Indian J.Sci.Res.2(3) :89-92, 2011

BRASSINOSTEROIDS STIMULATE SEED GERMINATION PARAMETERS AND CHLOROPHYLL CONTENT IN MOONGBEAN

KSHITIJ SRIVASTAVA[a1], N.RAGHAVA[b], SHAGUN[c] AND R.P.RAGHAVA[d]

P.G. Department of Botany, S.M.M. Town (P.G.) College, Ballia, U.P., India

[a]E-mail: kshitijsrivastava@yahoo.co.in

[b]E-mail: nisharaghava@yahoo.co.in

[c]E-mail: shgunsri@yahoo.co.in

[d]E-mail: raghavarp@rediffmail.com

ABSTRACT

The seeds of moong bean (*Phaseolus aureus* Rox.) Were used to observe the effect plant growth regulator (double brassinolide) which show the significant increase in seed germination and different seedling growth parameters viz. germination rate index, speed of germination, coefficient of velocity of germination, emergence index, relative seed germination, root and shoot length, relative root elongation, fresh and dry weights, seedling growth and vigour index. Maximum percent germination was observed on 11th day with the concentration of 0.4 ppm. Overall, 0.4 ppm showed optimum seedling growth.

KEY WORDS: Seed germination, Brassinosteroids, Moongbean

Brassinosteroids are growth promoting natural products found at low level in pollen, seeds and young vegetative tissues throughout the plant kingdom. The Brassinosteroids are a group of plant originated steroidal lactones that exert pronounced growth promoting activities. Brassinosteroids have strong and unique biological activities when applied to plant tissue at nano or micro molar levels. As result of extensive investigation Brassinosteroids were found to show characteristic physiological actions on the growth of plant in micro-quantities. Therefore, brassinosteroids can be regarded as a new class of plant hormone, in addition to auxin, gibberellin, cytokinin, abscisic acid and ethylene (Fujioka and Sakurai, 1997; Clouse and Sasse, 1998).

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).

Commonly known as moong bean, green gram or golden gram (*Phaseolus aureus* Rox.; family- Fabaceae) is one of the most important short duration pulse crop grown in India .It ranks third among all pulses grown in India after chickpea and pigeon pea. It makes a good manure, if enriches the soil by atmospheric nitrogen fixation through root nodules. The seeds are highly nutritious with protein (23-24%); carbohydrates, minerals and vitamins. Green gram is grown in about 3.3 million hac.in India with total production of 1.37 million tones (Pandey, 2000). Among the states growing this crop, Orissa rank first in area and production. It is the need to promote the production of moong in East UP. Therefore, the work has been selected to explore the potential use of this growth regulator, as positive or stimulatory effect on seed germination and different seedling growth parameters of this important pulse crop.

MATERIALS AND METHODS

Different concentrations of Brassinosteroids used were 0.1,0.2,0.4,0.6,0.8,1 ppm against control. The seeds of moong were sterilized washing in 0.1% $HgCl_2$ for 5 minutes, after rinsing in sterile distilled water for 30 minutes, the seeds were placed on sterile filter paper disc in Petridishes 15 cm diameter and 2 cm in depth. For screening, 7 Petridishes, each containing 10 seeds were allowed to germinate separately, at various concentrations against control. Percentage of germination, shoot length, root length, fresh and dry weight of shoot and root were measured at different growth parameters. Biochemical estimation on chlorophyll content was measured using the method of Arnon (1949) and carotenoid content was estimated following the method given by Jensen and Jensen (1972).

Germination parameters were determined according to following formulae at final days of observation:

[1]**Corresponding author**

A. **Germinability (%G)**=Total No. of Seeds Germinated X 100 /Total No. of Seeds Sown

B. **Standard Germination Test**: It was calculated as Germination Rate Index and Speed of Germination (Maguire, 1962)

(i) Germination Rate Index (**GRI**)= No. of normal seedlings of days x/ Days *X*

X= No. of days from seed soaking

(ii) Speed of Germination (**SpG**) = n/t

Where n = No. of seeds emerged on the day

t = time or days from soaking.

