SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 1, pp. 149-154, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

28-HOMOBRASSINOLIDE REGULATED MN-UPTAKE AND GROWTH OF *BRASSICA JUNCEA* L.

*Renu Bhardwaj, Priyanka Sharma, Hardesh Kumar Arora and Nitika Arora
Department of Botanical and Environmental Sciences
Guru Nanak Dev University, Amritsar – 143005, India

ABSTRACT

The present study describes the effects of 28-homobrassinolide (28-homoBL) on plant growth, metal (Mn) uptake and bioconcentration factor (BCF) in plants of *Brassica juncea* L. cv. PBR 91 under Mn stress. The seeds of *B. juncea* pre-treated with different concentrations of 28-homoBL were sown in earthen pots containing various concentrations of heavy metal (Mn) in soil. The observations were made for percent seed germination, shoot length and fresh weight, Mn-uptake and BCF after 30 days of sowing. The investigation revealed reduced rate of growth and germination with increasing concentration of metal. However pre-treatment of 28-homoBL was found significantly effective in lowering the growth inhibition and germination and metal uptake, indicating stress protective properties of this compound.

Keywords: 28-homobrassinolide, bioconcentration factor, heavy metal stress, Indian mustard.

INTRODUCTION

Brassinosteroids (BRs) represent a new class of plant hormones with wide occurrence in the plant kingdom in addition to auxins, gibberellins, cytokinins, abscisic acid and ethylene. They have unique biological effects on plant growth and development (Sasse, 1997; 1999; Bhardwaj *et al*., 2006, 2007a). BRs are growth-promoting plant hormones with structures similar to animal steroid hormones. In addition to their role in plant development, BRs have the ability to protect plants from various environmental stresses, including drought, extreme temperatures, heavy metals, herbicidal injury and salinity (Dhaubhadel *et al*., 1999, 2002; Bajguz 2000, Krishna, 2003; Kaur and Bhardwaj, 2004; Ozdemir *et al*., 2004; Upreti and Murti 2004).

Soil contamination with heavy metals is a worldwide problem, leading to agricultural losses and hazardous health effects as metals enter the food chain and being nondegradable in nature, keep on cycling from abiotic to biotic components. Toxicity of heavy metals depends upon their concentration experienced by plants in the environment. They interfere with the normal functioning of the plant body, which due to the stress, may result in loss in production, often leading to death. Among heavy metals, Mn at higher concentration leads to reduction in the relative growth rate (RGR), chlorophyll a and b content, photosynthetic O_2 evolution activity and photosystem II (PSII) activity in pea plants (Doncheva *et al*., 2005). Compared the treatment of normal Mn (10 μM), excess Mn significantly increased H_2O_2 concentration and lipid peroxidation. Excess Mn induced formation of brown spots and callose in the cell walls of leaves of cowpea and the plant growth was significantly inhibited (Fecht-Christoffers *et al*., 2003; Shi *et al*., 2005). BRs have been reported to possess the ability to regulate the uptake of heavy metals and radioactive elements in plants grown in polluted areas (Khripach *et al*., 2000; Janeczko *et al*., 2005) and can be used to reduce the accumulation of heavy metals and radioactive elements. The application of BRs in appropriate doses at an appropriate stage of development reduces the metals absorption (Zn, Cd, Pb and Cu) significantly in different agricultural plants such as barley, tomatoes, radish, sugar beet and winter rape (Volynets *et al*., 1997). Keeping in mind the anti-stress properties of BRs, the present piece of work was undertaken to observe the effect of 28-homobrassinolide (28-homoBL) on growth and metal uptake under Mn stress in *B. juncea* plants.

