

Effect of brassinolide on the growth of mustard crops grown in semi-arid tropics of Nizamabad

ABSTRACT

The effect of brassinolide (BL) on the shoot growth, root growth and foliar growth (plant fresh weight, leaves per plant and leaf area) of mustard plants grown in the semi-arid tropics of Nizamabad was studied. The soil in Nizamabad district is saline land black soil wherein the plants usually experience drought and saline stresses. BL stimulated the shoot growth, root growth as well as the foliar growth of mustard plants. The promotion of shoot, root and foliar growth is an indicator that BL mitigated the negative effect of the semi-arid conditions of the soil.

Key words: brassinolide; foliar growth; mustard; root growth; shoot growth

Abbreviations: Brassinolide=BL; Brassinosteroids =BRs

INTRODUCTION

Brassinosteroids (BRs) are a new type of polyhydroxy steroidal phytohormones with significant growth-promoting influence [1]. Mitchell and his co-workers [2] discovered BRs which were later extracted from the pollen of *Brassica napus* L. by Grove et al. [3]. BRs can be classified as C₂₇, C₂₈ or C₂₉ BRs according to the number of carbons in their structure [4]. However, Vardhini et al. [5] reported that brassinolide (BL), 28-homobrassinolide (28-HomoBL) and 24-epibrassinolide (24-EpiBL) are the three bioactive BRs being widely used in most physiological and experimental studies. The work with BR biosynthetic mutants in *Arabidopsis thaliana* [6] and *Pisum sativum* [7] have provided strong evidences that BRs are essential for plant growth and development and BR- signaling plays a positive in plant growth and development [8].

Rao et al. [9] stated that BRs are a new group of plant growth hormones that perform a variety of physiological roles like growth, seed germination, rhizogenesis, senescence, etc. and also confer resistance to plants against various abiotic stresses. Though, BRs were initially

identified based on their growth promoting activities, subsequent physiological and genetic studies revealed additional functions of BRs in regulating a wide range of processes, including source/sink relationships, seed germination, photosynthesis, senescence, photomorphogenesis, flowering and responses to different abiotic and biotic stresses [4].

Nizamabad district experiences a tropical dry and wet season with most of the rainfall in June to October. It usually experiences erratic rain fall. The soil is saline land black soil which is deep loamy to clay loam, moderately drained, neutral to alkaline in nature. The semi-arid condition directly poses a threat to the overall yield of the plants as they usually experience drought and saline stresses.

Mustard (*Brassica juncea*) is an oil yielding plant that belongs to the family *Brassicaceae* which is grown through the world. The plant has tiny seeds which are usually used as a condiment. Mustard oil is extracted from the seed which are used for cooking, massaging etc. Some kinds of mustard plants have edible leaves that are known as *mustard greens*. 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 [10]. The present study is undertaken to understand the effect of application of BL on the growth of mustard plants in terms of shoot, root and foliage grown in the semi - arid soils of Nizamabad.

MATERIALS AND METHODS

Chemicals and Plant Material

Brassinolide (BL: double) is a commercially available brassinosteroid was procured from Bahar Agrochem & Feeds Pvt. Ltd, Ratnagiri, Maharastra, India, Ltd. It is marketed by Godrej

Agrovet Ltd., Hyderabad, Andhra Pradesh, India. Brassinolide (Double) consists of 0.1% of brassinolide, 2.0% of emulsifier and 97.9 % of solvent IPA.

Mustard (*Brassica juncea* var tulasi) was purchased from National Seeds Corporation, Hyderabad, Andhra Pradesh, India.

Shoot Growth

Mustard seeds were sown in clay pots containing fresh sieved black soil mixed with farmyard manure. Plants were grown under natural day length. BL was supplied to the plants as foliar spray at three different concentration levels viz., 0.5 μ M, 1.0 μ M and 2.0 μ M on 35th, 40th, 45th and 50th day (from the day of sowing). Growth parameters in terms of shoot and root growth, diameter and fresh weight were recorded on the 55th day.

