

## Influence of brassinosteroids on metabolites of *Raphanus Sativus* L.

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### Abstract

The effect of 24-epibrassinolide and 28-homobrassinolide on the soluble proteins, nucleic acids (DNA and RNA) and carbohydrate fractions (reducing sugars, non-reducing sugars and starch) of radish was studied. Brassinosteroids elevated the soluble proteins of radish. Nucleic acids (DNA and RNA) were also increased by brassinosteroid- application. Increased levels of carbohydrates in terms of reducing sugars, non - reducing sugars and starch was also observed.

**Keywords:** carbohydrates, 24-epibrassinolide, 28-homobrassinolide, nucleic acids, soluble proteins

### INTRODUCTION

Radish (*Raphanus sativus*) is an edible root vegetable belonging to the family *Brassicaceae* which is grown throughout 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 [1] stressed the need of 'divert research' activities in improving the nutritional quality of plants with respect to nutrient content and composition.

Brassinosteroids are a new type of polyhydroxy steroidal phytohormones with significant growth promoting influence [2]. Brassinosteroids (BRs) were discovered in 1970 by Mitchell and his co-workers [3] and were later extracted from the pollen of *Brassica napus* L. [4]. 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 [5]. 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 [6, 7]. The present study is undertaken to understand the effect of application of 28-homobrassinolide (28-HomoBL) and 24-epibrassinolide (24-epiBL) on the metabolites of radish (*Raphanus Sativus* L.).

### MATERIALS AND METHODS

#### Chemicals and Plant Material

28-Homobrassinolide (28-HomoBL) and 24-epibrassinolide (24-EpiBL), 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.

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-Homobrassinolide (28-HomoBL) and 24-epibrassinolide (24-epiBL) was supplied to the plants as foliar spray at three different concentration levels viz., 0.5  $\mu$ M, 1.0  $\mu$ M and 3.0  $\mu$ M on 20<sup>th</sup>, 35<sup>th</sup> and 50<sup>th</sup> day (from the day of sowing). Water treated controls were also maintained. On 60<sup>th</sup> day leaf material was homogenized using 70% (v/v) ethanol and stored in deep freezer for further biochemical 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. [8] was used for protein estimation.

##### Nucleic acids

DNA and RNA in the alcohol homogenate were separated by Ogur and Rosen [9] method. DNA was estimated by Burton [10] method and RNA was estimated by the procedure of Schneider [11].

##### Carbohydrates

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of reducing sugars [12]. Non-reducing sugars were calculated by the formula given by Loomis and Shull [13]. The residue was used for the estimation of starch [14].

### RESULTS

Foliar application of 28-homobrassinolide (28-HomoBL) and 24-epibrassinolide (24-epiBL) to the radish plants resulted in substantial increment in protein contents (Table 1). 28-HomoBL was found to be more effective over 24-epiBL and control plants. It accounted for 65% increase in soluble proteins over control.

Exogenous application of 28-homobrassinolide (28-HomoBL) to the radish plants resulted in substantial increments in nucleic

Received: Dec 10, 2011; Revised: Jan 12, 2012; Accepted: Feb 05, 2012.

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acids (DNA and RNA) over 24- epibrassinolide (24-epiBL) and control plants (Table 1).

28-Homobrassinolide (28-HomoBL) and 24- epibrassinolide (24-epiBL) supplementation to the radish plants caused sharp rise in

the levels of all the three carbohydrate fractions (Table 2). 28-HomoBL at all three concentrations uniformly accounted for steep increments in reducing sugars, non-reducing sugars and starch contents.

**Table.1** Effect of 28-homobrassinolide and 24- epibrassinolide on soluble proteins and nucleic acids (DNA and RNA) of *Raphanus sativus*

Compounds	Treatments	Soluble proteins (mg g <sup>-1</sup> Fr. Wt)*	DNA content (mg g <sup>-1</sup> Fr. Wt)*	RNA content (mg g <sup>-1</sup> Fr. Wt)*
28-HomoBL	0.5µM	6.97 ± 0.09	6.05 ± 0.35	10.35 ± 0.19
	1.0µM	7.13 ± 0.04	6.79 ± 0.12	11.15 ± 0.45
	3.0µM	7.90 ± 0.03	7.54 ± 0.07	12.80 ± 0.50
24-EpiBL	0.5µM	6.22 ± 0.06	4.91 ± 0.02	10.12 ± 0.35
	1.0µM	6.99 ± 0.15	6.01 ± 0.33	10.97 ± 0.13
	3.0µM	7.77 ± 0.05	6.86 ± 0.15	12.00 ± 0.50
Control		4.77 ± 0.05	4.31 ± 0.06	8.26 ± 0.26

28-Homo BL = 28-Homobrassinolide

24-EpiBL = 24 - Epibrassinolide

Values are Mean ± S.E. (N=5)

**Table 2.** Effect of 28-homobrassinolide and 24- epibrassinolide on carbohydrate fractions of *Raphanus sativus*

Compounds	Treatments	Reducing sugars (mg g <sup>-1</sup> Fr. Wt)*	Non-reducing Sugars (mg g <sup>-1</sup> Fr. Wt)*	Starch (mg g <sup>-1</sup> Fr. Wt)*
28-HomoBL	0.5µM	3.83 ± 0.32	3.16 ± 0.07	4.60 ± 0.09
	1.0µM	4.30 ± 0.24	3.72 ± 0.24	4.99 ± 0.02
	3.0µM	4.92 ± 0.17	4.01 ± 0.17	5.71 ± 0.05
24-EpiBL	0.5µM	3.59 ± 0.54	3.01 ± 0.04	4.26 ± 0.17
	1.0µM	4.02 ± 0.35	3.71 ± 0.35	4.81 ± 0.03
	3.0µM	4.51 ± 0.21	3.86 ± 0.21	5.20 ± 0.06
Control		2.18 ± 0.19	2.78 ± 0.09	3.58 ± 0.07

28-Homo BL = 28-Homobrassinolide

24-EpiBL = 24 - Epibrassinolide

Values are Mean ± S.E. (N=5)

## DISCUSSION

Sasse [15] suggested that brassinosteroids can stimulate the synthesis of particular proteins associated with growth. Supplementing the culture media with 24-epibrassinolide increased cell division rate and amount of soluble proteins in Chinese cabbage protoplasts [16]. Exogenous application of 24-epiBL and 28-homoBL enhanced the total protein contents in the seedlings of *Brassica juncea* [17]. Further, the alleviating influence of brassinosteroids on salinity stress induced inhibition of growth in rice was found associated with elevated levels of proteins [18].

The results obtained in the present study with 28-homoBL and 24-epiBL are in confirmity with the observations made by Key [19] with regard to the then known phytohormones which influence the growth by regulating nucleic acid synthesis. The increase in the levels of nucleic acids might be due to enhanced synthesis and reduced degradation. Recently, Vardhini et al. [20] reported that foliar application of brassinolide increased the DNA and RNA contents of radish.

A spray application of 24-EpiBL to cucumber (*Cucumis sativus*) plants grown in a greenhouse resulted in increases in sucrose, soluble sugars and starch [21]. The increase might be due to enhanced photosynthetic capacity of the plants as influenced by the 28-homoBL and 24-epiBL application. Infact increase in CO<sub>2</sub> fixation and levels of reducing sugars was reported in wheat and mustard plants by the application of brassinolide [22]. Similarly, Vardhini et al. [23] reported increased carbohydrate fractions like reducing sugars and starch in the storage roots of radish by foliar supplementation of 28-homoBL and 24-epiBL.

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