Acta Physiol Plant (2007) 29:259–263
DOI 10.1007/s11738-007-0032-7

ORIGINAL PAPER

Effects of 24-epibrassinolide on growth and metal uptake in *Brassica juncea* L. under copper metal stress

Priyanka Sharma · Renu Bhardwaj

Received: 6 June 2006 / Revised: 13 November 2006 / Accepted: 3 January 2007 / Published online: 22 February 2007

Abstract The present investigation describes the effects of 24-epibrassinolide on plant growth, copper uptake and bioconcentration factor in the plants of *Brassica juncea* L. cv. PBR 91 under Cu metal stress. The study revealed that there was an improvement in the shoot emergence and plant biomass production under the influence of pre-germination treatment of 24-epibrassinolide (24-epiBL). In addition, 24-epiBL blocked copper metal uptake and accumulation in the plants.

Keywords Brassinosteroid · Heavy metal stress · Hormone · Indian mustard

Abbreviations

24-epiBL	24-Epibrassinolide
BCF	Bioconcentration factor
BRs	Brassinosteroids

Introduction

Brassinosteroids (BRs) represent a group of naturally occurring steroidal lactones widely distributed in plant kingdom. These compounds, which include the highly bioactive brassinolide (BL) and its derivatives, have been regarded as new plant growth regulators essential for plant development (Mandava 1988). In addition to stimulating growth, BRs have an anti-stress effect on plants. These compounds have wide-range of biological activities that increase the crop yields by changing plant metabolism and protecting plants from environmental stresses (Krishna 2003). BRs have been implicated in protecting plants from various types of stresses like drought (Upreti and Murti 2004), salt (Ozdemir et al. 2004), heat (Dhaubhadel et al. 2002) and heavy metals (Bajguz 2000b; Kaur and Bhardwaj 2004; Janeczko et al. 2005).

Among pollutants of agricultural soils, Cu has become increasingly hazardous due to its involvement in fungicides, fertilizers and pesticides (Chen et al. 2000). However, Cu at high levels become strongly phytotoxic and cause inhibition of plant growth or even death. BRs have ability to regulate the uptake of ions into the plant cells and can be used to reduce the accumulation of heavy metals and radioactive elements. The high biological activity of BRs suggests an important role in the regulation of physiological processes in plants and also in antistress activity. The application of BRs in low concentrations at a certain stage of development reduced the metal absorption in barley, tomatoes and sugerbeet significantly (Volynets et al. 1997). Keeping in mind the anti-stress properties of brassinosteroids, the present piece of work was undertaken to observe the involvement of 24-epibrassinolide in Cu metal stress in *B. juncea*, an important edible crop.

Communicated by S. Lewak.

P. Sharma · R. Bhardwaj (✉)
Department of Botanical and Environmental Sciences,
Guru Nanak Dev University,
Amritsar 143005 Punjab, India
e-mail: renu_bhardwaj@rediffmail.com

Materials and methods

Study material and treatments

The seeds of *B. juncea* L cv. PBR 91 were surface sterilized with 0.01% $HgCl_2$ and rinsed with double distilled water. The sterilized seeds were soaked for 8 h in different concentrations of 24-epiBL (0, 10^{-7}, 10^{-9} and 10^{-11} M). In order to study the effects of 24-epiBL on the plant growth and Cu metal uptake in the different parts (leaves, shoots and roots) of the *B. juncea* plant under Cu stress, a seasonal pot experiment was performed. The garden soil used for investigation was analysed for Cu ion content and filled in the experimental pots (approximately 9 kg in each pot). Different concentrations of copper ($CuSO_4 \cdot 5H_2O$) (0, 25, 50 and 100 mg kg^{-1} soil) were added to pots containing garden soil. The 24-epibrassinolide treated seeds (8 h) were sown in the pots that contained different concentrations of Cu metal. The plants were kept in natural seasonal conditions. After 30 days of sowing, the plants kept under various treatments were harvested and observations were made for shoot length, fresh and dry weights. Cu-metal uptake was studied in leaves, shoots and roots of the plants.

