Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Number 7, 2015, pp. 2947-2951
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

BRASSINOSTEROIDS ENHANCE THE SHOOT GROWTH, FOLIAR GROWTH AND CHLOROPHYLL PIGMENTS OF RADISH

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

[1]Department of Botany, Telangana University, Dichpally, Nizamabad -503322, India
[2]Department of Botany, Women's College, Osmania University, Koti, India
[3]Department of Botany, University College of Science, Osmania University, Hyderabad 500007, India.

Abstract

The effect of brassinolide, 28-homobrassinolide and 24-epibrassinolide on the shoot growth, foliar growth (leaves per plant and leaf area) and chlorophyll pigments (chl "a", chl "b" and total chlorophylls) of radish plants was studied. Brassinosteroids stimulated the shoot growth as well as the foliar growth of radish plants. The promotion of shoot and foliar growth was associated with increased levels chlorophyll pigments indicating the ability of brassinosteroids in positively monitoring photosynthesis.

INTRODUCTION

Radish (*Raphanus sativus*) is an edible root vegetable belonging to the family *Brassicaceae* which is grown throughout the world. It consists of ascorbic acid, folic acid, niacin and many important minerals apart from carbohydrates [1-3]. 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 minerals and organic nutrients to humans [4]. Therefore, Grusak and Dellapenna [5] emphatically stressed the need of 'divert research' activities in improving the nutritional quality of plants with respect to its nutrient content and composition.

Brassinosteroids (BRs) are a new type of polyhydroxy steroidal phytohormones with significant growth promoting influences which are now considered as the sixth group of phytohormones [6, 7]. BRs were discovered by Mitchell and his co-workers [8] and were later extracted from the pollen of *Brassica napus* L. [9]. 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 [10]. Dwarf and de-etiolated phenotypes and BR - deficient species of some *Arabidopsis* mutants were rescued by the application of BRs [11, 12]. The work with BR biosynthetic mutants in *Arabidopsis thaliana* [13] and *Hordeum vulgare* [14] provided strong evidences that BRs are essential for plant growth and development. Thus, 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 [15, 16]. The present study is undertaken to understand the effect of application of BRs on the shoot growth, foliar growth and chlorophyll pigments of radish plants.

MATERIALS AND METHODS

Chemicals and plant material

Brassinolide (BL), 28-homobrassinolide (28-homoBL) and 24-epibrassinolide (24-epiBL) 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.

Shoot 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 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). Shoot growth parameter in terms of shoot fresh weight was recorded on 60th day. The shoots were oven dried at 110 ^{0}C for 24 hours and dry weights were also recorded.

Foliar growth

Foliar growth was recorded in terms of leaves per plant and leaf area per plant. Average leaf area per plant was determined with the help of the equation developed by Kemp [17] (1960)

$A = (L \times B \times 0.9) \times n$,

Where A= leaf area, L= leaf length, B=leaf breadth, 0.9= constant factor and n= number of leaves.

Chlorophylls

Chlorophyll pigments were extracted and estimated by the procedure described by Arnon [18]. Leaves were homogenized with 80% (v/v) acetone and centrifuged at 4000 rpm for ten minutes. The acetone extract was used to calculate the chlorophyll "a", "b" and total chlorophylls employing the formula given below.

Chlorophylls a= [O.D at 663 nm× 12.7 –O.D at 645 nm × 2.69] × [v/1000) × w]

Chlorophyll b= [O.D at 645 nm × 22.9– O.D. at 663 nm× 4.68] × [v/1000) × w]

Total chlorophylls = [O.D at 663 nm × 8.2+O.D at 645nm × 20.2] × [v/1000) × w]

Where, v = volume of acetone extract and w = weight of leaves.

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 at 5% level of significance over control. The values were calculated employing SPSS 16.0 statistical software.

RESULTS

Exogenous application of BRs (BL, 28-homoBL and 24-epiBL) resulted in substantial increase in growth of radish shoots as reflected in increases in fresh weight and dry weight of the shoots (**Table 1**). Among the three BRs employed, 28-homoBL was found to be most effective in stimulating the shoot growth of radish plants. An increase of around 70% of shoot growth was observed in the plants treated with 3µM conc. of 28-homoBL over all the other treatments.

The radish plants treated with foliar application of BRs (BL, 28-homoBL and 24-epiBL) showed increased foliar growth in terms of number of leaves/plants and leaf area (**Table 1**). 28-HomoBL at 3µM conc. was more effective in increasing the foliar growth compared to its other concentrations as well as all the concentrations of 24-epiBL, BL treated plants as well as control plants. The shoot growth promotion by BRs (BL, 28-homoBL and 24-epiBL) was associated with increments in the levels of chlorophyll 'a', chlorophyll 'b' as well as total chlorophylls of the radish plants (**Table 2**). 3µM Conc. of 28-homoBL exhibited maximum elevated levels of chlorophyll "a", chlorophyll "b" and total chlorophylls compared to both the other treatments (BL and 24-epiBL) and also the untreated controls.

