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Effect of Modes of Application of 28-Homobrassinolide on Mung Bean

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Abstract: An experiment was conducted to assess the response of mung bean (*Vigna radiata* L. Wilczek) to 28-homobrassinolide (HBL) supplied to the seeds (1.0 µM) and/or to the foliage (0.01 µM) of the plants. Samples of the treated plant materials were assessed 30 and 50 days after sowing (DAS). It was observed that the activities of carbonic anhydrase (CA) and nitrate reductase (NR), leaf chlorophyll content (Chl), net photosynthetic rate (P_N), stomatal conductance (g_s), carboxylation efficiency (CE), dry mass (DM), pod number, and seed yield at harvest increased significantly over the control, irrespective of the mode of application. However, the plants raised by seed soaking also received foliar application of HBL generated maximum response. The order of response to HBL treatment was seed soaking + foliage application > foliage application only > seed soaking > control.

Key Words: Brassinosteroids, carbonic anhydrase, nitrate reductase, stomatal conductance

28-Homobrassinolitin Mung Bean Üzerine Uygulama Dozlarının Etkisi

Özet: Mung bean bitkisinin (*Vigna radiata* L. Wilczek) tohum ve/ veya yaprakların 28-homobrassinolide (HBL) uygulamalarına verdiği tepkiyi tayin etmek için bu çalışma yapılmıştır. Yüzeyi sterilize edilen tohumlar (i) 1.0 µM HBL içinde 8 h bekletilmiş (ii), yapraklara 0.01 µM HBL @ 5 cm^3 $bitki^{-1}$ olacak şekilde uygulanmış (iii), tohumlar 1.0 µM HBL de 8 h ve bitki karışımı 0.01 µM HBL de 15 gün bekletilmiştir. Bu muamel edilmiş örnekler ekildikten 30 ve 50 gün (DAS) sonra karbonik anhidraz (CA) ve nitrat redüktaz (NR) aktiviteleri; lif klorofil muhtevası (Chl); net fotosentetik oran (P_N); stomatal kondaktans (g_s); karboksilasyon verimliliği (CE); kuru ağırlık (DM); saksı sayısı ve hasatdaki tohum verimliliği belirlenmiştir. Tohum ıslatması ve aynı zamanda HBL uygulamasına tabi tutulma sonucu yetişen bitkiler yukarıdaki bütün parametrelerde 30 ve 50 gün sonra kontrole göre daha iyi sonuç vermiştir. HBL uygulamasına cevap sırasıyla tohumu ıslatma ve bitkiye uygulama > yalnız bitkiye uygulama > tohumun ıslatılması > kontrol şeklinde olmuştur.

Anahtar Sözcükler: Brassinosteroidler, karbonik anhidraz nitrat redüktaz, stomatal konductans

Introduction

Brassinosteroids (BRs) are naturally occurring steroidal plant hormone and regarded essential for the normal growth and development of plants (1). Their various forms, widely distributed in the plant kingdom, have a distinct role in stem elongation, pollen tube growth, leaf bending, ethylene biosynthesis, and xylem differentiation (2). The standing plants supplemented with BRs improved the productivity potential of economically important crops by activating cell elongation, vascular differentiation, and/or proton pump (3). Plants supplemented with BRs exhibited an increase in the activities of carbonic anhydrase and nitrate reductase (4,5), phosphoenolpyruvate carboxylase, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and the contents of soluble proteins (6-8). BRs treated plants also exhibit higher resistant to stress and produce more seeds in crop plants (3). However, in all the earlier studies (9) the application of BRs was either through seed soaking or to the foliage of the plants. The present experiment was designed to compare the impact of different modes of application of 28-homobrassinolide on the metabolic markers of photosynthesis and nitrogen metabolism, which can be used for predicting the yield potential of *Vigna radiata*.

Materials and Methods

The seeds of *Vigna radiata* (L.) Wilczek cv. T-44, which belongs to family Fabaceae, were obtained from the National Seed Corporation Ltd., New Delhi, India. Healthy seeds were surface sterilized with 0.01% mercuric chloride solution followed by repeated washing with double distilled water. The sterilized healthy seeds were soaked in water (control) or 1.0 μM aqueous solution of 28-homobrassinolide (HBL) for 8 h. Foliage of 15-day old seedlings was sprayed with water (control) or 0.01 μM HBL (5 cm^3 $plant^{-1}$). In the third set, the seedlings raised from the seeds pretreated with water (8 h) or 1.0 μM HBL (8 h) were sprayed with water (control) or 0.01 μM HBL. The seeds were sown in earthen pots (25 x 25 cm^2) filled with soil and farmyard manure (9:1). Inorganic fertilizers (urea, single superphosphate, and muriate of potash) were applied to each pot at the rate of 40, 295, and 73 mg. Irrigation was done with tap water as and when required. The experiment was conducted twice and 3 replicates were taken for each treatment. The pots were lined in the net house according to simple randomized block design. 28-Homobrassinolide was obtained from Godrej Agrovet Ltd. Mumbai, India. The plants were sampled 30 and 50 days after sowing (DAS) to study various characteristics.