Copper uptake analysis

Samples of ground, dried plant material were digested using digestion mixture (H_2SO_4:HNO_3:$HClO_4$ in 1:5:1 ratio) by following the method of Allen et al. (1976). The samples were digested till dry colourless residue obtained by evaporating all the acids completely. The dried colourless residue was dissolved in 20 ml double distilled water, which was fine filtered through Whatman # 1 filter paper. The heavy metal analysis was done using Atomic Absorption Spectrophotometer (AA-6200 Shimadzu, Japan). The bioconcentration factor (BCF) was calculated as follows:

$$\mathrm{BCF} = \frac{\text{Trace element concentration in plant tissues (mg kg}^{-1}\text{) at harvest}}{\text{Initial concentration of the element in the external nutrient solution (mg kg}^{-1}\text{)}}.$$

Results were statistically analyzed by calculating the mean value, standard deviation, standard error and student's *t*-test following Bailey (1995).

Results

Morphological parameters

The treatment of 24-epiBL to plants under Cu metal stress reduced the toxicity of metal by showing better growth (Table 1). The results indicated that the growth of *B. juncea* was significantly affected by different concentrations of Cu metal alone against the control. The reduced shoot length was observed in higher treatment of Cu (100 mg kg^{-1}). The pre-germination treatment of seeds with 24-epiBL increased the shoot length. The most effective treatment of 24-epiBL was 10^{-9} M (Table 1). As the concentration of Cu increased, 10^{-11} M was found to lower the toxicity of metal by increasing the shoot length of plants. It was revealed that fresh weight of whole plant was increased in all the concentrations of 24-epiBL as compared to control (Table 2). The most effective concentration of 24-epiBL was 10^{-11} M. Similar observations were made for dry weight of whole plant under the influence of brassinosteroid treatments (Table 2).

Metal uptake and bioconcentration factor (BCF)

The observations on metal ion content (Cu) in different plant parts (leaves, shoots and roots) revealed that the Cu^{2+} content of roots was higher, when compared to leaves and shoots in all concentrations of Cu^{2+} (Fig. 1). Various concentrations (25, 50 and 100 mg kg^{-1}) of Cu^{2+} in the presence of 24-epiBL treatments, showed a significant reduction in metal uptake and BCF content. In all plant parts (leaves, shoots and roots), uptake of Cu^{2+} and BCF content was significantly lowered under the effect of different concentrations of 24-epiBL. This compound at 10^{-7} M concentration was found to be most effective for low-

Table 1 Effects of 24-epiBL on shoot length (cm) of *B. juncea* plants under Cu metal stress after 30 days of sowing

Concentrations of 24-epiBL (M)	Concentrations of Cu metal (mg/kg)			
	0	25	50	100
0	2.60 ± 0.23	2.200 ± 0.100	2.067 ± 0.167	1.833 ± 0.167
10^{-7}	2.80 ± 0.35	2.833 ± 0.441*	2.667 ± 0.333	2.333 ± 0.441
10^{-9}	3.02 ± 0.15	2.167 ± 0.167	2.333 ± 0.167	2.400 ± 0.333
10^{-11}	2.90 ± 0.12	2.167 ± 0.167	2.667 ± 0.10*	2.667 ± 0.441

Mean ± SE

* Values significantly different at $P \le 0.05$ from control

Table 2 Effects of 24-epiBL on fresh and dry weight (g plant^{-1}) of *B. juncea* plants under Cu metal stress after 30 days of sowing

Concentrations of 24-epiBL (M)	Concentrations of Cu metal (mg/kg)							
	Fresh weight (g/plant)				Dry weight (g/plant)			
	0	25	50	100	0	25	50	100
0	2.23 ± 0.12	2.167 ± 0.296*	1.475 ± 0.048	3.634 ± 0.307	1.07 ± 0.06	1.511 ± 0.253	0.666 ± 0.030	1.492 ± 0.006
10^{-7}	4.08 ± 0.15	4.267 ± 0.170	1.737 ± 0.073	5.760 ± 0.246	0.78 ± 0.03	0.698 ± 0.188	0.456 ± 0.024*	2.044 ± 0.068*
10^{-9}	4.89 ± 0.09	5.124 ± 0.077*	5.012 ± 0.206	4.539 ± 0.416	1.35 ± 0.04	1.874 ± 0.086	1.711 ± 0.042	1.665 ± 0.062
10^{-11}	4.95 ± 0.07	5.977 ± 0.486	1.945 ± 0.045	5.608 ± 0.433	1.56 ± 0.10	2.138 ± 0.239	0.739 ± 0.039	2.034 ± 0.110

