Critical Stage of Boron Application for Reproductive Growth and Seed Quality of Bush Snap Bean (*Phaseolus Vulgaris* L.)

Ram Lal Shrestha[1] and Renato C. Mabesa PhD[22]

ABSTRACT

The experiment were conducted to determine the critical stage of boron(B) application on the seed development and quality of bush snap bean (Phaseolus vulgaris L) grown in the nutrient solution (hydroponic). Boron was applied @ 0.05g/l (50ppm) of water at weekly intervals according to treatments up to the seed physiological maturity stage. The number of flowers and pod set were increased at 0 days and 21 days after sowing (DAS) treatment and decreased continuously as time of B application was delayed. Boron was effective to increase the seed weight 18.8% greater at full B treatment as compared to 0-boron (-B). The quality of seed was greatly reduced in –B solution due to lack of B. Seed germination was 1.24 times greater in laboratory condition and 1.47 times greater under field condition at 0 day treatment than –B treatment. The normal seedling percent was 8.1 times greater in 0 day (8 time applied) treatment than –B under field condition. The seedling dry weight was higher (3.70g) at 21 DAS B applied seed as compared to control (1.73g). Seeds harvested from earlier B applied treatments were higher in quality and produced strong seedlings as compared to -B. The critical stage of B requirements to the Contender bean for reproductive growth and seed quality improvement was identified at 28 DAS. The quality of seed was decreased if B is applied later from 28 DAS. It can be concluded that earlier stage (Vegetative growth) of B application give better yield and quality of seed.

Key words: Boron, physiological maturity, vigor, tissue, germination, emergence, contender, bush-snap bean

INTRODUCTION

The production and quality of snap bean (*Phaseolus vulgaris* L) depends on the quality of seed. Seed quality embraces all physiological, biological, morphological and genetical attributes, which contribute to increase in the final yield of the crop (Basra, 1995). In general, poor quality seed have poor germination and production of abnormal seedlings (Powell *et al*., 1984) results decreased yield. One of the causes of low quality seeds is absent of B in the soil and seeds. Boron plays an important role in the vegetative and reproductive growth in the plant especially in the flowering, pollination, fertilization and seed formation stages. During flowering, low B reduces male fertility primarily by impairing micro-sporogenesis and pollen tube growth (Dell *et al*., 1997). Dear and Lipsett (1987) reported that, soil application of B increased the biomass and seed quality of legumes (clover). The significant effect of B was observed in seed yield of legume crops i.e. in soybean 50%, peanut 45% and black gram 93% (Rerksem *et al*., 1997). In peanut application of 300g boric acid /ha at vegetative growth stage that increased fresh pod yield by 31.7% (Ayo *et al*., 1998).

Sillanappa (1982) reported that B deficiency occurs in almost every country but deficiency was greater in several Asian countries like India, Korea, Nepal, Philippines and Thailand and also in Africa particularly Malawi, Nigeria, Sierra Leone and Zambia. In Nepal most

[1] Senior Technical Officer, National Citrus Research Program, Dhankuta; E-mail: rals_135@yahoo.com
[2] Associate Professor, Department of Horticulture, Collage of Agriculture, UPLB, Laguna, Philippines

legume crops are affected by B deficiency symptoms in vegetative growth stage (reduced apical growth) and reproductive stage (failure of fertilization and seed setting).

Bush snap bean is a short duration crop and can be grown four seasons in a year (winter, spring, summer and autumn) in Nepal. Its cultivation range is wide from Terai (100m) to Higher Hills (1600m). Most of the soils in this range are sandy and calcareous which are lacks of B. Seeds produced from these soils have poor in quality. There is serious problem of seed germination and abnormal seedling under field condition. Higher rate of seed germination is required to maintained optimal plant population. Therefore, there is high demand of bean seed in all of the seasons in Nepal. Most of the seeds are imported and millions of dollars are drained out every year. Imported seeds are more expensive and not always of higher quality. Application of B at the critical stage is more effective to improve the quality of seed and maximize the yield.

Bush snap bean yield is prolific when grown under hydroponics. There was difficulty in getting soil without B to study the effect of B in bush snap bean. So that hydroponics techniques is one of the best methods to study the effect of micronutrient for this crop.

The general objective of the study was to determine the effect of boron application in reproductive growth of bush snap bean. Specifically, the study aimed to determine the critical stage of boron application that improved the seed yield and quality of bush snap bean.