MATERIALS AND METHODS

Study material and treatments:

The surface sterilized seeds of *B. juncea* L. cv. PBR 91 were soaked for 8h in different concentrations of 28-homoBL (0, 10^{-7}, 10^{-9} and 10^{-11} M). In order to study the effects of 28-homoBL on the plant growth and Mn uptake in the different parts of the plant (leaves, shoots and roots), a seasonal field experiment was performed. The seeds treated with different concentrations of 28-homoBL were sown in earthen pots (26 cm diameter). Approximately 9 kg (in each pot) of garden soil (clay: sand: manure in the ratio of 2:1:1) was filled in the experimental pots. Different concentrations of heavy metal (Mn) (0, 25, 50 and 100 mg/kg soil) were added in pots containing garden soil. 28-HomoBL treated seeds

*Corresponding author email: renubhardwaj82@gmail.com

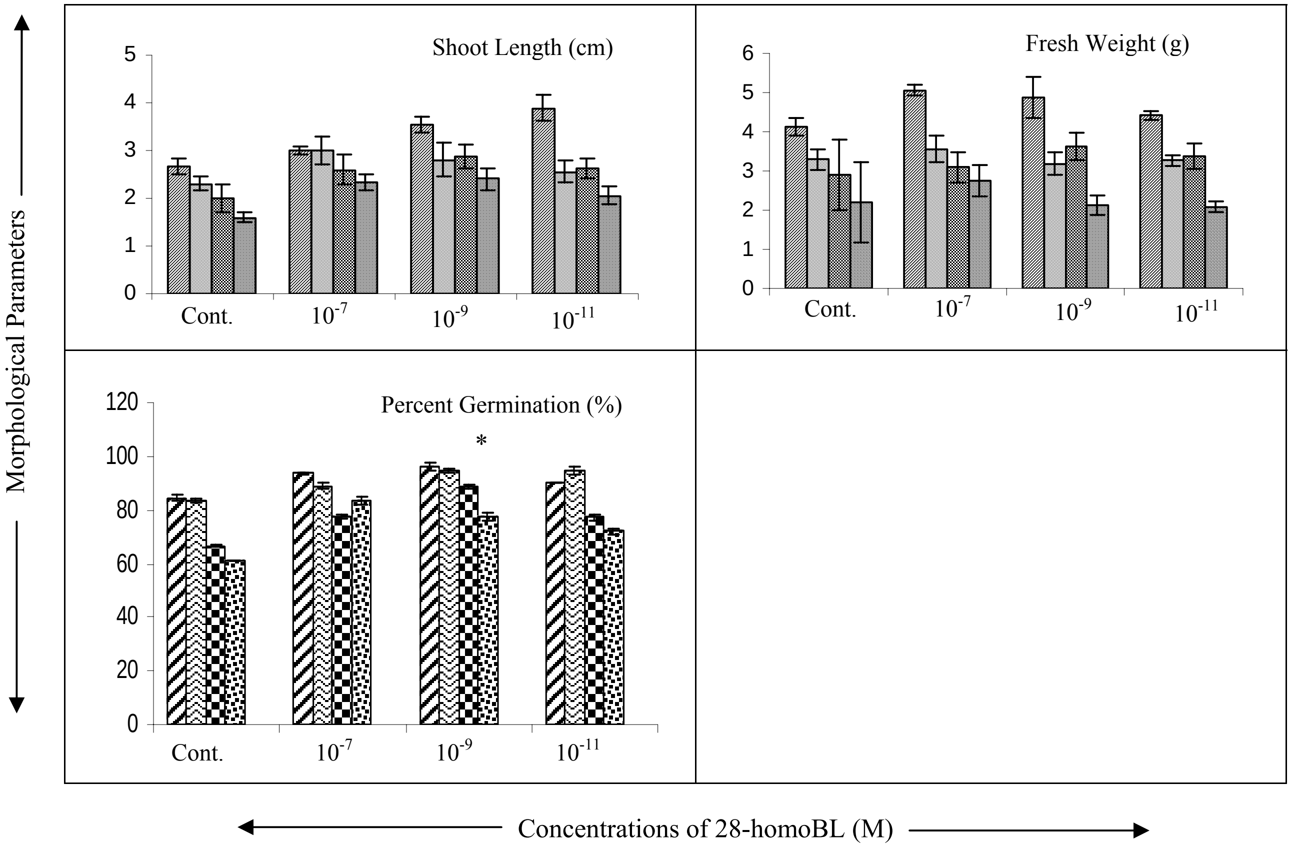

Fig. 1. Effect of 28-homobrassinolide (M) (seed-presowing treatment, 8h) on shoot length (cm) and fresh weight (g/plant) and percent germination of 30-days old *B. juncea* plants under different concentrations (25, 50 and 100 mg l^{-1}) of heavy metal (Mn). Bars represent the SE. *Indicate significant at $p \leq 0.05$