Mean ± SE

* Values significantly different at $P \le 0.05$ from control

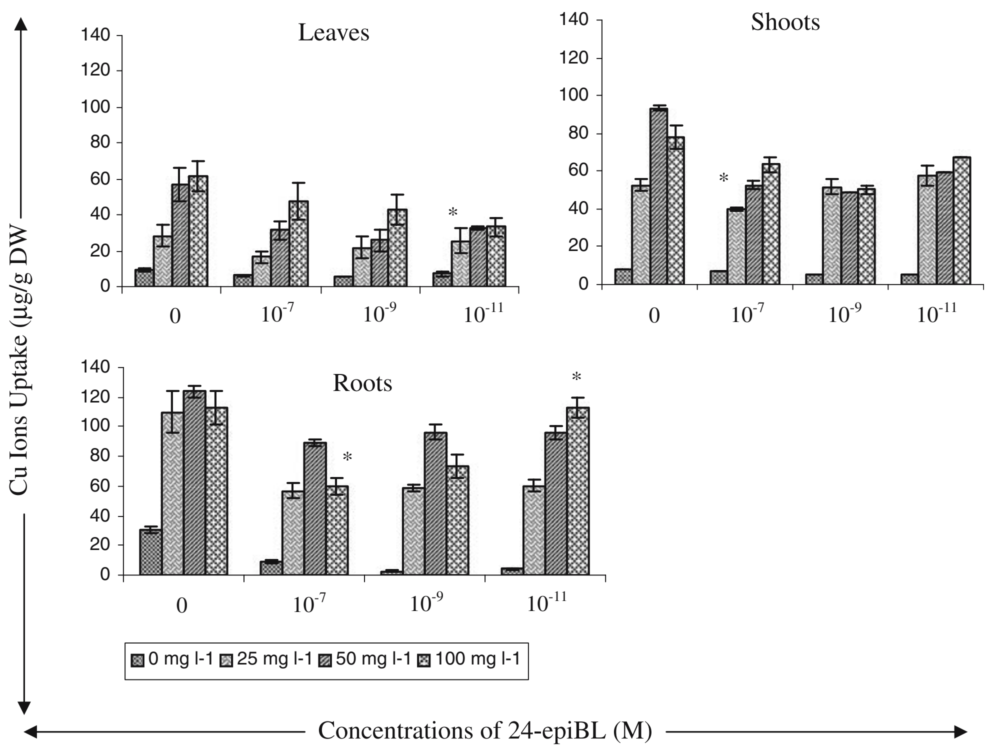

Fig. 1 Uptake of Cu metal (µg/g DW) in 30-day-old leaves, shoots and roots of *B. juncea* under the influence of 24-epibrassinolide. *Bars* represent the SE. *Asterisk* indicate statistically significant difference from control at $P \le 0.05$

ering the Cu uptake and accumulation of ions (Figs. 1, 2). Among all plant parts, the maximum reduction was reported in case of leaves.

Discussion

In the present investigation, 24-epiBL has been observed to improve the growth of *B. juncea* plant as compared to metal treated and non-treated plants. Similarly, BR stimulated leaf elongation of wheat and mustard plants (Braun and Wild 1984). In addition, Clouse et al. (1992) reported that BR (0.1 µM) induced measurable increase in length of soybean epicotyl. Franck-Duchenne et al. (1998) found that epibrassinolide at a concentration of 0.1 µM promoted stem induction and elongation, and produced large leaves of two cultivars of sweet peppers.