MATERIALS AND METHODS

The commercial variety of bush bean Contender was used in this experiment. The experiment was conducted in the screen house of the Vegetable Crop Division, University of the Philippines, Los Baños, Laguna from June to September 2002 with five treatments in four replications. Analysis of boron in the leaf and seed tissue was done in the chemistry laboratory of Institute of Plant Breeding (IPB). Three-hundred milligram of leaf sample ashed at 550^0C temperature overnight was dissolved in 5ml 0.10 N HCl, filtered and washed with 20ml distilled water in volumetric flask. One milliliter of the aliquot placed in evaporating dish with 4ml cur cumin-oxalic acid solution was evaporated to complete dryness in hot water bath at 55^0C, and was baked for 15 minutes. Then 25ml ethanol (95%) was added and absorbance readings were taken using automatic absorption Spectrophotometer (Model GBC-908). The amount of boron (B) concentration in the seed tissue was determined 14mg kg^{-1} of dry weight before seed sowing.

Plants were grown in the nutrient solution in Styrofoam box (50cm length x 40cm width x 17cm height). All the nutrient solutions were prepared without boron except T_1. Boron at the rate of 0.05g boric acid /l (50ppm) was added in the nutrient solution boxes at weekly intervals up to the seed physiological maturity stage. The treatments were: T_1=start B application from 0 day, T_2=start B application from 21 DAS or 5 times, T_3=start B application from 28 DAS (4 times), T_4=start B application from 35 DAS (3 times), T_5=start B application 42 DAS (2 times) and control without B (-B) in the nutrient solution i.e. included only for comparison.

Preparation of the Solution

The nutrient solution developed by John Mason (1990) for bush bean was used in this experiment. The required amount of reagents was calculated for 400*l* of solutions at initial stage. The required amount of reagents are; monoamonium phosphate 59.2g, potassium nitrate 386.2g, calcium nitrate 400.0g, ammonium sulfate 169.7g, magnesium sulfate

140.0g, iron sulfate 6.0g, copper sulfate 0.077g, zinc sulfate 0.087g, ammonium molybdate 0.037g and manganese sulfate 0.080g. All the chemicals were weighed individually and the required amount of chemicals were mixed in double distilled water in a plastic container and stirred thoroughly until fully dissolved. Micronutrients were added after the major chemicals are fully dissolved and pH was adjusted to 5.5 to 6. Nutrient solutions were placed in the Styrofoam boxes at the rate of 12*l* per box and boric acid 0.60g was added per box according to the treatment wise.

Two seeds were sown in the Styrofoam cup (6.5cm diameter x 9cm height) with coconut coir dust as the medium. The cups were placed in the nutrient solution box with only the base of the cups touching in solution. One healthy plant was maintained per cup and maintained four healthy plants per box or twenty plants per replication. The parameters taken for analysis were; days to 50% flowering, number of flower, pod, pod set efficiency, pod size, number of seed, 100 seed weight, seed B, normal seedling, seedling growth rate, and seed germination. Germination test was done in the laboratory condition and field (soil) condition. Seedling vigor was determined by accelerated ageing test (42^0C temperature, 100% RH for 72 hour) and seeds were subjected to germination in laboratory and field. Seed vigor was evaluated by rating of seedling growth in 3rd and 5th days after sowing in both lab and field condition. Seedlings were categorized in normal (strong >0.5g) medium (0.25-.05g) and abnormal (weak <0.25g) on the basis of weight and development of plumule and primary root. Vigor percent were evaluated from normal seedling under both conditions. Dry weight was taken by placing the seedling (with root) in electric oven at 85^0C temperatures for 24 hour.

The experiment was conducted in Complete Randomized Design (CRD) with four replications and five treatments. Data gathered on all the parameters were analyzed using Analysis of Variance (ANOVA) in the Statistical Analysis System (SAS) Software Package Program (6.12). Treatment means were compared with LSD (least significant different) values at 5 percent level of significance

RESULTS AND DISCUSSIONS

Reproductive Growth Stage

Flowering days and number of flowers

Effect of B on 50% flowering was significant between the 0 DAS and -B treatments. No significant effect of B was observed between the 28 DAS, 35 DAS and -B treatment (Table 1). However the results show that earlier application of B resulted in earlier flower opening of bush snap bean.

The effect of B on number of flowers was varies between the treatments. The number of flowers was two times greater at 0 day (19 plant^{-1}) than at 42 DAS treatment (9 plant^{-1}) and 3.1 times greater in 0 day treatment as compared to -B. Decreasing flower number was observed as times of B application decreased. The results shows that increase the time of B application that increased number of flowers. Singh *et al*. (1984) reported that, in leguminous crops 52-76% of flowers are drops, caused by B deficiency.