Shoot dry mass was determined after dehydrating the plants at 80 °C for 24 h. The activity of carbonic anhydrase (CA) was determined following the procedure described by Dwivedi and Randhava (10). The leaf samples were cut into small pieces and suspended in cysteine hydrochloride solution. The samples were incubated at 4 °C for 20 min. The pieces were blotted and transferred to the test tubes containing phosphate buffer (pH 6.8) followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tube was incubated at 5 °C for 20 min. The reaction mixture was titrated against 0.01 N HCl after addition of 0.2 ml of methyl red indicator. The results were expressed as mol (CO_2) kg^{-1} (leaf fresh mass) s^{-1}. The activity of nitrate reductase (NR) was determined in fresh leaf samples by the procedure explained by Jaworski (11). The fresh leaf samples were cut into small pieces and transferred to plastic vials, containing phosphate buffer (pH 7.5) followed by the addition of potassium nitrate and isopropanol solutions. The reaction mixture was incubated at 30 °C for 2 h followed by addition of sulphanilamide and *N*-1-naphthylethylenediamine dihydrochloride. The absorbance of the color was read at 540 nm and compared with that of calibration curve. The activity of NR (n mole NO_2 g^{-1} h^{-1}) was computed on fresh mass basis. Chlorophyll content was quantified by the procedure of Mackinney (12). The net photosynthetic rate and stomatal conductance were measured using an LI-6200 portable photosynthetic system (LI-COR, NE, USA). The measurements were made in the upper most fully expanded leaves, between 1100 to 1300 hours. The carboxylation efficiency was calculated by employing a formula given by Tiwari et al. (13). The yield characteristics were studied at harvest. The data were analyzed for variance by one-way ANOVA. Mean values were tested by the least significance difference at the 0.05 level of probability using the statistical package SPSS 7.5.

Results

Shoot dry mass increased as the growth progressed (Table 1). The plants, raised from the seeds soaked in HBL and also received foliar treatment of HBL, generated the maximum values that were 32% and 21% more than the control, at days 30 and 50, respectively. The activity of nitrate reductase was maximum at day 30 and decreased at day 50. HBL treatment significantly enhanced the level of NR at both stages. Leaf chlorophyll content in leaves was maximum at day 30 (Table 1). The plants, raised from HBL treated seeds and applied with hormone at day 15, had 42% and 29% more chlorophyll content than the control 30 and 50 days after sowing, respectively.

Leaf carbonic anhydrase (CA) activity, stomatal conductance, and net photosynthetic rate were maximum at day 30 and were favored by HBL treatment (Table 2). Moreover, younger leaves (30-day old) had higher carboxylation efficiency than older leaves (Table 3). Carboxylation efficiency increased by 39% and 38% over the control at 30- and 50-day-old plants, which received HBL as a presowing seed treatment and to their foliage 15 days after sowing.

Pod number and seed yield were favored by HBL treatment, irrespective of the mode of application (Table 3). The plants that received HBL as presowing treatment, and also at the foliage, at day 15 had the maximum pod number and seed yield, which is 38% and 32 % higher than the control, respectively.

Table 1. Effect of 28-homobrassinolide [presowing seed treatment (1.0 μM) and/or foliar application (0.01 μM)] on shoot dry mass, nitrate reductase (NR) activity, and leaf chlorophyll content in *Vigna radiata* plants.
HBL = 28-homobrassinolide; NS = Nonsignificant; S = Soaking; SP = Spray; W = water.

Mode of Treatment	Shoot dry mass (g) Plant^{-1}		NR activity (n mole NO_2 g^{-1} h^{-1} leaf f.m.)		Chlorophyll content [g kg^{-1} (f.m.)]	
	30 DAS	50 DAS	30 DAS	50 DAS	30 DAS	50 DAS
W (S)	2.40	5.85	316	290	1.17	1.06
HBL (S)	3.15	6.53	400	360	1.40	1.22
W (SP)	2.46	6.0	337	293	1.13	1.07
HBL (SP)	3.10	7.0	467	370	1.51	1.35
W (S) + W (SP)	2.45	5.83	330	279	1.20	1.09
HBL (S)+ HBL (SP)	3.23	7.07	468	350	1.71	1.41
LSD P = 0.05	0.17	0.19	25	32	0.09	0.10

Table 2. Effect of 28-homobrassinolide [presowing seed treatment (1.0 μM) and/or foliar application (0.01 μM)] on leaf carbonic anhydrase (CA) activity, stomatal conductance, and net photosynthetic rate in *Vigna radiata* plants.
HBL = 28-homobrassinolide; NS = Nonsignificant; S = Soaking; SP = Spray; W = water.

