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Studies on the Effect of Brassinosteroids on the Qualitative Changes in the Storage Roots of Radish

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ABSTRACT

The effect of 24-epibrassinolide and 28-homobrassinolide on the qualitative changes in the storage roots of radish was studied. Brassinosteroids stimulated the growth of radish roots which was associated with increased levels of carbohydrates in terms of reducing sugars and starch. The soluble proteins were also elevated. Minerals like phosphorous, calcium and iron increased whereas the levels of potassium and sodium decreased. Brassinosteroids also considerably increased the contents of vitamins i.e. ascorbic acid and niacin present in the roots indicating their ability to improve the quality of storage roots of radish as the roots are the consumable parts of the plant.

Keywords: Brassinosteroids, carbohydrates, minerals, proteins, root growth, storage roots, vitamins

INTRODUCTION

Brassinosteroids (BRs) are a new type of polyhydroxy steroidal phytohormones with significant growth-promoting influence (Vardhini *et al*. 2008, 2010). BRs were discovered in 1970 by Mitchell and his co-workers (1970) and were later extracted from the pollen of *Brassica napus* L. (Grove *et al*. 1979). BRs can be classified as C27, C28 or C29 BRs according to the number of carbons in their structure. Haubrick and Assmann (2006) reported that 60 related compounds have been identified. However, brassinolide (BL), 28-homobrassinolide (28-HomoBL) and 24-epibrassinolide (24-EpiBL) are the three bioactive BRs being widely used in physiological and experimental studies (Vardhini *et al*. 2006). BRs are considered ubiquitous in plant kingdom as they are found in almost all the phyla of the plant kingdom like alga, pteridophyte, gymnosperms, dicots and monocots (Bajguz 2009). BRs are a new group of phytohormones that perform a variety of physiological roles like growth, seed germination, rhizogenesis, sensecence, etc. and also confer resistance to plants against various abiotic stresses (Rao *et al*. 2002).

Dwarf and de-etiolated phenotypes and BR-deficient species of some *Arabidopsis* mutants were rescued by the application of BRs (Bishop and Yakota 2001; Zeng *et al*. 2009). The work with BR biosynthetic mutants in *Arabidopsis thaliana* (Li *et al*. 1996) and *Pisum sativum* (Nomura *et al*. 1997) provided strong evidences that BRs are essential for plant growth and development. Although BRs were initially identified based on their growth promoting activities, subsequent physiological and genetic studies revealed additional functions of BRs in regulating a wide range of processes, including source/sink relationships, seed germination, photosynthesis, senescence, photomorphogenesis, flowering and responses to different abiotic and biotic stresses (Deng *et al*. 2007). Du and Pooviah (2005) reported that BRs are plant-specific steroid hormones that have an important role in coupling environmental factors, especially light, with plant growth and development, but stated that the role of endogenous BRs changes in response to the environmental stimuli was still largely unknown.

Various data provided consistent evidence that exogenous BR treatment is effective in stressful rather than optimal conditions (Fujita *et al*. 2006). They have been further explored for stress-protective properties in plants against a number of stresses like chilling (Huang *et al*. 2006; Liu *et al*. 2009), salt (Anuradha and Rao 2007; Shahabaz and Ashraf 2007), heat (Singh and Shono 2005), water (Hnilička *et al*. 2007; Yuan *et al*. 2010), heavy metals (Cao *et al*. 2005; Bharadwaj *et al*. 2007) and oxidative (Xia *et al*. 2009a) stresses. Thus Xia *et al*. (2009b) aptly stated that BRs induce plant tolerance to a wide spectrum of stresses.