DISCUSSION

It is a well-established fact that BRs are a new group of plant growth regulators which play a positive role in the growth and development of plants. Earlier studies clearly emphasized that external supplementation of 0.01 and 0.05 mg/L of Biobras- 6, a synthetic BR improved the plant growth in the case of *Alluim cepa* [19] and *Oryza sativa* [20]. Supplementation of 40 mg/L, 20mg/L and 0.03mg/L of epiBL along with DA-6 (dialkyl amino ethyl alkoate) and GA_3 increased the shoot fresh weight, length and fresh weight of petiole of *Spinacia oleracea* [21].

Supplementation of 0.1 and 1.0 ppm of HomoBL increased the growth of two varieties of *Triticum aestivum* [22] whereas 0.5, 1.0 and 3.0 µM of 24-epiBLand BL application increased the shoot growth of *Arachis hypogaea* [23] which are in tune with the present study where the foliar application of BL, 28-homoBL and 24-epiBL substantially increased the shoot growth of radish plants.

The enhanced shoot growth was associated with increased foliar growth of the radish plants. Similarly, Arteca and Arteca [24] reported that 1.0 µM BL and 24-epiBL induce exaggerated growth in hydroponically grown *Arabidopsis thaliana*. Treatment of the BR-related mutants of *Arabidopsis thaliana* (L.) Heynh, det2 (de-etiolated2 = cro1) and dwf1 (dwarf1 = cro2) and wild-type plants with BL, reversed the mutation and restored the potential for growth to that of the wild type suggesting that BRs play a dual role in regulating cell expansion and proliferation [25]. Qayyam *et al*. [26] reported that foliar application of 0.125 or 0.025 mgL^{-1} of epiBL increased the total chlorophylls in S-24 cultivar of wheat (*Triticum aestivum*). EpiBL enhanced the rate of photosynthesis in *Cucumis sativus* plants by increasing not the total chlorophyll contents, but also by regulating various photosynthetic processes like O_2 assimilation and elevation of a variety of photosynthetic enzymes like RUBISCO, fructose 1-6-bisphosphatase, fructose1-6-bisphosphate aldolase etc. [27]. Further, Yu *et al*. [28] reported that 0.01, 0.1 or 1.0 mgL^{-1} of epiBL also enhanced the total chlorophylls of *Cucumis sativus* plants which is in agreement with the results obtained in the present study, where external supplementation of BL, 28-homoBl and 24-epiBL resulted in increased Chlorophylls "a", "b" and total chlorophylls of radish plants.

Table 1. Effect of brassinosteroids on the shoot growth and foliar growth of radish plants

Compounds	Treatments	Shoot fresh weight	Shoot dry weight	Foliar growth
BL	0.5µM	300.6 ± 2.42	18.8 ± 0.39	428 ± 3.54
	1.0µM	347.5 ± 1.48	20.1 ± 0.19	457 ± 6.03
	3.0µM	362.6 ± 1.68	22.5 ± 0.17	507 ± 2.48
28-HomoBL	0.5µM	322.3 ± 2.76a	20.4 ± 0.21a	470 ± 2.73a
	1.0µM	357.6 ± 1.18a	22.4 ±.0.34a	492 ± 4.73a
	3.0µM	372.6 ± 1.28a	23.6 ± 0.17a	554 ± 3.06a
24-EpiBL	0.5µM	312.3 ± 3.92	19.7 ± 0.33	432 ± 3.56
	1.0µM	350.0 ± 2.35	21.5 ± 0.23	492 ± 4.73
	3.0µM	367.6 ± 3.21	22.6 ± 0.23	540 ± 3.12
Control		218.0 ± 1.24b	15.2 ± 0.24b	344 ± 5.44b

BL= Brassinolide, 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 significant at 5% level of significance over control
Shoot fresh & dry Wt. = g, foliar growth = Sq.cm.

