GROWTH, FLOWERING AND YIELD OF *PHASEOLUS VULGARIS* L. AS INFLUENCED BY MICRONUTRIENT BORON

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ABSTRACT

The reason of low productivity in pulses appears to be primarily due to water stress, poor pod setting irrespective of large number of flower buds and flowers produced. The flower and pod drop are the major problem in pulses, in general, and French bean, in particular. An effort was made to investigate the basal and foliage applied boron (B) on growth, flowering and yield attributes of French bean (*Phaseolus vulgaris* L.). Boron in the form of boric acid, respectively, were applied in two split doses (half basal at the time of sowing and the rest of the half as foliar spray at the pre-flowering stage} to provide instant supply of nutrients. Three levels of B (i.e., 0.0, 2.0 and 3.0 mg kg $^{-1}$ soil) were taken. Different physio-morphological parameters including nitrate reductase activity and N_2-fixation showed an obvious improvement by B @ 2.0 mg kg^{-1} soil, respectively. In addition, decreased flower abortion and pod-drop out of large number of flowers produced were noted. As a result of this, improvements in different yield attributes were recorded. The lower B concentration, i.e., @ 2.0 mg kg^{-1} soil was found to be most suitable combination for this particular crop in the present agro-climatic conditions.

No: of Tables : 06 **No: of References : 43**

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) occupies a position amongst grain legume crops due to its ability for the production of high quality protein. The reason primarily is poor pod setting irrespective of large number of flower buds and flowers. The flower and pod drop is a major problem in some of the grain legume crops. So far limitation of photosynthesis, nitrogen availability, reduced light intensity in crop canopies, canopy temperature, gas exchange and humidity in canopies and hormonal/ soil/ water factors have been assigned to have their one or other role in affecting the link potential of plant in general. Boron (B) nutrient is essential for crop growth, development, production, and seed quality when applied on foliage too (Bellaloui *et al.,* 2013). Boron deficiency in soil due to biotic or abiotic stress factors results in yield loss and poor seed quality. Legume crops have unusual ability to be self sufficient in nitrogen supply. Biological nitrogen fixation by symbiotic associations of legume plant with microorganisms is economically sounder and environmentally more acceptable than use of nitrogen fertilizer in agriculture. Nutritional constraints on root nodule bacteria affects symbiotic nitrogen fixation (O'Hara, 2001). Not much work has been carried out on trace element nutrition with special reference to boron on grain legume crops as per our theme. Boron distribution in nitrogen-fixing pea plants were reported by several workers. Yamagishi and Yamamoto (1994) reported strong alterations in N_2 fixation in soybean plants with a low B supply. Bolanos *et al.* (1996) made a study of the boron effect on Rhizobium-legume cell-surface interaction and nodule development in pea. In boron-deficient plants, the number of Rhizobia infecting the host cells and the number of infection threads were reduced and the infection threads developed morphological aberrations. The cell walls of root nodules of boron-deficient plants showing structural aberrations are reported to lack the covalently bound hydroxyproline/proline rich proteins (Bonilla *et al.,* 1997), which contribute to an O_2, barrier, preventing inactivation of nitrogenase and associated decrease in nitrogen fixation. The present investigation reports the effect of individual application of B on nitrate reductase activity in leaves, total nitrogen and its uptake, number of flowers and pods dropped per plant, and yield attributes of French bean.

MATERIALS AND METHODS

Field studies were conducted in Agricultural Research Farm, Banaras Hindu University, Varanasi, in two successive years on a sandy loam soil. Soil test values, prior to the establishment of experiments are given in Table 1. Seeds of French bean (*Phaseolus vulgaris* L.) Var. PK- 327 treated with thiram @ 4.58 g. kg^{-1} seed inoculated with *Rhizobium phaseoli* @ 0.5 kg ha^{-1} seed rate were sown during *kharif* (season). Application of boron as boric acid @ 2.0 and 3.0 mg kg^{-1} soil were made with two modes of application (basal and foliar) in order to obtain a timely and adequate amount of this micronutrient. The plants were treated in two split doses. The first half of the dose of B was applied at the time of sowing as basal application and the rest half of the dose was applied as foliar spray application at the pre-flowering stage [i.e., 20 days after emergence (DAS)]. Basal application of N: P: K: S @ 40: 60: 40:20 kg ha^{-1} as N, P_2O_5, K_2O, and S respectively was made uniformly in all plots irrespective of B application. A control lot of plants were also maintained where no additional supply of B was made. To confirm the same, pot experiments were also carried out not only for two but for three years. However, depending more on field experimental data, the pot experiments data have been not included, which were, of course much improved under the controlled conditions than the field experiments.

