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

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## 4 ABSTRACT

5 The effect of brassinolide (BL) sprayed in three concentrations viz.,  $0.5 \,\mu$ M,  $1.0 \,\mu$ M and  $2.0 \,\mu$ M 6 on the shoot growth, root growth and foliar growth (plant fresh weight, leaves per plant and leaf 7 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 8 9 saline stresses. All the three concentrations of BL stimulated the shoot growth, root growth as well as the foliar growth of mustard plants. BL at 0.5µM was found most effective in increasing 10 11 the shoot and root growth. 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. 12

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

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14 Abbreviations: Brassinolide=BL; Brassinosteroids =BRs; 24-Epibrassinolide = 24-EpiBL; 28-
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15 Homobrassinolide =28-HomoBL

### 16 INTRODUCTION

Brassinosteroids (BRs) are a new type of polyhydroxy steroidal phytohormones with 17 significant growth-promoting influence [1]. Mitchell et al. [2] discovered BRs which were later 18 extracted from the pollen of *Brassica napus* L. by Grove *et al.* [3]. BRs can be classified as  $C_{27}$ , 19 C<sub>28</sub> or C<sub>29</sub> BRs according to the number of carbons in their structure [4]. However, Vardhini et 20 al. [5] reported that brassinolide (BL), 28-homobrassinolide (28-HomoBL) and 24-21 epibrassinolide (24-EpiBL) are the three bioactive BRs being widely used in most physiological 22 and experimental studies. The work with BR biosynthetic mutants in Arabidopsis thaliana [6] 23 and Pisum sativum [7] have provided strong evidences that BRs are essential for plant growth 24 and development and BR- signaling plays a positive in plant growth and development viz., 25 spatiotemporal control of BR pathways in plant development employing microscope lens turret 26 to study the pleiotropic phenotypes of the BR mutants at a higher magnification [8]. 27

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 reduction of growth of many plants by salinity and drought usually effects on dry matter production, ionic relations, metabolic variations, physiological processes, water contents. 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 42 which is grown throughout the world. The plant has tiny seeds which are usually used as a 43 condiment. Mustard oil is extracted from the seed which are used for cooking, massaging etc. 44 Some kinds of mustard plants have edible leaves that are known as *mustard greens*. It is a well-45 established fact from time immemorial that plants are the vital components of dietary food chains 46 in which they provide almost all the essential mineral and organic nutrients to humans [10]. The 47 present study is undertaken to understand the effect of application of BL on the growth of 48 mustard plants in terms of shoot, root and foliage grown in the semi - arid soils of Nizamabad. 49

50 MATERIALS AND METHODS

#### 51 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.

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

#### 58 **Shoot and Root Growth**

59 Mustard seeds were sown in clay pots containing fresh sieved black soil mixed with 60 farmyard manure. Plants were grown under natural day length. BL was supplied to the plants as 61 foliar spray at three different concentration levels viz.,  $0.5 \mu$ M,  $1.0 \mu$ M and  $2.0 \mu$ M on  $35^{th}$ ,  $40^{th}$ , 62  $45^{th}$  and  $50^{th}$  day (from the day of sowing). Growth parameters in terms of shoot weight diameter 63 and length were recorded on the  $55^{th}$  day. Growth parameters in terms of root weight, diameter 64 and length were recorded on the  $55^{th}$  day.

#### 65 Foliar growth

Foliar growth was recorded in terms of plant fresh weight, leaves per plant and leaf area
per plant were recorded on 55<sup>th</sup> 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).

73 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., 0.5µM, 1µM and 2µM 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.,  $0.5\mu$ M,  $1\mu$ M and  $2\mu$ M increased the shoot growth of mustard plants grown in semi-arid soils of Nizamabad over control plants. Application of BL at  $0.5 \mu$ M conc. was more pronounced in increasing the foliar growth of mustard plants compared to the other two treatments as well as control plants.

92 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<sup>-8</sup>, or 10<sup>-6</sup> 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 103 radish (*Raphanus sativus* L. var Pusa chetki long) in terms of root fresh weight, root dry weight, 104 105 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 (P. 106 sativum L.) cv. Climax seeds subjected to sodium chloride stress exhibited increased 107 108 germination, embryo axis length and most of the aspects of shoot and root growth at seedling 109 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 110 111 present study where foliar application of BL substantially increased the root growth of mustard plants. 112

113 The enhanced shoot and root growth of BL treated mustard plants was associated with 114 increased foliar growth. Arteca and Arteca [20] also reported that BRs not only induce 115 exaggerated growth in hydroponically grown *A. thaliana*, but also control the proliferation of its 116 leaf cells [21]. Kudryashova *et al.* [22] reported that supplementation of 24-epiBL played a 117 pivotal role in *in vitro* regeneration of highbush blueberry (*Vaccinium corymbosum* L., cv. 118 Brigitta blue).

119 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.

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# 191 Table 1. Effect of brassinolide on the shoot growth in *Brassica junceae*

Treatment	Shoot weight (gm / fr. wt.)*	Shoot diameter (cm)*	Shoot length (cm)*	
0.5 1.0 2.0	$8.093 \pm 1.925$ $6.610 \pm 0.100$ $4.510 \pm 0.238$	$6.400 \pm 0.213$ $6.427 \pm 0.019$ $5.098 \pm 0.036$	$61.66 \pm 3.179$ $57.10 \pm 1.000$ $47.66 \pm 7.881$	
Control	2.001 ± 1.041	4.330 ± 0.169	32.00 ± 4.582	

- 195 \*Values are Mean  $\pm$  S.E. (N=9)
- (gm / fr. wt.) = gram/fresh weight; (cm) = centimeters
- 197 Table 2. Effect of brassinolide on the root growth in *Brassica junceae*

Treatment	Root weight	Root diameter	Root length
( µM)	(gm / fr. wt.)*	(cm)*	(cm)*

0			
0.5	$1.130 \pm 0.430$	$4.850\pm0.087$	$11.02\pm0.288$
1.0	$0.830 \pm 0.100$	$4.370\pm0.134$	$10.50\pm0.288$
2.0	$0.710\pm0.058$	$4.435\pm0.131$	$10.33\pm0.033$
Control	$0.440\pm0.145$	$3.320\pm0.150$	$8.83 \pm 4.582$

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

(gm / fr. wt.) = gram/fresh weight; (cm) = centimeters

200	Table 3.	Effect of braz	ssinolide on	the foliar	growth of	Brassica	junceae
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Treatment	Plant fresh weight	Number of	Leaf Area
( µM)	(gm / fr. wt.)*	leaves/plant	(sq. cm)*
0.5	$9.550 \pm 2.224$	$12.33 \pm 1.850$	483.1 ± 11.26
1.0	$8.403 \pm 1.315$	$11.26 \pm 1.650$	$411.3\pm9.90$
2.0	$5.510 \pm 1.043$	$10.66 \pm 2.060$	398.2 ± 10.10
Control	$2.991 \pm 1.041$	$7.33 \pm 1.520$	133.1 ± 9.91

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

(gm / fr. wt.) = gram/fresh weight; (sq cm) = square centimeters