24-Epibrassinolide has also been found to reduce the Cu ion uptake and accumulation in *B. juncea* L plants. Earlier studies done on the accumulation of heavy metals under the influence of BRs also shown reduction in metal toxicity in barley, tomato and radish etc., (Volynets et al. 1997). Bajguz (2000b) revealed that 24-epibrassinolide blocked the heavy metal accumulation in *Chlorella vulgaris* cells. It has been observed that brassinosteroids in combination with Pb caused stimulation of phytochelatins synthesis in *C. vulgaris*. BRs stimulated growth and photosynthetic parameters after blocking the bioabsorption of lead in

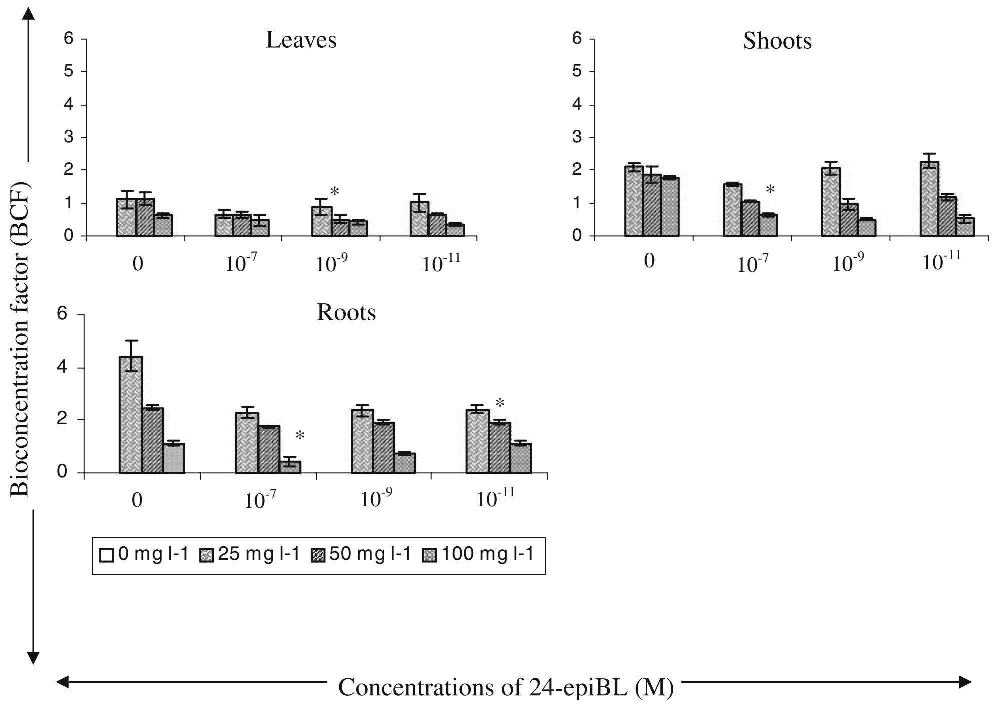

Fig. 2 Bioconcentration factor (BCF) of Cu metal in 30-day-old leaves, shoots and roots of *B. juncea* under the influence of 24-epibrassinolide. *Bars* represent the SE. *Asterisk* indicate statistically significant difference from control at $P \leq 0.05$

C. vulgaris (Bajguz 2002). The protective effect of epibrassinolide on winter rape plants under Cd stress was investigated by Janeczko et al. (2005). Bilkisu et al. (2003) reported that BL during Al-related stress stimulated growth in *Phaseolus aureus*. It was shown that changes in the ions/metal content were influenced by 24-epiBL 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).

The mechanism involved for reducing the toxicity may be the chelation of the metal ion by a ligand. Such ligand includes organic acids, amino acids, peptides or polypeptides (Bajguz 2002). Further, the BRs have also influence on electrical properties of membranes and transport of ions by altering their permeability and structure, stability and activity of membrane enzymes. The reduction of toxicity by BRs was associated with lesser uptake of ions and enhanced levels of soluble proteins and nucleic acids with the increasing activity of ATPase, an enzyme responsible for acid secretion and changes in membrane level (Bajguz 2000a). The proton pump generates an H^+ electrochemical gradient and provides a driving force for the rapid ion fluxes required for the uptake of various nutrients such as K^+, Cl^-, NO_3^-, amino acids and sucrose across the plasma membrane (Sze et al. 1999). The regulation of H^+-ATPase activity (Kasamo 2003) not only allows nutrient uptake in plant cells but also controls water fluxes (Sondergaard et al. 2004). However, little is known about the roles of ion transport systems during BR-induced cell expansion. Previous studies reported a proton secretion induced by BL when applied to azuki bean epicotyls or apical root segments of maize (Zhang et al. 2005). This proton secretion was accompanied by an early hyperpolarization of the plasma membrane, indicating that proton pumps could be targets of BRs (Zhang et al. 2005). The high biological activity of BRs suggests an important role in the regulation of physiological processes in plants and also in anti-stress activity. The present study thus reveals the involvement of 24-epiBL in lowering the uptake of Cu metal ions in *B. juncea* plants.