0 mg l^{-1} ; 25 mg l^{-1} ; 50 mg l^{-1} ; 100 mg l^{-1}

(8h) were sown in the pots that contained different concentrations of Mn (0, 25, 50 and 100 mg kg^{-1}). The plants were kept in natural seasonal conditions. After 30 days of sowing, the plants were harvested and observations were made on percent seed germination, shoot length and fresh weight. Mn-uptake was studied in leaves, shoots and roots of the plants.

Manganese uptake Analysis

The collected samples were oven dried at 80º C for 24 hours. The dried samples of leaves, shoots and roots of *Brassica juncea* were digested in H_2SO_4: HNO_3: $HClO_4$ (1:5:1) by following the method of Allen *et al*. (1976). The digested samples were fine filtered with double distilled water by using Whatman No. 1 filter paper and final volume was made up to 20 ml.

Heavy metal analysis was done using Atomic Absorption Spectrophotometer (AA-6200 Shimadzu). The bio-concentration factor (BCF) was calculated as follows:

$$\text{BCF} = \frac{\text{Tace element concentration in plant tissues (mg kg}^{-1}\text{) at harvest}}{\text{Initial concentration of the element in the applied external solution (mg l}^{-1}\text{)}}$$

Results were statistically analyzed by one-way analysis of variance (ANOVA), *Z*-test and comparisons with p values <0.05 were considered significantly different. Standard error due to replicates was also calculated.

RESULTS

Morphological parameters

The observation made on various morphological parameters revealed that the treatment of 28-homoBL to plants under Mn stress reduced the toxicity of metal by showing improved growth. The effects of seed pre-sowing treatments of 28-homoBL on morphological parameters (percent germination, shoot length and fresh weight) in field experiment are presented in fig 1. The percent