C. **Coefficient of Velocity of Germination** (**CVG**)- Kotowski (1926)

CVG=Sum of n x 100/ Sum of (nt)

Where n= No. of seeds emerged on the day

t = time or days of soaking

D. **Emergence Index** (**EI**) was calculated by the formula of by Baskin (1969).

EI = (n1/dn1)+(n2/dn2)+(n3/dn3)……. (nx /dnx)

Where n = No. of seeds emerged on the day 1^{st}, 2^{nd}, 3^{rd}, …… n^{th} day.

dn = No. of days from the day of sowing.

dnx = No. of days to the final count.

E. **Relative Seed Germination** (**RSG**)

$$\textbf{RSG} = \frac{\text{No. of seeds germinated in the extract x100}}{\text{No. of seeds germinated in the control}}$$

F. **Relative Root Elongation** (**RRE**)

$$\textbf{RRE} = \frac{\text{Mean root elongation in the extract x 100}}{\text{Mean root elongation in the control}}$$

G. **Growth Index** (**GI**) = % seed germination x % root elongation/100 (Tam and Tiquia, 1994)

H. **Seedling growth** (**SG**) =

$$\textbf{RRE} = \frac{\text{Total seedling Growth length in cm}}{\text{No. of germinated seeds}}$$

I. **Vigour Index (VI)** = Germination % x Seedling length (Abdul-Baki and Anderson, 1973).

All the data were recorded in triplicate and statistically analyzed using completely randomized block design (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

Present investigation revealed overall increase in seedling growth with brassinolide. Table-1, clearly revealed that the maximum germination percentage was recorded with 0.4 ppm i.e 100% and 0.2 ppm i.e 96.66% in comparison with control i.e. 80% only. The GRI was also recorded maximum with 0.4 ppm i.e 57.5 %, over control. The speed of germination were recoded in 0.4 ppm i.e 27.5 % and coefficient of velocity of germination in 0.4 ppm i.e 12.3 %. The maximum emergence index, relative seed germination, relative root elongation, and growth index were recorded maximum with 0.4 ppm i.e. 29.0%, 26.4 %, 34.5 %, 48.3 %, respectively, over control. The maximum seedling growth was also recorded with 0.4 ppm i.e. 78 % and vigour index was recorded with 0.4 ppm i.e. 82.38 %, over control. The maximum increase in carotenoids were recorded with 0.1 ppm i.e. 1.4 fold and chlorophyll 'a' with 0.6 ppm i.e. 4.2 fold; chlorophyll 'b' with 0.1 ppm i.e. 1.8 fold and total chlorophyll with 0.2 ppm i.e. 2 fold over control.

Shoot length was increased with all the concentrations .The maximum increases were recorded with 0.2 ppm i.e. 79.41 %, over control. The hypocotyl length was increased with all the concentrations. The maximum increase was recorded with 0.2 ppm i.e. 56 %, over control. The maximum root length was recorded with 0.6 ppm i.e. 39.29 %, over control. In case of fresh and dry weights of shoot, the maximum increases were recorded with 0.6 ppm i.e. 97.46 % and 23.64%, respectively over control. The maximum increase in fresh and dry weight of root was recorded with 0.4 ppm i.e. 5.49% and 11.11% respectively, over control (Table-2).

It is well documented that Brassinosteroids promote seed germination, like other hormones. The treatment of the seeds of *Lepidium sativus* (Jones Held et al., 1996) with brassinolide improved percent germination. Similarly, Brassinosteroids promoted seed germination in case of *Brassica napus* (Chang and Cai, 1988), rice (Dong et al., 1989), wheat (Hayat et al., 2003) and tobacco (Leubner-Metzger, 2001).

