by about 50 % in the presence of 10^{-8} M epibrassinolide. The protective effect of brassinosteroids on winter rape plants under Cd stress and thermal stress was investigated by Janeczko *et al*. (2005, 2007). Similarly Bilkisu *et al*. (2003) reported that brassinolide stimulated growth of Al stressed *Phaseolus aureus* seedlings.

Increasing concentrations of Ni decreased the protein content of seedlings. However it was enhanced at the treatment with HBL (Table 2). The decrease in protein content in Ni-treated seedlings (Table 2) was possibly due to a decrease in the metabolism of amino acids and that of nitrogen (El-Shintinawy and El-Ansari 2000). However, the treatment of seedlings with HBL alone or together with Ni treatment, increased protein content in seedlings is possibly the result of the well-documented effect of BRs on transcription and/or translation (Kalinich *et al*. 1985, Bajguz 2000b, Fariduddin *et al*. 2004).

It was further observed that activities of antioxidative enzymes mostly decreased with increasing concentration of Ni. The exception was SOD at 25 mg (Ni) dm^{-3} (Table 2). HBL alone enhanced the activities of antioxidative enzymes. In case of Ni-stressed seedlings, maximum activities of POD and APX were observed in case of seedlings treated with 25 mg(Ni) dm^{-3} with 100 nM of HBL. Seedlings treated with 50 mg(Ni) dm^{-3} with 100 nM HBL showed maximum CAT and GR activities. SOD activity showed maximum in seedlings treated with 100 mg(Ni) dm^{-3} with 100 nM HBL (Table 2). Ozdemir *et al*. (2004) reported an increased activity of POD, CAT and GR in combination of NaCl treatment with 24-epibrassinolide. In rice an increase in CAT activity was induced by a BR analogue (Nunez *et al*. 2003) and in tomato, increase in CAT activity depended on the structure of BR, dose and temperature (Mazorra *et al*. 2002).

The reduction of toxicity by BRs might be also associated with enhanced contents of soluble proteins and nucleic acids and with increasing activity of ATPase (Bajguz 2000b). BRs bind with the membrane proteins and scavenge ROS, which are generated by heavy metals thereby reducing the membrane oxidative damage. It seems that BRs improves the resistance in the plants.

These results clearly indicate a metal stress-ameliorative property of brassinosteroids. Improvement in the growth of seedlings, lowered uptake of Ni and enhanced activities of antioxidative enzymes support the stress protective properties of HBL.

References

Aebi, H.: Catalase. - In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. Pp. 673-684. Verlag-Chemie, Weinham 1974.

Ali, B., Hayat, S., Ahmad, A.: Response of germinating seeds of *Cicer arietinum* to 28-homobrassinolide and/or potassium. - J. gen. appl. Plant Physiol. **31**: 55-63, 2005.

Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby, C., Roberts, J.D.: Chemical Analysis. - In: Chapmann, S.B. (ed.): Methods in Plant Ecology. Pp. 424-426. Blackwell Scientific Publications, Oxford - London 1976.

Bailey, N.T.J.: Statistical Methods in Biology (3rd Ed). - The English University Press, London 1995.

Bajguz, A.: Blockage of heavy metal accumulation in *Chlorella vulgaris* cells by 24-epibrassinolide. - Plant Physiol.

Biochem. **38**: 797-801, 2000a.

Bajguz, A.: Effects of brassinosteroids on nucleic acids and protein in cultured cells in *Chlorella vulgaris*. - Plant Physiol. Biochem. **38**: 209-215, 2000b.

Bhardwaj, R., Arora, H.K., Nagar, P.K., Thukral, A.K.: Brassinosteroids – a novel group of plant hormones. - In: Trivedi, P.C. (ed.): Plant Molecular Physiology - Current Scenario and Future Projections. Pp. 58-84. Aaviskar Publisher, Jaipur 2006.

Bilkisu, A.A., Xiao-Gang, G., Qing-Lei, G., Yong-Hua, Y.: Brassinolide amelioration of aluminium toxicity in mungbean seedling growth. - J. Plant Nutr. **26**: 1725-1734, 2003.

Cao, X., Ma, L.Q., Tu, C.: Antioxidative responses to arsenic in the arsenic-hyperaccumulator Chinese brake fern (*Pteris vittata* L.). - Environ. Pollut. **128**: 317-325, 2004.