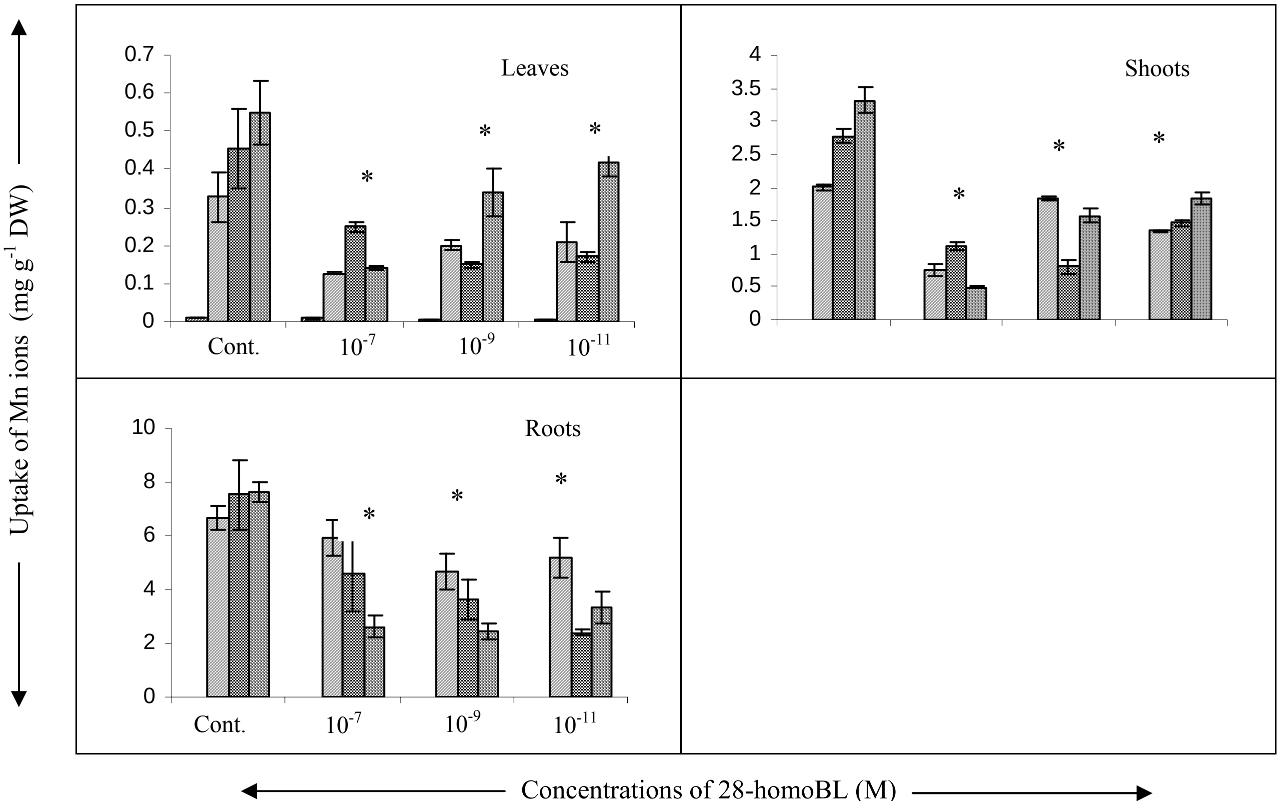

Fig. 2. Effect of 28-homobrassinolide (M) (seed-presowing treatment, 8h) on Mn ions uptake (mg g^{-1} DW) in leaves, shoots and roots of 30-days old *B. juncea* plants under different concentrations (0, 25, 50 and 100 mg l^{-1}) of heavy metal (Mn). Bars represent the SE. *Indicate significant at $p \leq 0.05$

0 mg l^{-1} ; 25 mg l^{-1} ; 50 mg l^{-1} ; 100 mg l^{-1}

germination and shoot length of plants got reduced as metal treatment alone was enhanced to *B. juncea* plants. Maximum reduction in percent germination and shoot length was observed in 100 mg kg^{-1} of Mn. The pre-sowing treatment of seeds with 28-homoBL alone increased percent germination and shoot length of plants. The most effective treatments were 10^{-9} M and 10^{-11} M for percent germination and shoot length of plants respectively. However in 25 mg kg^{-1} treatment of Mn, 10^{-11} M and 10^{-7} M of 28-homoBL showed maximum percent germination and shoot length respectively. In 50 mg kg^{-1} of Mn supplemented soil, 10^{-7} M of 28-homoBL showed maximum percent germination and shoot length. In case of 100 mg kg^{-1} of Mn supplemented soil, 10^{-7} M and 10^{-9} M of 28-homoBL showed maximum percent germination and shoot length respectively. Similar observations were made for fresh weight of whole plant under the influence of various concentrations of 28-homoBL treatments.

Metal uptake and bioconcentration factor (BCF)

The observations on Mn-uptake in different plant parts (leaves, shoots and roots) revealed that Mn^{2+} content of roots was higher, when compared to leaves and shoots of *B. juncea* plants given treatments of Mn^{2+} (Fig. 1). 28-homoBL pre-sowing treatments, resulted in a significant reduction in metal uptake and BCF content. In all plant parts (leaves, shoots and roots), uptake of Mn^{2+} and BCF content was significantly lowered under the effect of different concentrations of 28-homoBL. In 25 mg kg^{-1} of Mn supplemented soil, 10^{-7} M and 10^{-9} M of 28-homoBL showed maximum reduction in metal uptake and BCF in leaves, shoots and roots. In 50 mg kg^{-1} of Mn supplemented soil, 10^{-9} M and 10^{-11} M of 28-homoBL showed maximum reduction in metal uptake and BCF in leaves, shoots and roots. In case of 100 mg kg^{-1} of Mn supplemented soil, 10^{-7} M and 10^{-9} M of 28-homoBL showed maximum reduction in metal uptake and BCF in leaves, shoots and roots (Figs. 1, 2). Among all plant parts, maximum reduction was reported in case of leaves.