Number of pods and pod setting

The number of pod decreased as the time of B application was decreased. There was no effect of B on the number of pods between the 0, 21 and 28 DAS treatments but significant effects was observed during 35 and 42 DAS treatments (Table 1). Higher number of pod sets was observed at 0 days B application (8 plant^{-1}) and lowest number of pod sets (4 plant^{-1}) at 42 DAS treatments. Pod formation was 8 times greater in 0 day treatment as compared to -B treatment. Therefore, B application is more effective when applied during the vegetative stage rather than at the reproductive stage for higher pod setting. Zhang *et al.* (1994) reported that requirement of B is higher for the reproductive part (flower, anther and pistil) development than leaves and shoots.

Non significant effect of B was observed in the percent of pod setting on all treatments but higher percentage of pod sets as compared to –B treatment. It was not affected by the stage of the plant when B application was started. According with Gardener *et al*. (1985) failure of pod set in the plants would be due to flower abscission i.e. lack of pollination, lack of fertilization and abruption of flower.

Pod size and number of seeds

The effect of B on pod length was not significant between 0, 21 and 35 DAS treatment but significant with -B treatments (Table 1). The higher pod length (14.5cm) was observed in 0 day B treatment and lowest pod length (12.8cm) was observed at 28, 42 DAS and the control. In general there was no effect of B in pod size (length and width) at different days of application.

Effect of B on the number of seeds was significant in 0, 21 and 35, 42 DAS but not in -B treatments (Table 1). The number of seeds was 2.5 times greater at 0 day treatment as compared with -B. This result shows that application of B at earlier stage is more effective for number of pods and seed setting in bush snap beans.

Seed weight and boron concentration in seed tissue

The effect of B was not significant in seed weight between the 0 and 21 DAS treatments (Table 1). Highest seed weight (66.5g) was observed at 0 day and decreased 4.3% at 28 DAS, 5.2% at 35 DAS and 17% at 42 DAS and 19% at –B as compared with 0 day B treatment. Thus increase the times of B application that increases the seed weight of bush bean. An earlier application of B was effective to increase seed weight, which indicates that the seeds would be better in quality. Schoon and Voyest , (1990) also reported that B increased the seed weight of legume crops under applied through the leaves during flowering time. Most of the legumes produced abnormal seeds in B deficient condition.

Table 1: Reproductive growth of bush snap bean (*Contender) with* B applications

Growth parameters	Treatments (DAS)[1]						LSD (<0.05)
	0 (8)	21 (5)	28 (4)	35 (3)	42 (2)	Control (-B)	
Number of flowers plant $^{-1}$	19	15	15	10	9	6	2.8
Days to 50% flowering	28	29	30	30	31	30	1.8
Number of pods plant^{-1}	8	6	6	4	4	1	2.2
Percent of pod set	42.1	40.0	40.0	40.0	41.4	16.6	NS
Pod length (cm)	14.5	13.8	12.8	13.4	12.8	12.8	1.3
Pod width (cm)	1.2	1.2	1.2	1.2	1.2	1.1	NS
Number of seeds pod^{-1}	5	5	4	3	3	2	1.1
100 seed weight (g) [2]	66.5	65.3	63.6	63.1	55.2	54	2.8
Seed boron concentration (mg kg^{-1} dry weight)	6.4	4.3	4.2	4.1	3.4	0.3	0.2

[1]Numbers in parentheses refers to the number of B application, **[2]** Measured at 14% seed moisture level

Keerti-Lasikorn *et al*. (1991) reported that increases in pod and seed size of legume crop were depends on the xylem delivery of B. Those seeds that can acquire sufficient B can accumulate sufficient amount of assimilates and have bigger size, which is correlated with seedling growth. An increase in the seed size resulted to an increase in germination and seedling vigor.

Concentration of seed boron was higher at 0 day of application as compared to the other treatments (Table 1). The amount of seed B decreased when the times of B application decreased. B was more effective to increase the amount of seed B when it was applied from 0 day. A small amount of B was observed in the seed tissue (0.3mg kg^{-1} dry weight) in –B treatment. That could have the results of existing seed (14mg B kg^{-1} dry weight) before sowing. Therefore, the plant does not show the deficiency symptoms of B in vegetative stage, but there is greatly affect in the reproductive growth i.e. reduced in flower, pod, seed, seed weight and seed B, due to the in sufficient amount of B in the nutrient solution.