Arteca and Arteca (2001) reported that BRs induce exaggerated growth in hydroponically grown *A. thaliana* and also control the proliferation of its leaf cells. BRs promote the growth of apical meristems in potato tubers (Korableva *et al*. 2002), accelerate the rate of cell division in isolated protoplasts of *Petunia hybrida* (Ho 2003) and also induce callus growth and regeneration ability in *Spartina patens* of poaceae (Lu *et al*. 2003). BRs also play a prominent role in nodulation and nitrogenase activity of groundnut [*Arachis hypogaea*] (Vardhini and Rao 1999), French bean [*Phaseolus vulgaris*] (Upreti and Murti 2004) and soya bean [*Glycine max*] (Hunter 2001). They even play a pivotal role in the genes expressing male fertility in the anther and pollen development of *Arabidopsis thaliana* (Ye *et al*. 2010).

Radish (*Raphanus sativus*) is an edible root vegetable belonging to the family *Brassicaceae* which is grown through the world. It is a well established fact from time immemorial that plants are the critical components of dietary food chains in which they provide almost all the essential mineral and organic nutrients to humans. Grusak and Dellapenna (1999) stressed the need of 'divert research' activities in improving the nutritional quality of plants with respect to nutrient content and composition. The present study is undertaken to understand the effect of application of 28-homoBL and 24-epiBL on the qualitative changes in the storage roots of radish.

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MATERIALS AND METHODS

Chemicals and plant material

The two BRs employed in the study, viz., 28-homobrassinolide (28-HomoBL; 940100) and 24-epibrassinolide (24-epiBL; 940600) are the commercially available BRs for research purposes and manufactured by M/s. Beak Technologies Inc., Ontario, Canada. Seeds of radish (*Raphanus sativus* L. var Pusa chetki long) were obtained from National Seeds Corp., Hyderabad, Andhra Pradesh, India.

Root growth

The seeds were sown in clay pots containing fresh sieved red soil mixed with farmyard manure. Plants were grown in a glass house under natural day length. 28-HomoBL and 24-epiBL were supplied to the plants as foliar spray at three different concentration levels viz., 0.5, 1.0 and 3.0 µM on the 20th, 35th and 50th day (from the day of sowing). Root growth parameters in terms of root length (cm), root fresh weight (g) and root dry weight (g) were recorded on 60th day. On the 60th day root material was homogenized using 70% (v/v) ethanol and stored in deep freezer for further biochemical analysis. Fresh roots were used for the estimation of vitamins viz., ascorbic acid and niacin. Simultaneously roots were dried in a hot air oven at 110°C for 24 h and the dried material was used for mineral analysis.

Metabolite contents

Soluble proteins in the ethanol homogenate were precipitated by adding 20% (w/v) tricholoroacetic acid. The precipitate was dissolved in 1% (w/v) sodium hydroxide. The method of Lowry *et al*. (1951) was employed for protein estimation.

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of reducing sugars (Nelson 1944). The residue was used for the estimation of starch by the McCready *et al*. (1980) method.

1 g of oven-dried sample was digested with 10 ml of tri-acid mixture (3 ml of conc. nitric acid + 3 ml of conc. percholoric + 4 ml of conc. sulphuric acid). The digested mixture was used for the estimation of phosphorus by the molybdate-vandate method following Johnson *et al*. (1980), and potassium and sodium were estimated following Issac and Kerber's procedure (1971).

1 g of the oven-dried sample was taken into a test tube and digested by 5 ml of *aqua regia* and the amount of iron present was estimated following the method of Issac and Kerber (1971).

1 g of the oven-dried sample was placed in silica crucibles and ashed in a muffle furnace and the amount of calcium present was estimated by EDTA trimetric method of APHA (1984) and calculated using the formula:

$$\text{Amount of calcium present} = \frac{\text{ml of versenate used x normality of versenate}}{\text{ml of aliquot taken}} \text{x500}$$

The vitamins, ascorbic acid and niacin present in the fresh roots were estimated according to Sadasivam and Balasubraminan (1987).

The values were presented as Mean ± S.E. of 5 replicates. ANOVA one way revealed that the mean values of different activities of 28-homoBL are significant over the control at the 5% level of significance. The values were calculated employing SPSS 16.0 statistical software.