DISCUSSION

It is evident from the observations made on the present piece of work that the pre-treatment of different concentrations of brassinilode not only improved percent germination and plant growth but also reduced the toxicity of metal by lowering the metal uptake. The work is in continuation to our earlier studies done on copper

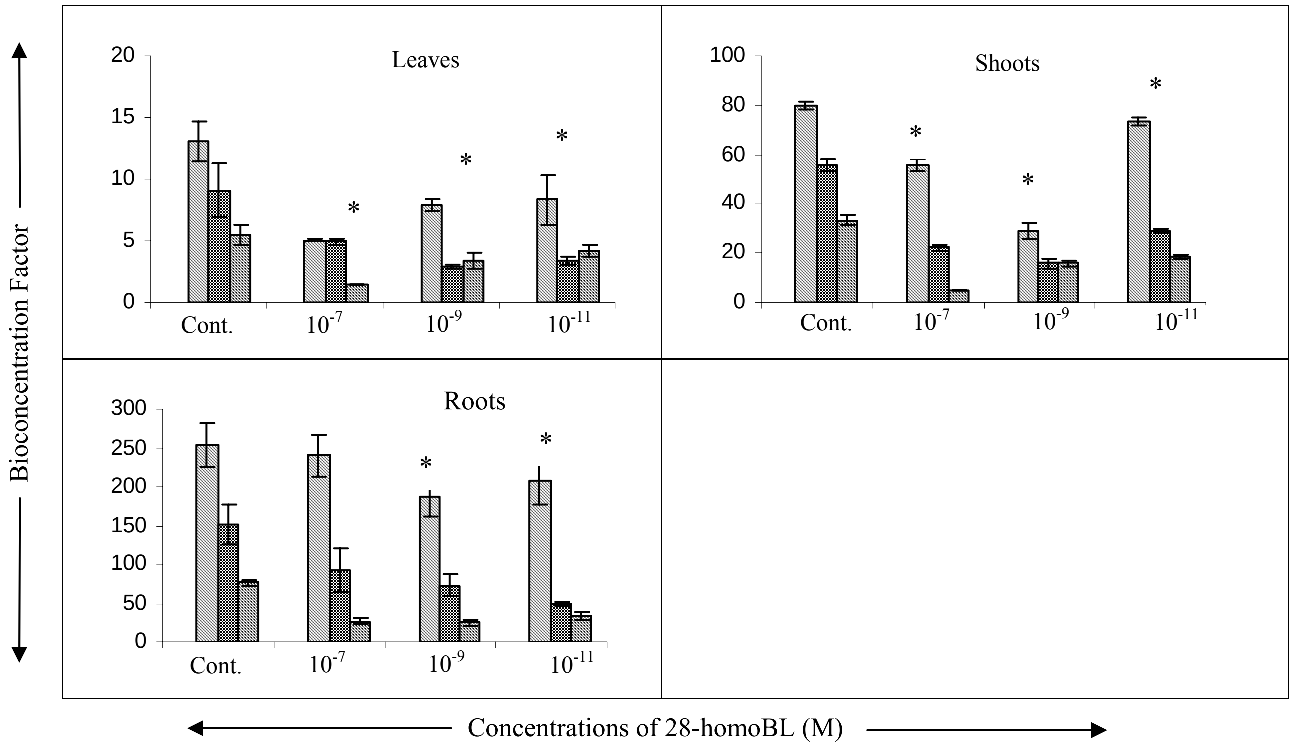

Fig. 3. Effect of 28-homobrassinolide (M) (seed-presowing treatment, 8h) on bioconcentration factor in leaves, shoots and roots of 30-days old *B. juncea* plants under different concentrations (0, 25, 50 and 100 mg l^{-1}) of heavy metal (Mn). Bars represent the SE. *Indicate significant at $p \leq 0.05$

25 mg l^{-1} ; 50 mg l^{-1} ; 100 mg l^{-1}

and nickel toxicity in *B. juncea* plants (Sharma and Bhardwaj, 2007a, 2007b). Other related reports also indicate stress-protective properties of BRs in other plants. Braun and Wild (1984) reported that BRs stimulated leaf elongation of wheat and mustard plants. In addition, Clouse *et al*. (1992) also found that BRs at a concentration of 0.1 μM induced measurable increase in length of soybean epicotyl.