Seed quality

Boron deficiency affects mainly the cotyledons of legume seed. However, in severe cases, it affects the plumule growth. After harvest, the quality of seeds was evaluated by accelerated aging test (AAT) and subjected the seeds for germination test and seedling growth under laboratory and field (soil) conditions.

Seed germination

Signs of seed germination (radical opening) were observed under laboratory condition at two days after sowing in 0 day and 21 DAS treatment. The higher percentage of seed germination (99.25%) was observed at 0 day and lowest percentage of germination (95.75%) was observed at 42 DAS (Table 2). There was no effect of B between the 0 day and 21 DAS treatments but significant difference was observed with 35 and 42 DAS treatments. The percentage of seed germination was reduced at the times of B application decreased. Higher concentration of B inside the seed would be high vigour and result in higher percentage germination.

Higher percentage of seed germination (98.25%) at 0 day and 21 DAS treatment and lowest at 42 DAS (68.5%) treatments were observed under field condition (Table 2). Germination % of harvested seeds was 1.2 times greater under laboratory condition and 1.4 times greater under field condition in 0 day treatment as compare –B. Under laboratory condition low vigour seeds were germinated well but under field condition low vigour seed could not germinated well. The critical stage of B application for germination and seedling emergence (visible of cotyledon leaf on the soil surface) was observed at 28 DAS.

Normal seedling

Percentage of normal seedlings was higher at 0 day treatment seed (81%) as compared to the 42 DAS treated seed (15.2%) under field condition (Table 2). There was no effect of B between 0 and 21 DAS treatment in terms of normal seedling growth. Variation of normal seedling was observed 10.5% at 28 DAS, 33.3% at 35 DAS and 65.8% at 42 DAS treatment as compared with 0 day. Normal seedling was 8 times greater under laboratory condition and 6.4 times greater under field condition in 0 day treatment compare with -B. It means decrease seedling vigor if B applied delay. The seedling vigour under laboratory condition was highest at 0 day treatment (91.5%) and lowest in 42 DAS treatment (56.75%) i.e. 37% greater strength of seedling in 0 days B treated seed as compared to 42 DAS.

Seedling vigor under laboratory condition was higher than field condition. Good quality seeds had higher emergence and produced strong seedlings under field condition. Bell *et* *al.* (1989) reported that B deficient seed shows normal in size but are weak physiologically. In this study earlier application of B was more effective to improve the seed quality.

Seedling growth

The growth of seedling was evaluated 8 days after seed sowing. Compared with 0 day application, shoot length of normal seedling were decreased 32% in 42 DAS (Table 2). The difference in shoot length was 2.3cm at 35 DAS and 4.5cm at 42 DAS as compared to 0-day treatment. Root length was not significantly different between 0 day and 21 DAS B treated seeds. The variation of root length was 2.5cm, 3.5cm and 6.0cm at 28, 35 and 42 DAS treatment respectively, as compared with 0-day treatment. The differences were 3 times greater at 0-day B treatment as compared to 42 DAS. As time of B application was decreased there was a corresponding decrease in root length.

The total dry matter of the seedlings was not significant between the 0 day treatment and 21 DAS treatment. The differences of seedling dry weight were 0.45g at 28 DAS, 0.87g at 35 DAS and 1.35g at 42 DAS. Compared with 0-day there was an increase in seedling dry weight at later application of B. It means seedling dry matter increased with earlier B application in the nutrient solution. Seedlings dry weight also 2.17 times greater in 0-day B treatment compare with -B. These results indicated that seed B is essential for seedling growth and development.

Vigorous seeds could result to better seedling growth and higher dry weight under field conditions. Vigorous seeds may store sufficient food materials and supply for seedling growth after germination. In this experiment earlier B applied seed was found more vigorous and have better seedling growth. Therefore application of B is essential to improve the seed quality and vigorous seedling production of bush snap bean if B is lacking in the soil.