References

Allen SE, Grimshaw HM, Parkinson JA, Quarmby C, Roberts JD (1976) Chemical analysis. In: Chapman SB (ed) Methods in plant ecology. Blackwell, Oxford, pp 424–426

Bailey NTJ (1995) Statistical methods in biology. The English University Press, London

Bajguz A (2000a) Effects of brassinosteroids on nucleic acids and protein in cultured cells in *Chlorella vulgaris*. Plant Physiol Biochem 38:209–215

Bajguz A (2000b) Blockage of heavy metal accumulation in *Chlorella vulgaris* cells by 24-epibrassinolide. Plant Physiol Biochem 38:797–801

Bajguz A (2002) Brassinosteroids and lead as stimulators of phytochelatins synthesis in *Chlorella vulgaris*. J Plant Physiol 159:321–324

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

Braun P, Wild A (1984) The influence of brassinosteroid on growth and parameters of photosynthesis of wheat and mustard plants. J Plant Physiol 116:189–196

Chen LM, Lin CC, Kao CH (2000) Copper toxicity in rice seedlings: changes in antioxidative enzyme activities, H_2O_2 level and cell wall peroxidase activity in roots. Bot Bull Acad Sci 41:99–103

Clouse SD, Zurek DM, McMorris TC, Baker ME (1992) Effect of brassinolide on gene expression in elongating soybean epicotyls. Plant Physiol 100:1377–1383

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

Franck-Duchenne M, Wang Y, Tahar BS, Beachy N (1998) In vitro stem elongation of sweet pepper in media containing 24-epibrassinolide. Plant Cell Tissue and Organ Cult 53:79–84

Janeczko A, Koscielniak J, Pilipowicz M, Szarek-Lukaszewska G, Skoczowski A (2005) Protection of winter rape photosystem 2 by 24-epibrassinolide under cadmium stress. Photosynthetica 43:293–298

Kasamo K (2003) Regulation of plasma membrane H^+-ATPase activity by the membrane environment. J Plant Res 16:517–523

Kaur S, Bhardwaj R (2004) Blockage of Zn-accumulation in seedlings of *Brassica campestris* L. by 24-epibrassinolide. Keystone symposium on plant responses to abiotic stresses, Abstract No. 216:64, pp 19–24

Khripach VA, Zhabinskii VN, de Groot AE (1999) Brassinosteroids: A new class of plant hormones. Academic, San Diego, p 456

Krishna P (2003) Brassinosteoids—mediated stress responses. J Plant Growth Regul 22:289–297

Mandava NB (1988) Plant growth promoting brassinosteroids. Annu Rev Plant Physiol Plant Mol Biol 39:23–52

Ozdemir F, Bor M, Demiral T, 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

Sondergaard TE, Schulz A, Palmgren MG (2004) Energization of transport processes in plants. Roles of the plasma membrane H^+-ATPase. Plant Physiol 136:2475–2482

Sze H, Li X, Palmgren MG (1999) Energization of plant cell membranes by H^+-pumping ATPases: regulation and biosynthesis. Plant Cell 11:677–689

Upreti KK, Murti GSR (2004) Effects of brassinosteroids on growth, nodulation, phytohormone content and nitrogenase activity in French bean under water stress. Biol Plant 48:407–411

Volynets AP, Pschenichanye LA, Khripach VA (1997) The nature of protective action of 24-epibrassinolide on barley plants. Plant Growth Regul Soc Am 24:133–137

Zhang Z, Ramirez J, Reboutier D, Brault M, Trouverie J, Pennarun AM, Amiar Z, Biligui B, Galagovsky L, Rona JP (2005) Brassinosteroids regulate plasma membrane anion channels in addition to proton-pumps during expansion of *Arabidopsis thaliana* cells. Plant Cell Physiol 46:1494–1504