Brassinolide application at ìM levels causes pronounced elongation of hypocotyls, epicotyls, and

peduncles of dicots, as well as coleoptiles and mesocotyls of monocots (Mandava, 1988; Sasse, 1990; Clouse, 1996). Young vegetative tissue is particularly responsive to Brassinolides, and, if endogenous Brassinolides are directly involved in the control of cell expansion, they must be present in such tissue. Approaches to establishing this include the analysis of levels in a Brassinolide-sensitive zone of pea stem (Sasse et al., 1992) and localization of an exogenously supplied 125I-BR, which accumulated in the elongating zone of mung bean epicotyls and the apex of cucumber seedlings (Xu et al., 1994). This experiment on the chemistry and morpho-physiology of Brassinolides provides a convincing body of evidence that these plant steroids in minute quantities are essential regulators of plant growth and development.

ACKNOWLEDGEMENTS

One of the author **(K)** thanks to Principal of the college, Head Botany department for providing necessary facilities to do the work and Dr. B.N. Vyas, Vice President Godrej Agrovet Ltd, Mumbai, India for the generous supply of brassinolide.

Table 1: Effect of various concentrations of brassinolide (Double) on different seed germination parameters and chlorophyll content (mg/g fresh weight of leaves) in Moongbean.All the data is an average of triplicates

Conc.	G%	GRI	SpG	CVG	EI	RSG%	RRE%	GI	SG (cm)	VI	Carotenoids	Chl-a	Chl-b	Total Chl.
CON	80	1.20	1.20	52.8	6.2	100	100	225.0	1.00	716.6	0.057	0.085	0.382	0.467
0.1ppm	93	1.42*	1.44*	57.1	7.3	116.8	104.9*	270.9	1.25*	1101.0	0.082	0.212	0.696	0.908
0.2ppm	97	1.53*	1.54	58.5	8.0	121.4	128.0	346.3	1.78	1281.3	0.069	0.271	0.667	0.938
0.4ppm	100	1.89	1.53	59.3	7.2	126.4	134.5	384.3	1.43	1307.0	0.067	0.269	0.613	0.882
0.6ppm	90	1.49*	1.36*	57.3	6.9*	114.5	126.9	317.7	1.31*	1060.0	0.065	0.358	0.316*	0.674*
0.8ppm	93	1.43*	1.31*	55.8	6.6*	110.5	118.1*	301.0	1.28*	1132.3	0.062*	0.068*	0.287	0.355*
1ppm	90	1.33*	1.28*	52.5*	6.6*	96.9*	114.8*	286.0	1.27*	1161.7	0.058*	0.063*	0.181	0.244*
CD at 5%	7.46	0.39	0.30	1.6	0.9	9.7	18.7	23.0	0.40	124.6	0.008	0 .084	0.080	0.282

Conc.= Concentrations; CON= Control; G% = Germinability; GRI=Growth Rate Index; SpG= Speed of germination; CVG = Coefficient of velocity of germination; EI = Emergence Index; RRE = Relative Root Elongation; RSG Relative Seed Germination; SG = Seedling Growth; VI = Vigour Index; *=Non-significant

Table 2: Effect of various concentrations of brassinolide (Double) on different seedling growth parameters in Moongbean.All the data is an average of triplicates

TREATMENT (ppm)	SL (cm)	RL (cm)	HL (cm)	FWS (g)	DWS (g)	FWR (g)	DWR (g)
CON	3.4	2.8	2.5	1.18	0.55	0.91	0.18
0.1	4.9	2.9*	3.7	1.64	0.61*	0.92*	0.19*
0.2	6.1	3.6	3.9	1.85	0.62	0.94*	0.20
0.4	5.9	3.8	3.8	1.88	0.64	0.96	0.20
0.6	5.7	3.9	3.6	2.33	0.68	0.93*	0.18*
0.8	5.5	3.5	3.1	2.21	0.66	0.93*	0.18*
1.0	5.0	3.5	3.0	1.83	0.62	0.92*	0.18*
CD at 5% level	0.6	0.6	0.5	0.12	0.07	0.04	0.01

SL= Shoot Length; RL= Root Length; FWS= Fresh weight of shoot; DWS= Dry weight of shoot; FWR= Fresh weight of root; DWR= Dry weight of root; *=Non-significant

REFERENCES

Abdul-Baki A.A. and Anderson J.D., 1973. Vigour determination in soybean and seed multiple criteria. Crop Sci., **13**: 630-633.

