Journal of Phytology 2012, 4(2): 45-47
ISSN: 2075-6240
Available Online: http://journal-phytology.com/

JOURNAL OF PHYTOLOGY

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

B. Vidya Vardhini*[1], E. Sujatha[2] and S. Seeta Ram Rao[3]

[*1,2]Department of Botany, Telangana University, Dichpally, Nizamabad -503322, India.
[2]Department of Botany, University College of Science, Osmania University, Hyderabad 500007, India.

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.

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

*Corresponding Author

B. Vidya Vardhini
Department of Botany, Telangana University, Dichpally, Nizamabad -503322, India.

Tel: +91-9959173377; Fax: +91-841 222212
Email: drvidyavardhni@rediffmail.com

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 µM, 1.0 µM and 3.0 µM on 20^{th}, 35^{th} and 50^{th} day (from the day of sowing). Water treated controls were also maintained. On 60th 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) tricholoroacetic 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 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-1 Fr. Wt)*	DNA content (mg g-1 Fr. Wt)*	RNA content (mg g-1 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-1 Fr. Wt)*	Non-reducing Sugars (mg g-1 Fr. Wt)*	Starch (mg g-1 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 junceae* [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_2 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.

REFERENCES

[1] Grusak, M.A. and D. Dellapenna. 1999. Improving the nutrient composition of plants to enhance human nutrition and health. *Ann. Review Plant Physiol. Plant Mol. Biol.* 50: 133-161.

[2] Vardhini B.V., S. Anuradha, E. Sujatha and S.S.R. Rao. 2010. Role of Brassinosteroids in Alleviating Various Abiotic and Biotic Stresses - A Review. In: N. A. Anjum, (Ed.), Plant Nutrition and Abiotic Stress Tolerance I. Plant Stress 4 (Special Issue 1), London: Global Science Books, pp.56-61.

[3] Mitchell, J. W., N.B. Mandava, J.E. Worley, J.R. Plimmer and M.V. Smith, 1970. Brassins : A family of plant hormones from rape pollen. *Nature* 225:1065-1066.

[4] Grove, M. D., G. F. Spencer., W. K. Rohwededer, N. B. Mandava, J.F.Worlet, , J.C. Warthen Jr., G.L. Steffens, J.L. Flippen-Andersen and J.C. Cook Jr. 1979. Brassinolide, a plant promoting steroid isolated from *Brassica napus* pollen. *Nature* 281:121-124.

[5] Bajguaz, A. 2009. Isolation and characterization of brassinosteroids from algal cultures of *chlorella vulgaris* Beijernick (Trebouxiophyceae). *J. Plant Physiol.* 166: 1946-1949.

[6] Rao, S.S.R., B.V., Vardhini , E. Sujatha and S. Anuradha. 2002. Brassinosteroids – A new class of phytohormones. *Curr. Sci.* 82:1239-1245.

[7] Vardhini, B.V., S. Anuradha and S.S.R. Rao. 2006. Brassinosteroids-New class of plant hormone with potential to

improve crop productivity. *Indian J. Plant Physiol.* 11: 1-12.

[8] Lowry, O.H., N. J. Rosenbrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with folin-phenol reagent. *J. Biol. Chem*. 193: 265-275.

[9] Ogur, M. and Rosen, G. 1950. The nucleic acids of plant tissue. 1. The extraction and estimation of deoxypentose nucleic acid and pentose nucleic acid. *Arch. Biochem*. *Biophysics*. 24: 262-276.

[10] Burton, K. 1968. Determination of DNA concentration with diphenyl amine. In: *Methods in enzymology* (eds) L. Grossman and M. Meidave (New York, Academic Press) Vol 12 pp 163-166

[11] Schneider, W.C.1957. Determiniation of nucleic acids in tissues by analysis. In: *Methods in Enzymology* (eds) S.P .Colowick and W.O. Kaplan (New York, Academic Press) pp 680-684.

[12] Nelson, N. 1944. A photometric adaption of the somagji method for determination of Glucose. *J Biol Chem* 154: 375-380.

[13] Loomis, W.E. and C.A. Shull. 1937. *Methods in plant physiology*. New York, Mc Graw Hill Book Co.

[14] Mc Cready, R.M., J. Guggloz, V. Silviora and H.S.Owens. 1950. Determination of starch and amalyase in vegetables. Application to peas. *Anal. Chem.* 29: 1156-1158.

[15] Sasse, J.M.1990. Brassinolide-induced elongation and auxin. *Plant Physiol*. 80:401-408.

[16] Nakajima, N., Atsubikoshida and S.Toyami. 1996. Effect of brassinosteroids on cell division and colony formation of Chinese cabbage mesophyll protoplasts. *Japanese Crop Sci.* 65: 114-118

[17] Sirhindi, G., S. Kumar, R. Bharadwaj and R. Kumar. 2009. Effects of 24-epibrassinolide and 28-homobrassinolide on the growth and antioxidant enzyme activities in the seedlings of *Brassica junceae* L. *Physiol. Mol. Biol. Plant.* 15: 335-341

[18] Anuradha, S. and S.S.R. Rao. 2001. Effect of brassinosteroids on salinity stress induced inhibition of germination and seedling growth of rice (*Oryza sativa*.L). *Plant Growth Regul.* 33:151-153

[19] Key, T.L. 1969. Hormones and nucleic acid metabolism. *Annu. Rev. Plant Physiol.* 20: 449-447.

[20] Vardhini, B.V., E. Sujatha, and S.S.R. Rao. 2011. Effect of brassinolide on biochemical composition of radish (*Raphanus sativus*). *Bioinfolet*, 8:404-406.

[21] Yu, J.Q., L. F. Huang, W.H. Hu, Y.H. Zhao, W.H. Mao, S.F. Ye and S. Nogues. 2004. A role for brassinosteroids in regulation in the photosynthesis in *Cucumis sativus*. *Journal of Experimental Biology* 55:1135-1143

[22] Braun, P. and A. Wild. 1984. The influence of brassinosteroid - A growth promotion steroidal lactone, on development and carbon-di-oxide fixation capacity of intact wheat and mustard seedlings; in *Advances in Photosynthesis Reseach. Proc. 6th Congr. Photosynthesis* (ed) C Sybesma (Hague, Nijhoff) pp 461-464.

[23] Vardhini, B.V., Sujatha, E and Rao, S.S.R. (2011). Studies on the effect of brassinosteroids on the qualitative changes in the storage roots of radish. *Asian and Australasian Journal of Plant Science and Biotechnology.* 5:27-30.