REFERENCES

1. YOUSEF, M., AND SHAFI, M., 1969, EVALUATION OF SOME QUICK MATURING EXOTIC VARIETIES OF RADISH (*RAPHANUS SATIVUS* L.), WEST PAKISTAN JOURNAL OF AGRICULTURAL RESEARCH, 7, 83-93.
2. NAUTITYAL, M.C., PRASAD, A., AND LAL, H., 1977, STUDIES ON VARIETAL PERFORMANCE OF RADISH. PLANT SCIENCE, 6,106-107.
3. PUJARI, M.M., JAIN, B.P., AND MISHRA, G.M., 1977, INVESTIGATIONS ON MORPHOLOGICAL FEATURES AND YIELD POTENTIAL OF PROMISING RADISH (*RAPHANUS SATIVUS* L.) CULTIVARS. HARYANA JOURNAL OF HORTICULTURAL SCIENCE, 6, 85-89.
4. MEUDT, W.J., THOMPSON, M.T., AND BENNETT, H.W., 1983, INVESTIGATIONS ON MECHANISM OF THE BRASSINOSTEROID RESPONSE. III. TECHNIQUES FOR POTENTIAL ENHANCEMENT OF CROP PRODUCTION. PROCEEDINGS OF PLANT GROWTH REGULATION OF SOCIETY OF AMERICA, 10, 312-318.

5. GRUSAK, M.A., AND DELLAPENNA, D., 1999, IMPROVING THE NUTRIENT COMPOSITION OF PLANTS TO ENHANCE HUMAN NUTRITION AND HEALTH. ANNUAL REVIEW OF PLANT PHYSIOLOGY AND PLANT MOLECULAR BIOLOGY, 50, 133-161.
6. VARDHINI, B.V., 2013, BRASSINOSTEROIDS, ROLE FOR AMINO ACIDS, PEPTIDES AND AMINES MODULATION IN STRESSED PLANTS - A REVIEW IN: PLANT ADAPTATION TO ENVIRONMENTAL CHANGE: SIGNIFICANCE OF AMINO ACIDS AND THEIR DERIVATIVES, (EDS.) ANJUM, N.A., GILL, S.S., AND GILL, R., CAB INTERNATIONAL OF NOSWORTHY WAY, WALLINGFORD OX10 8DE, UNITED KINGDOM. PP. 300-316.
7. VARDHINI, B.V., AND ANJUM, N.A., 2015, BRASSINOSTEROIDS MAKE PLANT LIFE EASIER UNDER ABIOTIC STRESSES MAINLY BY MODULATING MAJOR COMPONENTS OF ANTIOXIDANT DEFENSE SYSTEM. FRONTIERS IN ENVIRONMENTAL SCIENCES, 2: 67. DOI: 10.3389/FENVS.2014.0006.
8. MITCHELL, J.W., MANDAVA, N.B., WORLEY, J.E., PLIMMER, J.R., AND SMITH, M.V., 1970, BRASSINS: A FAMILY OF PLANT HORMONES FROM RAPE POLLEN. NATURE, 225, 1065-1066.
9. GROVE, M.D., SPENCER, G.F., ROHWEDEDER, W.K., MANDAVA, N.B., WORLET, J.F., WARTHEN JR, J.C., STEFFENS, G. L., FLIPPEN-ANDERSEN, J.L., AND COOK JR, J.C., 1979, BRASSINOLIDE, A PLANT PROMOTING STEROID ISOLATED FROM *BRASSICA NAPUS*. POLLEN, NATURE, 281, 121-124.
10. BAJGUZ, A., HAYAT, S., 2009, EFFECTS OF BRASSINOSTEROIDS ON PLANTS EXPOSED OR SUBJECTED TO VARIOUS STRESSES. PLANT PHYSIOLOGY AND BIOCHEMISTRY, 47, 1–8.
11. BISHOP, G.J., YAKOTA, T., 2001, PLANT STEROID HORMONES, BRASSINOSTEROIDS: CURRENT HIGHLIGHTS OF MOLECULAR ASPECTS ON THEIR SYNTHESIS/METABOLISM, TRANSPORT, PERCEPTION AND RESPONSE. PLANT AND CELL PHYSIOLOGY, 42, 114-120.
12. ZENG, H., TANG, Q., AND HUE, X., 2010, *ARABIDOPSIS* BRASSINOSTEROID MUTANTS *DEL 2-1* AND BIN 2-1 DISPLAY ALTERED SALT TOLERANCE. JOURNAL OF PLANT GROWTH REGULATION, 29, 44-52.
13. KIM, S.Y., KIM, B.H., LIM, C.J., LIM, C.O., AND NAM, K.H., 2010, CONSTITUTIVE ACTIVATION OF STRESS-INDUCIBLE GENES IN A *BRASSINOSTEROID-INSENSITIVE 1* (*BRI1*) MUTANT RESULTS IN HIGHER TOLERANCE TO COLD. PHYSIOLOGIA PLANTARUM, 138, 191-204.
14. GODDARD, R., PERALDI, A., RIDOUT, C.J., AND NICHOLSON, P., 2014, ENHANCED DISEASE RESISTANCE CAUSED BY BRI1 MUTATION IS CONSERVED BETWEEN *BRACHYPODIUM DISTACHYON* AND BARLEY (*HORDEUM VULGARE*). MOLECULAR PLANT MICROBE INTERACTIONS, 27, 1095-1106.
15. RAO, S.S.R., VARDHINI, B.V., SUJATHA, E., AND ANURADHA, S., 2002, BRASSINOSTEROIDS – A NEW CLASS OF PHYTOHORMONES. CURRENT SCIENCE, 82, 1239-1245.
16. DENG, Z., ZHANG, X., TANG, W., OSES-PRIETO, J. A., SUZUKI, N., GENDRON, J.M., CHEN, H., GUAN, S., CHALKEY, R.J., PETERMAN, T.K., BURLINGAME, A.L., WANG, Z.Y. (2007). A PROTEOMICS STUDY OF BRASSINOSTEROID RESPONSE IN *ARABIDOPSIS*. MOLECULAR AND CELLULLAR PROTEOMICS, 6, 2058-2071.
17. KEMP, C.D., 1960, METHODS OF ESTIMATING LEAF AREA OF GRASSES FROM LINEAR MEASUREMENT. ANNALS OF BOTANY, 24, 491-512.
18. ARNON, D.I., 1949, COPPER ENZYMES IN ISOLATED CHLOROPLASTS. POLYPHENOL OXIDASE IN *BETA VULGARIS*. PLANT PHYSIOLOGY, 24, 1–15.
19. NUNEZ, M., BENITEZ, B., DOMINGOS, J.P., TORRES, W., COLL, F., AND JOMARRON, I., 1994, INFLUENCIA DE ANALOGOS DE BRASSINOSTEROIDES EN EL RENDIMIENTO DE DIFFERENTES CULTIVOS HORTICOLAS. CULTIVOS TROPICALES, 15, 87.
20. FRANCO, I., MORALES, O., AND SOCORRO, M., 2002, *MEMORIAS 2DO*, ENCUENTRO INTERNACIONAL DE ARROZ, LA HABANA, 273 PP.