Mode of Treatment	CA activity [mol (CO_2) kg^{-1} s^{-1}]		Stomatal conductance (mol m^{-2} s^{-1})		Net photosynthetic rate [μmol (CO_2) m^{-2} s^{-1}]	
	30 DAS	50 DAS	30 DAS	50 DAS	30 DAS	50 DAS
W (S)	1.87	1.56	0.325	0.317	13.58	11.37
HBL (S)	2.32	1.92	0.412	0.395	17.00	14.69
W (SP)	1.81	1.52	0.343	0.305	14.01	12.00
HBL (SP)	2.47	1.97	0.483	0.402	18.66	15.97
W (S) + W (SP)	1.87	1.71	0.340	0.307	13.61	11.50
HBL (S)+ HBL (SP)	2.57	2.27	0.495	0.431	19.31	15.48
LSD P = 0.05	0.11	0.13	0.015	0.016	1.03	0.81

Table 3. Effect of 28-homobrassinolide [presowing seed treatment (1.0 μM) and/ or foliar application (0.01 μM)] on carboxylation efficiency and number of pods, number of seeds, and seed yield at harvest in *Vigna radiata* plants. HBL = 28-homobrassinolide; NS = Nonsignificant; S = Soaking; SP = Spray; W = water

Mode of Treatment	Carboxylation efficiency (mol m^{-2} s^{-1})		Number of pods Plant^{-1}	Number of seeds Pod^{-1}	Seed yield (g) Plant^{-1}
	30 DAS	50 DAS			
W (S)	0.045	0.034	25.30	8.30	3.95
HBL (S)	0.054	0.041	31.70	8.37	4.70
W (SP)	0.040	0.034	25.10	8.36	3.90
HBL (SP)	0.055	0.044	32.22	8.41	4.97
W (S) + W (SP)	0.043	0.034	25.61	8.45	3.97
HBL (S)+ HBL (SP)	0.060	0.047	34.25	8.40	5.18
LSD P = 0.05	0.003	0.003	1.35	NS	0.19

Discussion

It has become a general practice to employ phytohormones to explore the full potential of plants. They are involved in the regulation of photosynthetic processes and partitioning of assimilates, resulting in the alteration in plant size. However, the response varies with the type of plant, the hormone, and the mode of application. In the present investigation, the seedlings raised from the seeds pretreated with HBL and/or applied to the foliage had significantly higher net photosynthetic rate than the control (Table 2). Braun and Wild (6) also reported an increase in the photosynthetic parameters in BR-treated wheat and mustard plants because of the enhancement in CO_2 fixation. The photosynthesizing tissues of C_3 and C_4 plants contain carbonic anhydrase that makes CO_2 available to Rubisco by catalyzing reversible hydration of CO_2. The distribution pattern of CA is, therefore, very much comparable with that of Rubisco (14). However, in the present study, the seedlings raised from seeds treated with HBL and the foliage of these seedlings also received HBL had the maximum activity of CA compared to the control (Table 2). This was also linked with observed increase in the values of leaf chlorophyll content (Table 1), which is supported by Sairam (15) and Bhatia and Kaur (16). This response of the plants to HBL may be explained on the basis of the findings of Kalinich et al. (17) where BRs had an impact on the transcription, leading to an increase in protein content in *Phaseolus vulgaris* and *Phaseolus aureus*.

Plants obtained from HBL treated seeds and/or supplied HBL to the foliage generated a significant increase in carboxylation efficiency (CE) than the control (Table 3). One of the possible reasons for the increase in CE may be due to the enhanced fixation of CO_2 accompanied by higher CO_2 availability to Rubisco in the chloroplast [Table 3 and Badger and Price (18)]. This metabolic state had possibly been generated because the plants contain more chlorophyll [(Table 1 and Singh et al (19)] and stomatal conductance (Table 2) facilitating the free exchange of gases. The cumulative effect of more chlorophyll and CA could have improved the availability of CO_2 under high g_s (Table 2) and its rate of reduction by Rubisco whose activity is generally enhanced by brassinolide (6). Moreover, the elevated leaf NR activity (Table 1) might additionally support the healthy metabolic state, which is evident from increased dry mass of the plants received HBL either through seeds and/or to the foliage (Table 1). Moreover, high nitrogen content (20) might be involved in increased NR activity as nitrate is known to induce functional NR, essential for nitrate reduction (21), by producing nitrate sensing protein of unknown nature, which possibly binds with the regulatory region at the NR-genes and turns on the expression of NR-mRNA (22). The plants received HBL as seed soaking and/or foliar spray acquired higher P_N (Table 2), which was reflected in the form of healthy growth of the plants and increased dry mass production (Table 1). These healthy plants will naturally bear strong inflorescence axis with more pods per plants and seed yield (Table 3). Similar results were also reported in other plants (4, 16). This is probably a result of improved fruit set and grain filling as proposed earlier by Luo et al. (23).

Conclusion

The present study revealed that the plants raised from seeds presoaked in HBL and also supplied HBL to the foliage generated best response for all the parameters studied. The order of response to various methods is similar for most of the parameters (Seed soaking + foliar application > foliar application > seed soaking > control).

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