RESULTS

Exogenous application of BRs (28-homoBL and 24-epiBL) resulted in a substantial increase in growth of radish roots as reflected in increases in length, fresh weight and dry weight of the roots (**Table 1**). Among the two BRs employed, 28-homoBL was most effective in stimulating the root growth of radish plants. An increase of around 60% was observed in the plants treated with 3 µM of 28-homoBL in both the treatments over the control plants.

Table 1 Effect of brassinosteroids on the root growth of *Raphanus sativus*.

Compounds	Treatments (µM)	Root length	Root fresh weight	Root dry weight
28-Homo BL	0.5	13.4 ± 0.13	281.6 ± 2.73	11.7 ± 0.38
	1.0	13.8 ± 0.68	372.3 ± 3.18	15.6 ± 0.22
	3.0	15.6 ± 0.27 a	382.3 ± 1.98	16.9 ± 0.53
24-EpiBL	0.5	12.0 ± 0.43	271.6 ± 3.35	11.6 ± 0.17
	1.0	13.9 ± 0.22	356.3 ± 2.59	15.2 ± 0.22
	3.0	14.8 ± 0.18	380.6 ± 2.70	16.8 ± 0.14
Control		9.2 ± 0.21 b	239.3 ± 2.90	9.6 ± 0.39

28-HomoBL =28-Homobrassinolide; 24-EpiBL= 24-epibrassinolide
Mean ± S.E (N=5). One-way ANOVA employing SPSS 16.0 statistical software revealed that the mean values of different activities of 28-homoBL are significantly different to the control at 5% level of significance (indicated by different letters). Root length (cm), root fresh and dry weight = g

Table 2 Effect of brassinosteroids on the metabolites of *Raphanus sativus*.

Compounds	Treatments (µM)	Soluble proteins	Reducing sugars	Starch
28-Homo BL	0.5	2.33 ± 0.14	7.61 ± 0.14	4.25 ± 0.18
	1.0	2.71 ± 0.04	8.10 ± 0.31	4.78 ± 0.28
	3.0	3.17 ± 0.09 b	8.83 ± 0.13	5.18 ± 0.42
24-EpiBL	0.5	2.18 ± 0.10	7.21 ± 0.22	4.13 ± 0.31
	1.0	2.60 ± 0.07	7.71 ± 0.09	4.54 ± 0.23
	3.0	3.04 ± 0.05	8.18 ± 0.32	4.99 ± 0.48
Control		1.87 ± 0.07 a	5.71 ± 0.08	3.02 ± 0.21

28-HomoBL =28-Homobrassinolide; 24-EpiBL= 24-epibrassinolide
Mean ± S.E (N=5). ANOVA one way employing SPSS 16.0 statistical software revealed that the mean values of different activities of 28-homoBL are significantly different to the control at 5% level of significance (indicated by different letters).
Soluble proteins, reducing sugars and starch = g (mg g^{-1} fresh weight)*

The root growth promotion by 28-homoBL and 24-epiBL was associated with increments in the levels of soluble proteins present in the roots of radish (**Table 2**). 28-HomoBL at 3 µM was more effective in increasing the soluble protein content than all concentrations of 24-epiBL treatments as well as untreated control plants.

The radish roots treated with foliar application of BRs (28-homoBL and 24-epiBL) showed increased contents of carbohydrate fractions viz., reducing sugars and starch (**Table 2**). 3 µM of 28-homoBL exhibited maximum elevated levels of reducing sugars and starch compared to other treatments and also the untreated controls.

Foliar application of 28-homoBL and 24-epiBL showed diversified changes in the minerals present in radish roots. Supplementation of BRs caused a marked rise in the levels of the minerals like phosphorus and calcium but only a slight enhancement in the mineral, iron present in the radish roots (**Table 3**). Application of 28-HomoBL at 3 µM increased the minerals in the radish roots more than 24-epiBL as well as the control plant roots. But the contents of potassium and sodium were reduced in the BR-supplemented plant roots of radish (**Table 3**). The roots treated with 28-homoBL at 3 µM exhibited less potassium and sodium among all treatments.

