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Effect of brassinolide on nodulation, nodular protein and leghaemoglobin in moongbean

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Abstract

Effect of growth regulator (Double-brassinolide) was studied on nodulation, nodular protein and leghaemoglobin at three different stages of plant growth in moongbean (Vigna radiata (L.) R Wilczek syn. Phaseolus aureus Rox). Significant increase in nodulation, viz. number, weight and size of nodules was observed. Nodular protein and leghaemoglobin were also enhanced till later stage of growth. The enhancement effect was observed with lower concentrations (0.1, 0.2, 0.4 and 0.6 ppm), against control.

Key words- Brassinolide, nodulation, nodular protein, leghaemoglobin, moongbean

Introduction

Plants need hormones in order to regulate various growth activities, to send signals from one part to another and respond to the environmental stimulus. Besides, five major plant growth hormones; a new growth-regulating hormone from certain pollen extracts cause growth promotion paved the way for the discovery of Brassinosteroids (BR) in plants. BRs were first isolated and characterized from the pollen of rape plant and have significant growth-promoting activity (Sakurai and Fujioka, 1993; Clouse, 1996; Fujioka and Sakurai, 1997; Clouse and Sasse, 1998). This growth regulator is known to play major role in growth and yield of many vegetables and cereal crops (Sivakumar *et al*., 2002; Vardhini and Rao, 2001, 2003; Vardhini *et al*., 2006). Extensive investigation on the distribution of brassinolide like active substances in plant led to them being found not only in pollen but also in insect galls, immature seeds, shoots and leaves of a wide variety of plants (Rao *et al.,* 2002; Haubrick and Assmann, 2006). Subsequently, they have so far been reported from 44 plants and are regarded probably ubiquitous in the plant kingdom. BRs are considered as hormones with pleiotropic effects, as they influence varied developmental processes, like growth, germination of seeds, rhizogenesis, flowering and senescence. BRs also confer resistance to plant against various abiotic stresses. So, keeping in view, the present experiment was conducted with moongbean to test efficiency of BR on nodulation, nodular protein and leghaemoglobin (Lb), a key regulator in N_2-fixation.

Materials and Methods

The pot experiment was setup in the month of March (2008-2010) and the average temperature recorded was 15° C - 30° C. The pots were filled with sandy loam (4 kg/pot). The soil had pH 6.8 and contains 0.51% organic carbon, 13.5 kg/ hectare phosphate and 240-kg/ ha potash. According to the soil testing

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report, the cowdung (200 g/pot) was added. The viable seeds of moong bean were sown at equal distance in the pot (3/pot). For each treatment triplicate pots were maintained. The pots were watered, whenever required.

Different concentrations of BR (Brassinolide-Double-Godrej Agrovet Ltd, Mumbai) used were 0, 0.2, 0.4, 0.6, 0.8, and 1 ppm. The treatments were given as foliar spray on moongbean plant, at three stages of development i.e. pre-flowering. (20 days after sowing-DAS), flowering (40 DAS) and post flowering (60 DAS). After five days of BR spraying (25, 45 and 65 DAS) number, fresh weight and size of nodules, nodular protein and Lb content were recorded. Size of nodules was measured by using Vernier Callipers (Least Count=0.1mm and Zero error=0). Total Protein and Lb content were determined by following the procedures described by Lowery *et al*. (1951) and Hartee (1955), respectively. Data were statistically analyzed using randomized block design (Panse and Sukhatme, 1985).

Results and Discussion

Present investigation revealed that BR treatment significantly increased the nodulation except 0.8 and 1.0 ppm. Number of nodules increased from 3.94 to 71.43% at 25 DAS, 3.01 to 68.65% at 45 DAS and 16.57 to 132.74% at 65 DAS with different concentrations of BR over control (Table-1). The maximum increase was recorded with 0.4 ppm at all the three stages of growth. The increase in number nodules were more between 45 and 65 DAS, as compared to 25 and 45 DAS in both treated and control. Fresh weight of nodules showed an increase from pre-flowering (25 DAS) to flowering (45 DAS) in both control and treated plants and thereafter reduced from flowering (45 DAS) to post-flowering (65 DAS), in control plants, while treated plants showed an increase, except 1 ppm. Overall, fresh weight of nodules was increased with all concentrations except 0.1 and 0.8 ppm at 45 DAS, 1 ppm, at all the three stages of growth over control. The maximum weight was recorded with 0.4-ppm i.e. 95.88 %, 26.88 %, 267.40 % (3.6 fold) at 25, 45, and 65 DAS, respectively over control (Table-1). The size of nodules recorded as minimum-maximum (MIN-MAX) also increased with different concentrations, at all the three stages of growth (Table-1). The maximum size enhanced with 0.4 ppm i.e. 1.3-2.6 mm at 25 DAS, 1.6-3.3 mm at 45 DAS, and 1.0-4.0 mm at 65 DAS, as compared to control (1.0-2.0, 1.6-2.3 and 1.2-2.3, respectively). The increase in fresh weight from 25 to 45 DAS is correlated with the increase in both number and size of nodules and the reduction from 45 to 65 DAS, younger nodules with smaller sizes were observed with 0 ppm. In treated plants more increase was observed from 45 to 65 DAS than 25 to 45 DAS, associated with the increase in number and the size of nodules (Singh *et al*. 1999).