Arnon D.I., 1949.Copper enzyme in isolated chloroplast, polyphenol oxidase in *Beta vulgaris*. Plant Physiol., **129**: 13-18.

Baskin C.C., 1969. GADA and Seedling at tests for seed quality. Proc. Seedsmaen Short Comm. Mississippi State Univ.: 59-69.

Chang J. Q. and Cai D.T., 1988. The effects of brassinolide on seed germination and cotyledons tissue culture in *Brassica napus* L. Oil Crops China, **3**: 18-22.

Clouse SD.,1996. Molecular genetic studies confirm the role of brassinosteroids in plant growth and development. Plant J., **10**:18.

Clouse S.D. and Sasse J.M., 1998. Brassinosteroids essential regulators of plant growth and development. Annu. Rev. Plant Physiol. Plant. Mol. Biol., **49**: 427-451.

Dong J.W., Lou S.S., Han B.W., He Z.P. and Li P.M., 1989. Effects of brassinolide on rice seed germination and seeding growth. Acta Agriculture University Pekinensis ,**15**: 153-156.

Fujioka S. and Sakurai A., 1997. Biosynthesis and metabolism of brassinosteroids. Physiologia Plantarum, **100**: 710-715.

Hayat S., Fariduddin Q. and Ahmad A., 2003. Homobrassinolide affect germination and α-amylase activity in wheat seeds. Seed Technology, **25** (1): 45-49.

Haubrick L.L. and Assmann S.M.; 2006. Brassinosteroids and plant function: some clues and puzzles. Plant Cell And Environment, **29**:446-457.

Jones-Held S., Van Doren M. and Lockwood T.,1996. Brassinolide application to *Lepidium sativum* seeds and the effects on seeding growth. Journal of Plant Growth Regulation, **15**: 63-67.

Jensen S.L. and Jensen A., 1972. Quantitative determination of carotenoids in photosynthetic tissue. In: San Pietro (ed). Methods of Enzymology, **23**: 218-228.

Kotowski, F., 1926. Temperature relations to germination of vegetable seeds. Proc. American Soc. Hort. Sci., **23**: 176-177.

Leubner-Metzger G., 2001. Brassinosteroids and gibberellins promote tobacco seed germination by distinct pathways. Planta, **213** (3)**:** 758-763.

Magurie J.D., 1962. Speed of germination aid in selection and evaluation of seedling emergence and vigour. Crop Sci., **2**: 176-177.

Mandava NB., 1988. Plant growth promoting brassinosteroids. Annu. Rev. Plant Physiol. Plant Mol. Biol., **39**:23-52

Pandey B.P., 2000. Vegetable Crops. In: Economic Botany, S. Chand and Company Ltd. : 69-70.

Panse V.G. and Sukhatme P. V., 1985. Statistical Methods for Agricultural Workers. ICAR. N. Delhi, India.

Rao S.S.R. Vardhini B.V., Sujatha E. and Anuradha S., 2002. Brassinosteroids-A new class of phytohormone. Curr. Sci., **82**: 1239-1245.

Sasse JM., 1990. Brassinolide-induced elongation and auxin. Physiol. Plant,. **80**: 401-408.

Sasse J.M. ,Yokota T., Taylor P.E. ,Griffiths P.G. and Porter Q.N., 1992. Brassinolide induced elongation. In: Progress in Plant Growth Regulation.Edieted by C.M.Karssen, L.C.V.Loon and D.Vreugdenhil. (Kluwer), Dordrecht :319-325

Tam N. F. Y. and Tiquia S. M., 1994. Assessing toxicity of " Spent saw dust pig litter" using seed germination technique. Resource Conservation Recycling..**2**: 261-274.

Xu R-J., He Y-J., Wang Y-Q. and Zhao Y-J., 1994. Preliminary study of brassinosterone binding sites from mung bean epicotyls. Acta Phytophysiol. Sin., **20**:298-302.