Ascorbic acid and niacin contents present in the roots of radish plants were slightly elevated following the foliar application of BRs (28-homoBL and 24-epiBL) (**Table 4**). 28-HomoBL at 3 µM was more effective in increasing the ascorbic acid and niacin contents compared to 24-epiBL treatments as well as untreated control plants.

DISCUSSION

Humans face problems due to nutrient deficiencies. An adequate dietary intake of all vital nutrients is important. Application of plant growth regulators not only increases the quantative but also the qualitative yields of several crops. Vardhini and Rao (2003) reported that exogenous application of 3 µM of 28-homoBL and 24-epiBL to tomato (*Lycopersicon esculentum*) plants resulted in enhanced root growth. Schilling *et al*. (1991) examined the effects of homoBL on sugar beet (*Beta vulgaris*) under drought stress

Table 3 Effect of brassinosteroids on the mineral contents of *Raphanus sativus* roots.

Minerals (mg/100 g)	Control	28-HomoBL			24-EpiBL		
		0.5 µM	1.0 µM	3.0 µM	0.5 µM	1.0 µM	3.0 µM
Phosphorus	24.1	26.2	27.9	29.4	25.9	27.1	28.7
Potassium	295	260	249	221	275	256	235
Calcium	33.8	37.8	38.9	41.3	36.3	38.1	40.7
Iron	0.41	0.49	0.72	1.19	0.45	0.68	0.93
Sodium	40.1	35.8	33.5	32.6	37.7	36.38	34.8

28-HomoBL =28-Homobrassinolide; 24-EpiBL= 24-epibrassinolide. Values are Mean ± S.E (N=5).

Table 4 Effect of brassinosteroids on the vitamins of *Raphanus sativus*.

Compounds	Treatments (µM)	Ascorbic acid (mg/100 g)	Niacin (mg/100 g)
28-Homo BL	0.5	17.45 ± 0.02	0.345 ± 0.04
	1.0	18.36 ± 0.01	0.398 ± 0.05
	3.0	19.19 ± 0.04 a	0.423 ± 0.07
24-EpiBL	0.5	17.27 ± 0.03	0.338 ± 0.04
	1.0	18.20 ± 0.05	0.381 ± 0.04
	3.0	18.94 ± 0.02	0.408 ± 0.06
Control		14.68 ± 0.03 b	0.322 ± 0.05

28-HomoBL =28-Homobrassinolide; 24-EpiBL= 24-epibrassinolide
Mean ± S.E (N=5). One-way ANOVA employing SPSS 16.0 statistical software revealed that the mean values of different activities of 28-homoBL are significant over control at 5% level of significance.

and found an increase of tap root mass. The studies conducted by Bao *et al*. (2004) on *Arabidopsis thaliana* revealed that BL at 0, 1, 2, 5, 10, 50 and 100 nm not only promoted acropetal auxin transport but further enhanced lateral root development. Kartal *et al*. (2009) also reported that increasing concentrations of homoBL from 0.1, 0.5 and 1.0 µM not only increased the root growth of barley (*Hordeum vulgare*), but also showed enlarged root tips compared to the control materials. The present study also showed that foliar application of 3 µM of BRs (28-homoBL and 24-epiBL) to radish plants increased the length, fresh and dry weight of radish roots.