Carlberg, I., Mannervik, B.: Purification of the flavoenzyme glutathione reductase from rat liver. - J. biol. Chem. **250**: 5475-5480, 1975.

Clouse, S.D., Sasse, J.M.: Brassinosteroids: essential regulators of plant growth and development. - Annu. Rev. Plant Physiol. Plant mol. Biol. **49**: 427-451, 1998.

Dhaubhadel, S., Browning, K.S., Gallie, D.R., Krishna, P.: Brassinosteroid functions to protect the translational machinery and heat-shock protein synthesis following thermal stress. - Plant J. **29**: 681-691, 2002.

Dhaubhadel, S., Chaudhary, S., Dobinson, K.F., Krishna, P.: Treatment with 24-epibrassinolide (a brassinosteroid) increases the basic thermotolerance of *Brassica napus* tomato seedlings. - Plant mol. Biol. **40**: 333-342, 1999.

El-Shintinawy, F., El-Ansari, A.: Differential effect of Cd^{2+} and Ni^{2+} on amino acid metabolism in soybean seedling. - Biol. Plant. **43**: 74-84, 2000.

Fariduddin, Q., Ahmad, A., Hayat, S.: Response of *Vigna radiata* to foliar application of 28-homobrassinolide and kinetin. - Biol. Plant. **48**: 465–468, 2004.

Hayat, S., Ahmad, A.: Soaking seeds of *Lens culinaris* with 28-homobrassinolide increased nitrate reductase activity and grain yield in the field in India. - Ann. appl. Biol. **143**: 121-124, 2003.

Janeczko, A., Koscielniak, J., Pilipowicz, M., Szarek-Lukaszewsa, G., Skoczowspi, A.: Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. - Photosynthetica **43**: 293-298, 2005.

Janeczko, A. Gullner, G., Skoczowski, A., Dubert, F., Barna, B.: Effects of brassinosteroid infiltration prior to cold treatment on ion leakage and pigment contents in rape leaves. - Biol. Plant. **51**: 355-358, 2007.

Kalinich, J.F., Mandava, N.B., Todhunter, J.A.: Relationship of nucleic acid metabolism on brassinolide-induced responses in beans. - Plant Physiol. **120**: 207-214, 1985.

Kaur, S., Bhardwaj, R.: Brassinosteroids regulated heavy metals uptake in *Brassica campestris* L. - In: Annual Meeting of the American Society of Plant Biologists "Plant Biology 2003". P. 628. Honolulu 2003.

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

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

Lin, Y.C., Kao, C.H.: Proline accumulation induced by excess nickel in detached rice leaves. - Biol. Plant. **51**: 351-354, 2007.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.L.: Protein measurement with Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Maksimović, I., Kastori, R., Krstić, L., Luković, J.: Steady presence of cadmium and nickel affects root anatomy, accumulation and distribution of essential ions in maize seedlings. - Biol. Plant. **51**: 589-592, 2007.

Mandava, N.B.: Plant growth promoting brassinosteroids. - Annu. Rev. Plant Physiol. Plant mol. Biol. **39**: 23-52, 1988.

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

Meharg, A.A.: The role of the plasmalemma in metal tolerance in angiosperms. - Physiol. Plant. **88**: 191-198, 1993.

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

Nakano, Y., Asada, A.: Hydrogen peroxide is scavenged by ascorbate specific-peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

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

Ozdemir, F., Bor, M., Demiral, T., Turkan, I.: 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, 2004.

Panda, S.K., Chaudhury, I., Khan, M.H.: Heavy metals induced lipid peroxidation and affect antioxidants in wheat leaves. - Biol. Plant. **46**: 289-294, 2003.

Putter, J.: Peroxidase. - In: Bergmeyer, H.U. (ed): Methods of Enzymatic Analysis. Pp. 685-690. Verlag-Chemie, Weinham 1974.

Sharma, P., Bhardwaj, R.: Effect of 24-epibrassinolide on growth and metal uptake in *Brassica juncea* L. under copper metal stress. - Acta Physiol. Plant. **29**: 259-263, 2007a.

Sharma, P., Bhardwaj, R.: Effect of 24-epibrassinolide on seed germination, seedling growth and heavy metals uptake in *Brassica juncea* L. - J. gen. appl. Plant. Physiol. **33**: 59-73, 2007b.

Vahala, J., Keinanen, M., Schutzendubel, A., Polle, A., Kangasjarvi, J.: 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, 2003.