Foliar growth

Foliar growth was recorded in terms of plant fresh weight, leaves per plant and leaf area per plant were recorded on 55th day. Average leaf area per plant was determined with the help of the equation developed by Kemp [11].

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

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

The data is represented in terms of Mean \pm S.E (n=9).

RESULTS

Exogenous application of BL resulted in substantial increase in shoot growth of mustard plants as reflected in increases in length, fresh weight and diameter of the shoots (Table 1.). All the three concentrations applied viz., 0.5 μ M, 1 μ M and 2 μ M increased the shoot growth of

mustard plants grown in semi-arid soils of Nizamabad over control plants. BL at 0.5µM was found most effective in substantial increase in shoot growth compared to the other two concentrations as well as control plants.

The mustard plants grown in semi-arid soils of Nizamabad and treated with foliar application of BL showed increased root growth in terms of length, fresh weight and diameter (Table 2.). All the three concentrations of BL applied viz., 0.5µM, 1µM and 2µM increased the shoot growth of mustard plants grown in semi-arid soils of Nizamabad over control plants. 0.5µM Conc. of BL exhibited maximum enhanced root growth compared to the other treatments as well as untreated controls.

Foliar application of BL exhibited improved foliage in terms of plant fresh weight, leaves/plant and leaf area of mustard plants grown in semi-arid tropics of Nizamabad (Table 3.). All the three concentrations applied viz., 0.5µM, 1µM and 2µM increased the shoot growth of mustard plants grown in semi-arid soils of Nizamabad over control plants. Application of BL at 0.5 µM conc. was more promotive in increasing the foliar growth of mustard plants compared to the other two treatments as well as control plants.

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. BRs increased the height of two field-grown inbred lines of maize (*Zea mays* L.) during the vegetative and early reproductive phases of plant development during the early weeks after their application [12]. Fariduddin et al. [13] reported that cucumber seedlings sprayed with 10^{-8} , or 10^{-6} M of 28-homoBL exhibited improved growth at the 30-day stage after treatment plants were exposed for 18 h to chilling temperature (10/8°C, 5/3°C). BL applied to the sunflower seedlings significantly increased the

hypocotyl growth under different light qualities [14]. Even in the present study, application of BL significantly increased the shoot growth in mustard plants grown in the semi –arid soils of Nizamabad.

Vardhini et al. [15] reported that application of BRs increased the qualitative growth of radish in terms of root fresh weight, root dry weight, oxalic acid, ascorbic acid, folic acid and niacin. Hayat et al. [16] reported that 28-homoBL and 24-epiBL increased the growth of *Lycopersicon esculentum*. Imbibition of 24-epiBL to pea (*Pisum sativum* L.) cv. Climax seeds subjected to sodium chloride stress exhibited increased germination, embryo axis length and most of the aspects of shoot and root growth at seedling stage [17]. Earlier studies clearly emphasized that external supplementation/application of BRs improved plant growth in the case of wheat [18] and groundnut [19] which is in tune with the present study where foliar application of BL substantially increased the root growth of mustard plants.

The enhanced shoot and root growth of BL treated mustard plants was associated with increased foliar growth. Arteca and Arteca [20] also reported that BRs not only induce exaggerated growth in hydroponically grown *Arabidopsis thaliana*, but also control the proliferation of its leaf cells [21]. Kudryashova et al. [22] reported that supplementation of 24-epiBL played a pivotal role in *in vitro* regeneration of highbush blueberry (*Vaccinium corymbosum* L., cv. Brigitta blue).

Conclusion

The present study reveals that application of BL on mustard plants as foliar spray promoted the shoot, root and foliar growth of mustard plants grown in semi-arid soils of Nizamabad. The soils of Nizamabad are saline and dry in nature inhibiting the growth of plants. BRs have the ability to promote growth of plant under stressful conditions. The present study

reveals a new insight that application of BL overcame the negative effect of the semi-arid conditions of the soil (reflected in the control plants) and promoted the shoot, root and foliar growth (reflected in the BL-treated plants) of mustard.