The pleiotropic effects of BRs also indicate their ability to regulate the uptake of ions into the plant cell and hence have the potential to reduce the accumulation of heavy metals and radioactive elements when plants are grown in polluted areas (Khripach *et al*., 1996; Zhang *et al*., 2005). Barley plants (Cv. Zazersky) treated with epibrassinolide in the booted stage at a dose of 10 mg/ha showed that the diminution of metal content in the plant was 40-98 % in comparison to control. The accumulation of metals (Cd, Cu, Zn and Pb) under the influence of epibrassinolide had been studied in different agricultural plants such as barley, tomatoes, radish and sugar beet (Volynets *et al*., 1997). In tomato seeds, soaking treatment for 12h in 10^{-8} M solution of epibrassinolide before sowing was found more efficient in decreasing the content of Zn and Cd in tomato fruits than spraying treatment. It was shown that changes in the ions/metal content were influenced by 24-epibrassinolide and dependent on the stage of plant development when the seeds were treated. The content of ^{137}Cs in the plants at the flowering stage was even higher than in the untreated control. Fully matured plants showed some decrease of ^{137}Cs content especially in the vegetative organs probably as a result of vegetative dilution (Khripach *et al*., 1999).

It was observed that brassinosteroids in combination with Pb caused stimulation of phytochelatins synthesis in *Chlorella vulgaris*. BRs stimulated growth and photosynthetic parameters after blocking the bio-absorption of lead in *Chlorella vulgaris* (Bajguz, 2002). Bilkisu *et al*. (2003) reported that BL during Al-related stress stimulated growth in *Phaseolus aureus*. The protective effect of epibrassinolide on winter rape plants under Cd stress was investigated by Janeczko *et al*. (2005). The changes in plant growth, photosynthesis, carbonic anhydrase, nitate reductase and antioxidative enzymes by giving cadmium and 28-homobrassinolide to *Brassica juncea* were studied in 60-days old plants by Hayat *et al*. (2007). They observed that the toxic effects generated by Cd were reduced with homobrassinolide spraying treatment due to increase in plant growth and enhanced enzyme activities. Xia *et al*. (2006) found that 24-epibrassinolide pretreatment enhanced the resistance of cucumber seedlings to pesticides by increasing CO_2 assimilation capacity and activities of antioxidant enzymes. The finding of Alam *et al*. (2007) revealed that when plants of *Brassica juncea* L. cv. T-59 were supplied with 50 or 100 μM nickel (Ni_{50}, Ni_{100}) in 10-days after sowing and sprayed with 28-homobrassinolide at 20 days after sowing, 28-homoBL treatments overcame the toxic effect of Ni metal by increasing net photosynthetic rate, content of chlorophyll and the activities of nitrate reductase. Our earlier studies had also shown that 24-epiBL treatments (presowing) improved the shoot emergence and plant biomass production in *Brassica juncea* seedlings and plants under heavy metal stress (Cu, Zn, Mn, Co and Ni). 24-EpiBL has also been found to reduce the heavy metal uptake (Cu and Zn) and accumulation in *B. juncea* and *B. campestris* seedlings and plants. (Kaur and Bhardwaj 2004; Sharma and Bhardwaj, 2007a, 2007b). Further our studies on heavy metal stress indicate that 28-HomoBL ameliorate the Ni toxicity by increasing the activities of antioxidative enzymes like superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, catalase and glutathione reductase (Bhardwaj *et al*., 2007b).

