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# Brassinolide improves nutrient value in storage roots of *Raphnus sativus* L. var Pusa chetki long

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**Abstract**
The effect of brassinolide on the qualitative changes in the storage roots of radish was studied. Brassinolide 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. Brassinolide further increased the contents of ascorbic acid and niacin present in the roots indicating their ability to improve the over all quality of storage roots of radish.

**Keywords:** Brassinolide, carbohydrates, minerals, oxalic acid, proteins, root growth, storage roots, vitamins.

## INTRODUCTION

Brassinosteroids are a new type of polyhydroxy steroidal phytohormones with significant growth promoting influence [1]. Brassinosteroids (BRs) were discovered in 1970 by Mitchell and his co-workers [2] and were later extracted from the pollen of *Brassica napus* L. [3]. 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 [4]. 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 [5, 6].

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 [7] 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 brassinolide (BL) on the qualitative changes in the storage roots of radish.

## MATERIALS AND METHODS

### Chemicals and Plant Material

Brassinolide (BL) employed in the study was 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.

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### 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. BL was 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) tricholoroacetic acid. The precipitate was dissolved in 1% (w/v) sodium hydroxide. The method of Lowry *et al*. [8] 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 [9]. The residue was used for the estimation of starch by Mc Cready *et al*. [10] method.

### Mineral contents

1gm of oven-dried sample was digested with 10ml of tri acid mixture (conc. Nitric acid + conc. Percholoric + conc. Sulphuric acid). The digested mixture was used for the estimation of phosphorus by molybdate- vandate method according to Johnson *et al*. [11], and potassium and sodium by Issac and Kerber's procedure [12]

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 [12]

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 trimetric method of APHA [13] and calculated using the formula:-
ml of versenate used x normality of versenate x 500/ 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 Balasubraminan [14]. The values are presented Mean ± S.E. of 5 replicates.

## RESULTS

Exogenous application of BL 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.). An increase of around 60% of root growth was observed in the plants treated with 3µM conc. over the control plants.

The root growth promotion by BL was associated with increment in the levels of soluble proteins present in the roots of radish (Table 2). BL at 3µM conc. was more effective in increasing the soluble protein content compared to the untreated control plants i.e. around

The radish roots treated with foliar application of BL showed increased contents of carbohydrate fractions viz., reducing sugars and starch (Table 2). 3µM Conc. of BL exhibited maximum elevated levels of reducing sugars and starch compared to the untreated controls.

Foliar application of BL showed diversified changes in the minerals present in radish roots. Supplementation of BL 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 BL at 3 µM conc. was more promotive in increasing the minerals in the radish roots than the control plant roots. But the contents of potassium and sodium were reduced in the BL - supplemented plant roots of radish. (Table 3).The roots treated with BL at 3µM conc. exhibited fewer amounts 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 BL to radish plants (Table 4). BL at 3µM conc. was more effective in increasing the ascorbic acid and niacin contents compared to the untreated control plants.

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

| Compounds | Treatments | Root length (cm)* | Root Fr. Wt. (g)* | Root Dry Wt. (g)* |
|---|---|---|---|---|
| BL | 0.5µM | 10.9 ± 0.23 | 269.0 ± 2.63 | 11.3 ± 0.18 |
| | 1.0µM | 13.8 ± 0.28 | 342.3 ± 3.18 | 14.9 ± 0.24 |
| | 3.0µM | 14.6 ± 0.37 | 379.3 ± 1.78 | 16.1 ± 0.43 |
| Control | | 9.2 ± 0.21 | 239.3 ± 2.90 | 9.6 ± 0.39 |

BL = Brassinolide
Values are Mean ± S.E. (N=5)

Table 2. Effect of brassinolide 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.)* |
|---|---|---|---|---|
| BL | 0.5µM | 2.09 ± 0.12 | 6.79 ± 0.14 | 3.95 ± 0.16 |
| | 1.0µM | 2.71 ± 0.09 | 7.57 ± 0.31 | 4.38 ± 0.27 |
| | 3.0µM | 3.07 ± 0.08 | 8.01 ± 0.13 | 4.98 ± 0.43 |
| Control | | 1.87 ± 0.07 | 5.71 ± 0.08 | 3.02 ± 0.21 |

BL = Brassinolide
Values are Mean ± S.E. (N=5)

Table 3. Effect of brassinolide on the mineral content of *Raphanus sativus* roots (mg/100g)

| Minerals | Control | 0.5µM | 1.0µM | 3.0µM |
|---|---|---|---|---|
| Phosphorus | 24.1 | 25.2 | 26.9 | 27.4 |
| Potassium | 285 | 260 | 269 | 241 |
| Calcium | 33.8 | 34.8 | 36.9 | 39.3 |
| Iron | 0.41 | 0.43 | 0.66 | 0.99 |
| Sodium | 40.1 | 38.8 | 37.5 | 35.6 |

BL = Brassinolide
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)* |
|---|---|---|---|
| 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 |
| Control | | 14.68 ± 0.03 | 0.322 ± 0.05 |

BL = Brassinolide
Values are Mean ± S.E. (N=5)

## 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 quantative but also the qualitative yields of several crops. The studies conducted by Bao *et al*. [15] on *Arabidopsis thaliana* revealed that BRs promote the acropetal auxin transport and promote the lateral root development. Vardhini and Rao [16] reported that exogenous application of BRs to tomato plants resulted in enhanced root growth. 24 Epibrassinolide and 24-epicatasterone were found to promote the root growth of *Arabidopsis thaliana* [17]. Kartal *et al*. [18] 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 [19] reported that BRs increased the protein contents of *Chlorella vulgaris* as the cultured medium showed increases in the cell number. Similarly Vardhini *et al*. [20] stated 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 junceae* [21] which is in tune with the experiments on effect of BRs on the 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. Similarly, soaking the seeds of *Triticum aestivum* for around one day in homoBL significantly enhanced the soluble sugars in the seedlings [22] and the results obtained in the present study also enhanced the carbohydrates present in the storage roots of radish.

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 [23] observed enhanced translocation of phosphorus, but lowered calcium contents after BR-treatment to the leaves of mulberry. Even Bajguz and Czerpak [24] observed that the supplementation of BRs increased the phosphorus content in *Chlorella vulgaris*. Earlier Pirogovskya *et al*.[25] and Ronsch *et al*. [26] 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 another plant growth regulator, salicylic acid, resulted in increased content of minerals like potassium, calcium and iron in the strawberry roots [27].

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 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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