REFERENCES

1. Vardhini BV, Anjum NA. Brassinosteroids make plant life easier under abiotic stresses mainly by modulating major components of antioxidant defense system. *Frontiers in Environmental Sciences*. 2015; 2: 67. doi: 10.3389/fenvs.2014.0006.
2. Mitchell JW, Mandava NB, Worley JE, Plimmer JR, Smith MV. Brassins: a family of plant hormones from rape pollen. *Nature*. 1970; 225: 1065-1066.
3. Grove MD, Spencer GF, Rohwedder WK, Mandava NB, Worlet JF, Warthen Jr JC, Steffens GL, Flippen-Andersen JL, Cook Jr JC. Brassinolide, a plant promoting steroid isolated from *Brassica napus* pollen. *Nature*. 1979; 281: 121-124.
4. Vardhini BV. Brassinosteroids, Role for amino acids, peptides and amines modulation in stressed plants - A review. In: Anjum NA, Gill SS, Gill R, editors. *Plant Adaptation to Environmental Change: Significance of Amino Acids and their Derivatives*, United Kingdom: CAB International of Nosworthy Way, Wallingford OX10 8DE. 300-316; 2013.
5. Vardhini BV, Anuradha S, Rao SSR. Brassinosteroids - New class of plant hormone with potential to improve crop productivity. *Indian J. Plant Physiol*. 2006; 11: 1-12.
6. Tao YZ, Zheng J, Xu ZM, Zhang XH, Zhang K, Wang GY. Functional analysis of ZmDWF1, a maize homolog of *Arabidopsis* brassinosteroids biosynthetic DWF1/DIM gene. *Plant Sci*. 2004; 167: 743-751.

- 142 7. Nomura T, Nakayama N, Reid JB, Takeuchi Y, Yokota T. Blockage of brassinosteroid
143 biosynthesis and sensitivity cause dwarfism in *Pisum sativum*. Plant Physiol. 1997; 113:
144 31-37.
- 145 8. Fábregas N, Caño-Delgado AI. Turning on the microscope turret: a new view for the
146 study of brassinosteroid signaling in plant development. Physiol Plant. 2014; 151: 172-
147 183.
- 148 9. Rao SSR, Vardhini BV, Sujatha E, Anuradha S Brassinosteroids – new class of
149 phytohormones. Curr Sci. 2002; 82: 1239-1245.
- 150 10. Grusak MA, Dellapenna D. Improving the nutrient composition of plants to enhance
151 human nutrition and health. Annu Rev Plant Physiol Plant Mol Biol. 1999; 50: 133-161.
- 152 11. Kemp CD. Methods of estimating leaf area of grasses from linear measurement. Ann Bot.
153 1960; 24: 491-512.
- 154 12. Hola D, Rothova O, Kocova M, Kohout L, Kvasnica M. The effect of brassinosteroids on
155 the morphology, development and yield of field-grown maize. Plant Growth Regul. 2010;
156 61: 29-43.
- 157 13. Fariduddin Q, Yusuf M, Chalkoo S, Hayat S, Ahmad A. 28- Homobrassinolide improves
158 growth and photosynthesis in *Cucumis sativus* L. through an enhanced antioxidant system
159 in the presence of chilling stress. Photosynthetica. 2011; 49: 55–64.
- 160 14. Kurepin LV, Joo SH, Kim SK, Pharos RP, Back TG. Interaction of brassinosteroids with
161 light quality and plant hormones in regulating shoot growth of young sunflower and
162 *Arabidopsis* seedlings. J Plant Growth Regul. 2012; 31: 156-164.
- 163 15. Vardhini BV, Sujatha E, Rao SSR. Studies on the effect of brassinosteroids on the
164 qualitative changes in the storage roots of radish. Bulg J Agric Sci. 2012; 18: 63-69.

