

STUDIES ON THE EFFECT OF BRASSINOSTEROIDS ON THE QUALITATIVE CHANGES IN THE STORAGE ROOTS OF RADISH

B. VIDYA VARDHINI*¹, E. SUJATHA¹ and S. SEETA RAM RAO²

¹Telangana University, Department of Botany, Dichpally, Nizamabad 503322, India

²Osmania University, Department of Botany, University College of Science, Hyderabad 500007, India

Abstract

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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. The root growth promotion was associated with increased levels of reducing sugars, starch, soluble proteins and minerals like phosphorous, potassium, calcium, iron and sodium. 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.

Key words: Brassinosteroids; carbohydrates; minerals; proteins; root growth; storage roots; vitamins

Introduction

Brassinosteroids are a new type of polyhydroxy steroidal phytohormones with significant growth promoting influence (Vardhini et al., 2010; Vardhini et al., 2008). Brassinosteroids (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 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, senescence 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.

Arteca and Arteca (2001) reported that BRs induce exaggerated growth in hydroponically grown *Arabidopsis thaliana* and also control the proliferation of its leaf cells. BRs are a new group of phytohormones that perform a variety of physiological roles like growth, seed germination, rhizogenesis,

*E-mail: drvidyavardhini@rediffmail.com

senescence etc. and also confer resistance to plants against various abiotic stresses (Vardhini et al., 2006; Rao et al., 2002). 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 (Vardhini and Rao, 1999), french bean (Upreti and Murti, 2004) and soya bean (Hunter, 2001).

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-homobrassinolide (28-homoBL) and 24-epibrassinolide (24-epiBL) on the qualitative changes in the storage roots of radish.

Materials and Methods

Chemicals and Plant Material

The two brassinosteroids (BRs) employed in the study, viz., 28-homobrassinolide and 24-epibrassinolide were purchased from M/s. Beak Technologies Inc., Brampton, Ontario, Canada. Seeds of radish (*Raphanus sativus* L. var Pusa chetki long) were obtained from National Seeds Corporation, 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. BRs were supplied to the plants as foliar spray at three different concentration levels viz., 0.5 μM , 1.0 μM and 3.0 μM on 20th, 35th and 50th day (from the day of sowing). Root growth parameters were recorded on 60th day. On 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 hours and the dried material was used for mineral analysis.

Metabolite Contents

Soluble proteins

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

Reducing sugars and starch

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 McCready et al. (1980) method.

Mineral contents

- 1Gm of oven - dried sample was digested with 10ml of tri acid mixture (conc. Nitric acid + conc. Perchloric + conc. Sulphuric acid). The digested mixture was used for the estimation of phosphorus by molybdate- vandate method according to Johnson et al. (1980), and potassium and sodium by Issac and Kerber's procedure (1971).
- 1 Gm of the oven - dried sample was taken into a test tube and digested by 5ml of aquaregia and the amount of iron present was estimated employing the method of Issac and Kerber (1971).
- 1 Gm 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 titrimetric method of APHA (1984) and calculated using the formula:

$$\text{ml of versenate used} \times \text{normality of versenate} \times \frac{500}{\text{ml of aliquot taken}}$$

Vitamins (ascorbic acid and niacin)

The vitamins, ascorbic acid and niacin present in the fresh roots were estimated according to Sadasivam and Balasubramanian (1987). The values are presented Mean \pm S.E. of 5 replicates.

Results

Exogenous application of BRs resulted in 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 found to be most effective in stimulating the root growth of radish plants. An increase of around 60% was observed in the plants treated with 3 μ M conc. in both the treatments over the control plants.

The root growth promotion by BRs was associated with increment in the levels of soluble proteins present in the roots of radish (Table 2). 28-HomoBL at 3 μ M conc. was more effective in increasing the soluble protein content compared to 24-epiBL treatments as well as untreated control plants

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

Foliar application of BRs 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). Applica-

tion of 28-HomoBL at 3 μ M conc. was more promotive in increasing the minerals in the radish roots 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 μ M conc. exhibited less amount of potassium and sodium among all the other treatments.

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

Discussion

Man has been facing a lot of problems due to nutrient deficiencies. An adequate dietary intake of all vital nutrients is the need of the hour. Application of plant growth regulators not only increased the quantitative but also the qualitative yields of several crops. Vardhini and Rao (2003) reported that exogenous application of BRs to tomato plants resulted in enhanced root growth. Schilling et al. (1991) examined the effects of homobrassinolide on sugar beet under drought stress and found an increase of tap root mass. The studies conducted by Bao et al. (2004) on *Arabidopsis thaliana* revealed that BRs promote the acropetal auxin transport and promote the lateral root development. Kartal et al. (2009) also reported that the increasing concentrations of homoBL application not only increased the root growth of barley, but also showed enlarged root tips compared to the control materials. The present study also showed that foliar application of BRs to radish plants increased the root length, fresh and dry weight of radish roots.