Foliar application of BRs resulted in a substantial increase in soluble proteins of radish roots. Bajguz (2009) reported that 10 nm of BL increased the protein contents of *Chlorella vulgaris* as the cultured medium showed an increase in cell number. Sairam *et al*. (1996) also observed enhanced soluble protein content in wheat (*Triticum aestivum*) plants applied with 0.1 and 1.0 mg/l of homoBL. Similarly, Vardhini and Rao (2008) reported that supplementation of 0.5, 1.0 and 3.0 µM of BL as a foliar spray increased the protein content in the leaves of tomato plants whereas the exogenous application of 10^{-8} M of 24-epiBL and 28-homoBL enhanced the total protein contents in the seedlings of *Brassica junceae* (Sirhindi *et al*. 2009). Those results are in agreement with the present experiments on the effect of 28-homoBL and 24-epiBL on the contents of soluble proteins present in the storage roots of radish.

The present study revealed that the application of BRs (28-homoBL and 24-epiBL) resulted in substantial increases in the carbohydrate fractions like reducing sugars and starch in the storage roots of radish. These increases might have been due an enhanced photosynthetic capacity of the plants as well as an efficient source-sink translocation resulting from the foliar application of BRs. Soaking the seeds of *Triticum aestivum* for around one day in 10^{-10}, 10^{-8} or 10^{-6} M of homoBL significantly enhanced the soluble sugars present in the seedlings (Hayat *et al*. 2003). A spray application of 0.01, 0.1 or 1.0 mg L^{-1} 24-EpiBL to cucumber (*Cucumis sativus*) plants grown in a greenhouse resulted in increases in sucrose, soluble sugars and starch (Yu *et al*. 2004). The results obtained in the present study also exhibited that foliar application of 28-homoBL and 24-epiBL enhanced the carbohydrates present in the storage roots of radish. Further, BR-deficient *Arabidopsis thaliana* mutant has been found to have decreased starch and sugar contents (Schluter *et al*. 2002).

The content of phosphorus and calcium in the roots of BR-treated plants was higher than in untreated controls. An important observation in this study is that the iron content increased after BR treatment. On the other hand, the contents of potassium and sodium were low in the storage roots of 3 µM of 28-homoBL- and 24-epiBL-treated radish plants. Kuno (1997) observed enhanced translocation of phosphorus, but lowered calcium contents after BL-treatment to the leaves of mulberry (*Morus nigra*). Even Bajguz and Czerpak (1998) observed that the supplementation of 10^{-12}, 10^{-8}, 10^{-7} or 10^{-6} M of BL, 24-epiBL, homoBL, castasterone (CS), 24-epicastasterone (24-epiCS) and homocastasterone (homoCS) increased the phosphorus content in *Chlorella vulgaris*. Earlier, Pirogovskya *et al*. (1996) suggested that BRs can be employed to plants for effective absorption of minerals from the soil. Similar results were also observed where, external supplementation of 0.25, 0.50 or 1.00 mM of salicylic acid, another plant growth regulator, resulted in increased contents of minerals like potassium, calcium and iron in strawberry (*Fragaria x ananassa*) roots (Karlideg *et al*. 2009)

BR application slightly improved the vitamin C as well as niacin content in the radish storage roots. Thus the present study revealed that BR-supplementation resulted in favourable enhancement of vitamin C and niacin contents. The ability of BRs to enhance the growth and metabolism of the shoot system is a well established fact (Kamuro and Takatsuto 1999; Vardhini *et al*. 2008), but the present study revealed that foliar application of 28-homoBL and 24-epiBL might have resulted in enhanced plant shoot (source) growth which reflected in enhanced contents of not only the root (sink) growth but also the metabolites like soluble proteins and carbohydrates present in the roots. Recently, Vardhini *et al*. (2011) reported that application of 0.5, 1.0 and 3.0 µM of BL increased the biochemical compounds (soluble proteins, nucleic acids and carbohydrates) of *Raphanus sativus* plants. Apart from this, this paper also shows promising effects of BRs in increasing the mineral nutrients, especially iron, vitamin C and niacin, which provides new insight into another physiological role of BRs which has been neglected for around two decades i.e., its ability to enhance the quantity of minerals and vitamins.

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