- 165 16. Hayat S, Yadav S, Wani AS, Irfan M, Ahmad A. Comparative effect of 28-
166 homobrassinolide and 24-epibrassinolide on the growth, carbonic anhydrase activity and
167 photosynthetic efficiency of *Lycopersicon esculentum*. Photosynthetica. 2011; 49: 397-
168 404.
- 169 17. Shahid MA, Pervez MA, Balal RM, Mattson NS, Rashid A, Ahmad R, Ayyub CM,
170 Abbas T. Brassinosteroid (24-epibrassinolide) enhances growth and alleviates the
171 deleterious effects induced by salt stress in pea (*Pisum sativum* L.). Aust J Crop Sci.
172 2011; 5: 500-510.
- 173 18. Sairam RK. Effect of homobrassinolide application on plant metabolism and grain yield
174 under irrigated and moisture – stress conditions of two wheat varieties. Plant Growth
175 Regul. 1994; 14: 173-181.
- 176 19. Vardhini BV, Rao SSR. Effect of brassinosteroids on growth, metabolite content and
177 yield of *Arachis hypogaea*. Phytochemistry. 1998; 48: 927-930.
- 178 20. Arteca JM, Arteca RN. Brassinosteroid-induced exaggerated growth in hydroponically
179 grown *Arabidopsis* plants. Physiol Plant. 2001; 112: 104–112.
- 180 21. Nakaya M, Tsukaya H, Murakami N, Kato M. Brassinosteroids control the proliferation
181 of leaf cells of *Arabidopsis thaliana*. Plant Cell Physiol. 2002; 43: 239-244.
- 182 22. Kudryashova OA, Volotovich AA, Vasilevskaya TI, Varavina NP, Rupasova ZA,
183 Khripach VA. Effects of 24-epibrassinolide on in vitro micropropagation of highbush
184 blueberry. Russ J Plant Physiol. 2012; 59: 586-593.

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186 Table 1. Effect of brassinolide on the shoot growth in *Brassica juncea*
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Treatment (μM)	Shoot weight (gm / fr. wt.)*	Shoot diameter (cm)*	Shoot length (cm)*
0.5	8.093 ± 1.925	6.400 ± 0.213	61.66 ± 3.179
1.0	6.610 ± 0.100	6.427 ± 0.019	57.10 ± 1.000
2.0	4.510 ± 0.238	5.098 ± 0.036	47.66 ± 7.881
Control	2.001 ± 1.041	4.330 ± 0.169	32.00 ± 4.582

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190 *Values are Mean \pm S.E. (N=9)191 Table 2. Effect of brassinolide on the root growth in *Brassica juncea*

Treatment (μM)	Root weight (gm / fr. wt.)*	Root diameter (cm)*	Root length (cm)*
0.5	1.130 ± 0.430	4.850 ± 0.087	11.02 ± 0.288
1.0	0.830 ± 0.100	4.370 ± 0.134	10.50 ± 0.288
2.0	0.710 ± 0.058	4.435 ± 0.131	10.33 ± 0.033
Control	0.44 ± 0.145	3.320 ± 0.150	8.83 ± 4.582
			32.00 ± 4.582

192 *Values are Mean \pm S.E. (N=9)

193 Table 3. Effect of brassinolide on the foliar growth of *Brassica juncea*

Treatment (μM)	Plant fresh weight (gm / fr. wt.)*	Number of leaves/plant	Leaf Area (sq. cm)
0.5	9.550 ± 2.224	12.33 ± 1.850	483.1 ± 11.26
1.0	8.403 ± 1.315	11.26 ± 1.650	411.3 ± 9.90
2.0	5.510 ± 1.043	10.66 ± 2.060	398.2 ± 10.10
Control	2.991 ± 1.041	7.33 ± 1.520	133.1 ± 9.91

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195 *Values are Mean \pm S.E. (N=9)

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