Foliar application of BRs resulted in substantial increase in soluble proteins of radish roots. Bajguz (2009) reported that BRs increased the protein contents of *Chlorella vulgaris* as the cultured me-

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

Compounds	Treatments	Root length, cm*	Root Fr. Wt., g*	Root Dry Wt., g*
28-Homo BL	0.5µM	13.4 ± 0.13	281.6 ± 2.73	11.7 ± 0.38
	1.0µM	13.8 ± 0.68	372.3 ± 3.18	15.6 ± 0.22
	3.0µM	15.6 ± 0.27	382.3 ± 1.98	16.9 ± 0.53
24-EpiBL	0.5µM	12.0 ± 0.43	271.6 ± 3.35	11.6 ± 0.17
	1.0µM	13.9 ± 0.22	356.3 ± 2.59	15.2 ± 0.22
	3.0µM	14.8 ± 0.18	380.6 ± 2.70	16.8 ± 0.14
Control		9.2 ± 0.21	239.3 ± 2.90	9.6 ± 0.39

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

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

Compounds	Treatments	Soluble proteins, mg g ⁻¹ Fr.Wt.*	Reducing sugars, mg g ⁻¹ Fr.Wt.*	Starch, mg g ⁻¹ Fr.Wt.*
28-Homo BL	0.5µM	2.33 ± 0.14	7.61 ± 0.14	4.25 ± 0.18
	1.0µM	2.71 ± 0.04	8.10 ± 0.31	4.78 ± 0.28
	3.0µM	3.17 ± 0.09	8.83 ± 0.13	5.18 ± 0.42
24-EpiBL	0.5µM	2.18 ± 0.10	7.21 ± 0.22	4.13 ± 0.31
	1.0µM	2.60 ± 0.07	7.71 ± 0.09	4.54 ± 0.23
	3.0µM	3.04 ± 0.05	8.18 ± 0.32	4.99 ± 0.48
Control		1.87 ± 0.07	5.71 ± 0.08	3.02 ± 0.21

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

Table 3
Effect of brassinosteroids on the mineral content of *Raphanus sativus* roots, mg/100g

Minerals	Control	28-HomoBL			24 EpiBL		
		0.5µM	1.0µM	3.0µM	0.5µM	1.0µM	3.0µM
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	36.8	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	Ascorbic acid, mg/100mg*	Niacin, mg/100mg*
28-Homo BL	0.5µM	17.45 ± 0.02	0.345 ± 0.04
	1.0µM	18.36 ± 0.01	0.398 ± 0.05
	3.0µM	19.19 ± 0.04	0.423 ± 0.07
24-EpiBL	0.5µM	17.27 ± 0.03	0.338 ± 0.04
	1.0µM	18.20 ± 0.05	0.381 ± 0.04
	3.0µM	18.94 ± 0.02	0.408 ± 0.06
Control		14.68 ± 0.03	0.322 ± 0.05

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

dium showed increases in the cell number. Sairam (1994) also reported observed enhanced soluble protein content in wheat plants applied with homobrassinolide. Similarly Vardhini and Rao (2008) reported that supplementation of BL as foliar spray increased the protein content in the leaves of tomato plants where as the exogenous application of 24-epiBL and 28-homoBL enhanced the total protein contents in the seedlings of *Brassica juncea* (Sirhindi et al., 2009) which are in tune with the experiments on effect of BRs on the contents of soluble proteins present in the storage roots of radish.

The application of brassinosteroids resulted in substantial increase in the carbohydrate fractions like reducing sugars and starch in the storage roots of radish. The increase might be due to the enhanced photosynthetic capacity of the plants and an efficient source –sink translocation by the foliar application of BRs. Soaking the seeds of *Triticum aestivum* for around one day in homoBL significantly enhanced the soluble sugars in the seedlings (Hayat et al., 2003). 24-Epibrassinolide spray application to cucumber plants grown in a green house resulted in increases in sucrose, soluble sugars and starch (Yu et al., 2004) and the results obtained in the present study also showed that foliar application of brassinosteroids 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 roots from BR-treated plants was more compared to 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 BR-treated radish plants. Kuno (1987) observed enhanced translocation of phosphorus, but lowered calcium contents after BR-treatment to the leaves of mulberry. Even Bajguz and Czerpak (1998) ob-

served that the supplementation of BRs increased the phosphorus content in *Chlorella vulgaris*. Earlier Pirogovskaya et al. (1996) and Ronsch et al. (1993) 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 a salicylic acid, another plant growth regulator, resulted in increased contents of minerals like potassium, calcium and iron in the strawberry roots (Karlidge 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 favorable enhancement of vitamin C and niacin contents. The ability of BRs on the enhancement of growth and metabolism of the shoot system is a well established fact, but the present study reveals that foliar application of BRs showed the ability of monitoring the source – sink translocation which reflected in enhanced contents of not only the root growth but also the metabolites like soluble proteins and carbohydrates present in the roots. Apart from this, this paper also shows promising effect of BRs in increasing the mineral nutrients especially iron and as well as vitamin C and niacin which gives a new insight on another physiological role of BRs which is neglected for around two decades i.e. its ability in imparting enhancements in minerals and vitamins. This paper reveals the '*Role of BRs in increasing the qualitative changes of the storage root of radish*' where root is the consumable part of the plant.

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