The mechanism involved for reducing toxicity may be the chelation of the metal ions by the ligands. Such ligands include organic acids, amino acids, peptides or polypeptides (Bajguz, 2002). Further plant growth *via* cell elongation and cell division requires the coordination of several processes, some of them appears to be influenced by BRs. Plasticity of the cell wall is increased when proton extrusion by H^+- ATPases acidifies the apoplast, thereby activating the cell wall loosening enzymes. It increases the synthesis of new cell wall and membrane materials. BRs have been found to increase the ATPase activity in Azuki bean epicotyls and maize roots, leading to proton extrusion, and induced cell wall relaxation (Cerana *et al.,* 1983, 1984; Haubrick and Assmann, 2006). Further BRs also affect membrane permeability. Lowered uptake of metal ions could have been controlled at membrane level by BRs. The high biological activity of BRs therefore suggests an important role of BRs in protecting plants under stressed conditions.

ACKNOWLEDGEMENT

Financial assistance from University Grants Commission, New Delhi, India is duly acknowledged.

REFERENCES

Alam, MM., Hayat, S., Ali, B. and Ahmad, A. 2007. Effect of 28-homobrassinolide treatment on nickel toxicity in *Brassica juncea*. Photosynthetica. 45: 139-142.

Allen, SE., Grimshaw, HM., Parkinson, JA., Quarmby, C. and Roberts, JD. 1976. Chemical Analysis. In: Methods in Plant Ecology. Ed. Chapmanm, S.B. Blackwell Scientific Publications. Oxford – London, pp.424-426.

Bajguz, A. 2000. Effect of brassinosteroids on nucleic acids and protein content in cultured cells of *Chlorella vulgaris*. Plant Physiology and Biochemistry. 38: 209-215.

Bajguz, A. 2002. Brassinosteroids and lead as stimulators of phytochelatins synthesis in *Chlorella vulgaris.* Journal of Plant Physiology. 159: 321-324.

Bhardwaj, R., Arora, HK., Nagar, PK. and Thukral, AK. 2006. Brassinosteroids-A novel group of plant hormones. In: Plant Molecular Physiology-Current scenario and future Projections. Ed. Trivedi, P.C. Aaviskar Publisher. Jaipur, pp.58-84.

Bhardwaj, R., Kaur, S., Nagar, PK. and Arora, HK. 2007a. Isolation and characterization of brassinosteroids from immature seeds of *Camellia sinensis* (O) Kuntze. Plant Growth Regulation. 53: 1-5.

Bhardwaj, R., Arora, N., Sharma, P. and Arora, HK. 2007b. Effects of 28-homobrassinolide on seedling growth, lipid peroxidation and antioxidative enzyme activities under nickel stress in seedlings of *Zea mays* L. Asian Journal of Plant Sciences. 6(5): 765-772.

Bilkisu, AA., Xiao-Gang, G., Qing-Lei, G. and Yong-Hua, Y. 2003. Brassinolide amelioration of aluminium toxicity in mungbean seedling growth. Journal of Plant Nutrition. 26: 1725-1734.

Braun, P. and Wild, A. 1984. The influence of brassinosteroid on growth and parameters of photosynthesis of wheat and mustard plants. Journal of Plant Physiology. 116**:** 189-196.

Cerana, R., Bonetti, A., Marre, MT., Romani, G., Lado, P. and Marre, E. 1983. Effects of a brassinosteroid on growth and electrogenic proton extrusion in *Azuki bean* epicotyls. Physiolgia Plantarum. 59: 23-27.

Cerana, R., Lado, R., Anastasia, M., Ciuffreda, P. and Allevi, P. 1984. Regulating effects of brassinosteroids and sterols on growth and proton secretion in maize roots. Plant Flanzenphysiol. 114: 221-225

Clouse, SD., Zurek, DM., McMorris, TC. and Baker, ME. 1992. Effect of brassinolide on gene expression in elongating soybean epicotyls. Plant Physiology. 100: 1377-1383.

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

Dhaubhadel, S., Browning, KS., Gallie, DR. and Krishna, P. 2002. Brassinosteroid functions to protect the translational machinery and heat-shock protein synthesis following thermal stress. The Plant Journal. 29(6): 681-691

Doncheva, S., Georgieva, K., Vassileva, V., Stoyanova, Z., Popov, N. and Ignatov, G. 2005. Effects of Succinate on Manganese Toxicity in Pea Plants. Journal of Plant Nutrition. 28: 47-62.