Nodular proteins showed a pattern of increasing from pre-flowering (25 DAS) to flowering (45 DAS) and thereafter reduced from flowering (45 DAS) to post-flowering (65 DAS), in control as well treated plants. Present studies revealed that moongbean followed the common pattern that the nodulation (fresh weight of nodules) and nodular proteins were increased with plant growth to reach maximum at flowering (45 DAS) and decreased thereafter during maturity (Bethlenfalvay *et al.,* 1978; Bharti *et al*., 1987; Raghava, 1990; Raghava *et al.,* 1993). This reduction is due to the decreased availability of photosynthates to the nodules and diverted towards the developing pods and seeds, correspond to the reduction in nodules metabolic activities. It has been reported that photosynthetic efficiency has been increased in *Vigna radiata* (Bhatia and Kaur, 1997; Fariduddin *et al*., 2003) and *Brassica juncea* (Hayat *et al*., 2001). The total proteins in the nodules was also increased with the concentrations upto 0.6 ppm and thereafter either reduced or showed non-significant increase at all the three stages of growth. The maximum increased was recorded with 0.4 ppm i.e. 40.00 %, 83.82 % and 90.55 % at 25, 45, and 65 DAS, respectively over control (Table-1). The Lb content also showed the same pattern as that of total proteins i.e. increased from 25 to 45 DAS and then reduced from 45 to 65 DAS. Initially it showed an enhancement with 0.2 to 0.6 ppm at 25 DAS, then with all concentrations, except 1 ppm at 45 DAS and with 0.2 to 0.8 ppm at 65 DAS. The maximum increase was recorded with 0.4 ppm 206.98 %, 108.89% and 133.33% at 25, 45 and

65DAS, respectively over control (Table-1). In total proteins the reduction was more from 45 to 65 DAS in treated plants than control, but still higher than that of control at 65 DAS. Similarly, the Lb content showed the reduction from 45 to 65 DAS, but still it was more at 65 DAS, in treated plants as compared to control.

Table-1- Effect of Brassinolide on number and fresh weight of nodules of moongbean size of nodules (mm), total protein (mg/100 mg fresh weight of nodule) and leghaemoglobin content (mg/100mg fresh weight of nodules) of moongbean (All the values are an average of nine replicates and three-season crop).

	Number of Nodules/plant			Fresh weight of Nodules (mg)		
Concentration/ DAS	25	45	65	25	45	65
CON	4.06	9.96	16.9	77.7	148.8	123.3
0.1 ppm	4.25*	11.40	25.6*	107.7	154.4*	260.0
0.2 ppm	5.13	13.50	28.4	118.7	167.7	410.0
0.4 ppm	6.96	16.30	39.3	152.2	188.8	453.3
0.6 ppm	5.20	14.96	34.8	127.7	165.1	280.0
0.8 ppm	4.96*	10.70*	23.7*	110.0	157.7*	178.8
1 ppm	4.20*	10.26*	19.7*	82.2*	150.2*	137.2*
CD at 5% level	1.06	1.07	10.6	12.9	12.8	42.8

DAS	**25**		**45**		**65**	
Concentration/ DAS	MIN	MAX	MIN	MAX	MIN	MAX
00 ppm	1.0	2.0	1.6	2.3	1.4	2.3
0.1 ppm	1.0	2.0	1.0	2.6	1.2	3.0
0.2 ppm	1.0	2.0	1.3	2.6	1.0	3.0
0.4 ppm	1.3	2.6	1.6	3.3	1.0	4.0
0.6 ppm	1.0	2.3	1.0	2.6	1.0	3.0
0.8 ppm	1.0	2.3	1.0	2.6	1.0	3.6
1 ppm	1.0	2.0	1.0	3.3	1.0	4.0

Parameters	Total protein			Leghaemoglobin		
Concentration/ DAS	25	45	65	25	45	65
00 ppm	6.40	6.80	6.35	0.86	3.18	1.35
0.2 ppm	8.60	12.20	10.50	1.69	5.35	2.19
0.4 ppm	8.96	12.50	12.10	2.64	6.61	3.15
0.6 ppm	6.90	9.73	8.20	1.76	5.77	2.59
0.8 ppm	4.50	7.10	6.60	1.34	5.80	1.90
1.0 ppm	3.70	5.50	4.16	1.05	3.44	1.18
CD(P=0.05)	0.25	0.42	0.72	0.66	0.48	0.46

These observations clearly showed that despite of reduction, BRs showed more fresh weight and nodular proteins, specifically Lb. Earlier findings also indicated that the foliar application of BR (0.25 ppm) at preflowering and flowering stages can bring about changes in assimilatory component and thereby improving growth and productivity (Sairam, 1994; Cevahir et al., 2008; Maity and Bera, 2009). These studies correlate that BR through promoting the growth, might improve the active supply of more photosynthates to the developing nodules till productive stage of the plant, thus improving the nodule metabolism by enhancing Lb content. The nodular growth is directly dependent on the synthesis of proteins, which is very much clear from the studies, that nodular weight and total proteins are enhanced even with as low as 0.2 ppm and maximum upto 0.4 ppm. The minimum size of nodule observed in

treated plants at 65 DAS (post-flowering stage) was 1.0 mm (new emerging) and maximum, which supports the increase in nodular proteins and Lb content till later stage of growth.
Currently, it has been reported in different plants that BR treatment improved the growth and induced the antioxidative defense system in plants against stresses (Ali et al., 2008; Arora et al., 2010). These studies suggest a possible role of BRs to overcome ageing stress mechanism by increasing protein and Lb synthesis, in turn provide longevity to N_2–fixation till later stages of growth.

Conclusion

The present study, therefore, reveals that the growth promotory activity of brassinolide and optimum concentration to be 0.4 ppm, which has enhanced nodulation, nodular proteins, fresh weight and Leghaemoglobin content. The use of optimum concentration of Brassinolide with particular crop plant will be fruitful.

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