Table 2: After harvest seed quality and seedling growth of bush snap bean (*Contender*)

Seed vigor parameters		Treatments (DAS) [1] 0 (8)	21 (5)	28 (4)	35 (3)	42 (2)	Control	LSD (<0.05)
Germination (%)[2]	- Field (soil) condition	98.2	98.2	84.7	77.5	68.5	66.6	2.8
	- Laboratory condition	99.2	99.0	97.2	95.7	95.7	80.0	1.9
Normal seedling %	- Laboratory condition	91.5	90.2	78.0	70.7	56.7	14.2	4.2
	- Soil (field) condition	81.0	80.2	70.5	44.7	15.2	10.0	3.7
Seedling growth rate[3]	- Shoot length (cm)	14.0	13.7	12.0	11.7	9.5	11.3	2.8
	- Root length (cm)	18.2	17.7	15.7	14.7	12.2	9.5	2.2
	- Dry weight (g $plant^{-1}$)	3.7	3.7	3.2	2.8	2.3	1.7	0.3

[1]Number in parentheses refers to the number of B application, **[2]**Total No. of seed germinated/ Total No. of seed placed x 100, [3] eight days after sowing under field condition.

CONCLUSIONS AND SUGGESTIONS

Based on the above results, positive effect B on reproductive growth and seed quality improvement was observed. The quality and quantity of pod and seed could improve by the application of B at critical stage. Seed quality was poorer when the B application was later. Without B, reproductive growth is inhibited and seed quality also reduced drastically. The critical stage of B requirement for reproductive growth and seed quality improvement was observed to be at 28 DAS. If B not applied at 28 DAS the quality of seed decreased rapidly. This study proved that B is essential for seed development and application of B in earlier growth stage is more effective.

Findings of this study would be highly applicable to commercial growers to increase the yield and seed enterpriser to improve the seed quality and yield. It would also guide to scientists for further research. Research on the appropriate rates of B application for bush snap bean is still needed. In field grown plants, foliar application of B at different stages of application can also be tried.

ACKNOWLEDGEMENT

The author would like to thank Dr. Ofelia K Bautista, Dr. P.J.A. Santos and Dr. Elvira S. Talatayod for their valuable comments and suggestions and Mr. Felicito M. Rodriguez for boron analysis.

LITERATURE CITED

Ayo, E., R. Ulibas and S. Arai, 1998. Micronutrient management for crops in Ultisol. Soils and Fertilizers, Technical reports of soils research and development center (phase II), pp.141-151. BSWM, Quezon City.

Basra, S. Amarjit, 1995. Seed quality, basic mechanism and agricultural implication. Food Product Press.

Bell, R. W., L. Mmelay, D. Plaskatt, B. Dell and J. F. Loneragon, 1990. Germination and vigor of black gram (*Vigna mungo* L) seed form plant grown with and without boron. Aust. J. Agri. Res., 40:273-279.

Dear, B. S. and J. Lipsett, 1987. The effect of boron supply on the growth and seed production of subterranean clover (*Trifolium subterraneum* L.). Aust. J. Agric. Research, 38:537-547.

Dell R. and L. Huang, 1997. Physiological response of plant for low Boron (B), Plant and Soil, 193:103-120.

Gardner, F. P., R. B. Pearce and R.L. Mitchell, 1985. Physiology of Crop Plants. Ames. Iowa State University Press.

Haung, L., Z. Ye and R. Bell, 1996. The importance of sampling immature leaves for diagnosis of boron deficiency in oil seed rape (*Brassica napus* cv. Eureks). Plant and Soil, 183:187-198.

Keerti-Lasikorn, P., R.W. Bell and J. F. Loneragon, 1991. Response of two peanut (*Arachus hypogea*) cultivars to B and Ca. Plant and Soil, 138:61-66.

Mason. J., 1990. Directory of Vegetable Crops. Commercial Hydroponics, pp.112-115.

Powell, A. A., M. A. De Oliveria and S. Matthews, 1984. Seed quality in grain legumes. Advance in Applied Biology, 10:217-285.

Rerksem, B. R., W. Bell, S. Lordkaew and J. F. Loneragan, 1997. Relationship of seed boron concentration to germination and growth of soybean (*Glycine max L* Merr.). Nutr. Cycling in Agrosystem.

Schoon, A., V. Hovel and O. Voysest, 1990. Common bean research for crop improvement CIAT, pp.199-286. Cali, Colombia.

Sillanapaa, M., 1982. Micronutrient and nutrient status of soils: a global study. FAO Soil Bullitin No. 48, pp. 44. FAO, Rome.

Singh, V., and S. P., Singh, 1984. Study on boron toxicity in lentil and barley. Indian J. agron., 29(4):545-546.

Zhang X., Z. Shen and K. Shen, 1994. The effect of boron on the development of floral organ and seed yield of rape. Acta Pedologica Sinica, 31:146-151.