Fecht-Christoffers, MM. Braun, HP., Lemaitre-Guillier, C., VanDorsselaer, A. and Horst, WJ. 2003. Effect of Manganese Toxicity on the Proteome of the Leaf Apoplast in Cowpea. Plant Physiology. 133: 1935–1946

Haubrick, LL. and Assmann, SM. 2006. Brassinosteroids and plant function: some clues, more puzzles. Plant, Cell and Environment. 29: 446-457

Hayat, S., Ali, B., Aiman Hassan, S. and Ahmad, A. 2007. Brassinosteroids enhanced antioxidants under cadmium stress in *Brassica juncea.* Environmental and Experimental Botany. 60(1): 33-41.

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

Kaur, S. and Bhardwaj, R. 2004. Brassinosteroids regulated heavy metals uptake in *Brassica campestris* L. In: Annual Meeting of the American Society of Plant Biologists. Plant Biology. p. 628.

Khripach, VA., Zhabinskii,VN. and de Groot, AE. 1999. Brassinosteroids: A new class of plant hormones, Academic Press, San Diego - CA.

Khripach, VA., Zhanbinskii, VN., Litvinoskaya, RP., Zavadskaya, MI., Saveleva, EA., Karas II. and Vakul, VV. 1996. A method of increasing of potato value. Pat. Appl. BY. 960: 345.

Khripach, VA., Zhabinskii, VN. and de-Groot, AE. 2000. Twenty years of brassinosteroids**:** steroidal plant hormones warrant better crops for the XXI century. Annals of Botany. 86: 441-447.

Krishna, P. 2003. Brassinosteoids – mediated stress responses. Journal of Plant Growth Regulation. 22: 289-297.

Ozdemeir, 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 Regulation. 42: 203-211.

Sasse, JM. 1997. Recent progress in brassinosteroids research. Physiolgia Plantarum. 100: 696-701.

Sasse, J.M. 1999. Physiological actions of brassino-steroids. In: Brassinosteroids: Steroidel Plant Hormones. Eds. Sakurai, A., Yokota, T. and Clouse, S.D. p. 137-161.Springer-Verlag. Tokyo.

Sharma, P. and Bhardwaj, R. 2007a. Effect of 24-epibrassinolide on seed germination, seedling growth and heavy metals uptake in *Brassica juncea* L. General and Applied Plant Physiolgy. (In Press).

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

Shi, Q., Bao, Z., Zhu, Z., He, Y., Qian, Q. and Yu, J. 2005. Silicon-mediated alleviation of Mn toxicity in *Cucumis sativus* in relation to activities of superoxide dismutase and ascorbate peroxidase. Phytochemistry. 66:1551-1559.

Upreti, KK. and Murti, GSR. 2004. Effects of brassinosteroids on growth, nodulation, phytochrome content and nitrogenase activity in French bean under water stress. Biologia Plantarum. 48: 407-411.

Volynets, AP., Pschenichanye, LA. and Khripach, VA. 1997. The nature of protective action of 24-epibrassinolide on barley plants. Plant Growth Regulation Society America. 24: 133-137.

Xia, X J., Huang, YY., Wang, L., Huang ,L F., Yu, YL., Zhou, YH. and Yu, J Q. 2006. Pesticides-induced depression of photosynthesis was alleviated by 24-epibrassinolide pretreatment in *Cucumis sativus* L. Pesticide Biochemistry and. Physiology. 86: 42-48.

Zhang, Z., Ramirez, J., Reboutier, D., Brault, M., Trouverie, J., Pennarun, AM., Amiar, Z., Biligui, B., Galagovsky, L. and Jean-Pierre Rona, JP. 2005. Brassino-steroids Regulate Plasma Membrane Anion Channels in Addition to Proton Pumps During Expansion of *Arabidopsis thaliana* Cells. Plant Cell Physiology. 46: 1494-1504.