21. LIANG, G.J., LI, Y.Y., AND SHOA, L., 1998, EFFECT OF DA-6 AND BR+GA_3 ON GROWTH AND PHOTOSYNTHETIC RATE IN SPINACH. ACTA HORTICULTURAE SINICA, 25, 356-360.
22. SAIRAM, R.K., 1994, EFFECT OF HOMOBRASSINOLIDE APPLICATION ON PLANT METABOLISM AND GRAIN YIELD UNDER IRRIGATED AND MOISTURE – STRESS CONDITIONS OF TWO WHEAT VARIETIES. PLANT GROWTH REGULATION, 14, 173-181.
23. VARDHINI, B.V., AND RAO, S.S.R., 1998, EFFECT OF BRASSINOSTEROIDS ON GROWTH, METABOLITE CONTENT AND YIELD OF *ARACHIS HYPOGAEA*. PHYTOCHEMISTRY, 48, 927-930.
24. ARTECA, J.M., ARTECA, R. N., 2001, BRASSINOSTEROID-INDUCED EXAGGERATED GROWTH IN HYDROPONICALLY GROWN *ARABIDOPSIS* PLANTS. PHYSIOLOGIA PLANTARUM, 112, 104–112.
25. NAKAYA, M., TSUKAYA, H., MURAKAMI, N., AND KATO, M., 2002, BRASSINOSTEROIDS CONTROL THE PROLIFERATION OF LEAF CELLS OF *ARABIDOPSIS THALIANA*. PLANT AND CELL PHYSIOLOGY, 43, 239-244.
26. QAYYAM, Z., SHAHBAZ, M., AND AKRAM, N.A., 2007, INTERACTIVE EFFECT OF FOLIAR APPLICATION OF 24-EPIBRASSINOLIDE AND ROOT ZONE SALINITY ON MORPHO PHYSIOLOGICAL ATTRIBUTES OF WHEAT (*TRITICUM AESTIVUM* L.). INTERNATIONAL JOURNAL OF AGRICULTURAL BIOLOGY, 9, 584-589.
27. XIA, X-J., HUANG, L-F., ZHAU, Y-H., MAO, W-H., SHI, K., AND YU, J-Q., 2009, BRASSINOSTEROIDS PROMOTE PHOTOSYNTHESIS AND GROWTH BY ENHANCING ACTIVATION OF RUBISCO AND PHOTOSYNTHETIC GENES IN *CUCUMIS SATIVUS*. PLANTA, 230, 1185-1196.
28. YU, J.Q., HUANG, L.F., HU, W.H., ZHAO, Y.H., MAO, W.H., YE, S.F., AND NOGUES, S., 2004, A ROLE FOR BRASSINOSTEROIDS IN REGULATION IN THE PHOTOSYNTHESIS IN *CUCUMIS SATIVUS*. JOURNAL OF EXPERIMENTAL BIOLOGY, 55, 1135-1143.