Plant Tissue Analysis: The uppermost mature leaves (fourth leaf from the top) were taken from 15 to 20 plants of each plot at 30, 60 and 90 DAS. The samples were dried at 70 $\pm$ 1° C and ground to pass through a 2 mm sieve. Nitrogen was estimated by using the modified micro- kjeldahl method as described by Lang (1958).***Assay of nitrate reductase (NR) activity in vivo in leaves:*** The NR activitiy was measured in fresh leaves at 30, 60 and 90 days after sowing (DAS). The third leaf from the top was taken for use in the assay of enzymatic activities. The third leaf from the top represents both the mature and young leaves with respect to growth and age. Moreover, it was also convenient to keep sample of leaf position on the plant constant throughout the study.NR activity in leaf samples was determined by *in vivo* method as described by Nicholas *et al.* (1976). ***Flower and Pod Drop***: Observations pertaining to number of flowers dropped per plant were made at 60, 70, 90 and 105 DAS because flower drop begins at 60 DAS and continues up to 105 DAS, since thereafter no flowers on plants and plants were senescing. Number of pods dropped per plant were recorded at 75, 90, 105 DAS and finally at harvest, because flower drop begins at 75 DAS and continues till harvest.
Yield Attributes: Yield attributes, *viz.*, length of pods (cm), number of seeds per pod, test weight of 100 seeds (g), seed weight per plant (g) were recorded at harvest.

Statistical Analysis: The experimental design was a randomized complete block with three replications. The results were statistically processed for calculating Least Significant Difference (LSD) at P = 0.05 (Gomez and Gomez, 1984). LSD value written as NS indicates statistically non-significant effect of treatments.

RESULTS AND DISCUSSION

In general, plants treated with B showed better growth and development at various growth stages when compared with untreated control plants. However due to evasion of bulk of data regarding the growth attributes of plants, only tables on effects of these micronutrients studied on compositional determination, enzyme activity, flowering, pod-setting at different growth stages and important yield attributes at harvest have been given to understand the physiological significance of B in a particular soil status (Tables 1-6).Determination of total leaf nitrogen of French bean (*Phaseolus vulgaris* L.) revealed an increase by the application of B (Table 2) up to 60-day stage. A decrease at 90-day stage well indicates the greater utilization of leaf nitrogen during the seed development stage than the control. B at the threshold individual levels of 2.0 mg kg^{-1} significantly increased NR activity in leaves (Tables 3), that indicates the involvement of B in the activity of this enzyme either directly or indirectly. Maximum activity recorded in coincidence with the pod setting stage, a crucial phase for improving yield of this proteinaceous crop implicitly indicates the utility of B for nitrogen metabolism. Previously, foliar spray of B to B-deficient sorghum at reproductive stage increased the nitrogen content and NR activity (Misra *et al.*, 1991) as found in the present study by splitting the dose. In B-deficient sugar beet and tomato plants a decrease in the NR activity (Carpena *et al.*, 1978; Bonilla *et al.*, 1980; Ramon *et al.*, 1989) is in conformity with the present findings. A decline in nitrogen content at higher level of B fertilization was noted which might be due to decreased nitrate nitrogen (Starovoitova, 1983) or total nitrogen (Dami *et al.*, 1970).

TABLE 1. Soil Test Values Prior to the Study

Sl. No.	Soil Properties	Amount Present		Method	References
		First Year	Second Year		
1.	Soil Extractable Nutrients (mg kg^{-1} soil)				
(a)	Nitrogen	84.50 Permanganate	88.50 (1956)	Alkaline	Subbiah and Asifa
(b)	Phosphorous	11.00 extractable	10.75	0.5 N $NaHCO_3$	Oslen *et al.* (1954)
(c)	Potassium	104.00	102.00	Ammonium acetate extractable flame photometer	Jackson (1973)
(d)	Sulfur	8.50	8.25 (1950)	Turbidity	Chesnin and Yien
(e)	Boron	0.89	0.80	Hot water soluble boron	Sippola and Ervco (1977)
(f)	Zinc	1.20	1.00	DTPA	Lindsay and Norvell (1978)
2.	Soil pH (1: 2.5 soil water ratio)	7.48	7.50	Glass electrode pH meter	Jackson (1973)
3.	Electrical Conductivity d Sm^{-2} (1:2.5 soil water ratio)	0.15	0.16	Systronies electrical conductivity meter	Jackson (1973)

TABLE 2. Effect of Boron (B) on Nitrogen Content (per cent) in Leaves of French bean (*Phaseolus vulgaris* L.) at Different Growth Stages

Treatments B (mg kg^{-1} soil)	First Year Age of plants (DAS)				Second Year Age of plants (DAS)			
	30	60	90	At harvest	30	60	90	At harvest
0.0	3.35	3.74	2.79	2.35	3.28	3.65	2.72	2.28
2.0	3.38	3.79	2.85	2.41	3.30	3.70	2.82	2.36
3.0	3.30	3.71	2.76	2.33	3.25	3.63	2.70	2.25
LSD (P=0.05)	0.024	0.036	0.025	0.019	0.021	0.019	0.019	0.022

TABLE 3. Effect of Boron (B) on Nitrate Reductase (NR) Activity (μ mol.NO_2.hr^{-1}.g^{-1}) in Fresh Leaves of French bean (*Phaseolus vulgaris* L.) at different growth stages

Treatments B (mg kg^{-1} soil)	First Year Age of plants (DAS)			Second Year Age of plants (DAS)		
	30	60	90	30	60	90
0.0	1.620	3.090	1.610	1.690	3.190	1.750
2.0	2.810	3.506	1.790	1.870	3.430	1.910
3.0	1.200	2.560	1.070	1.270	2.660	1.200
LSD (P=0.05)	0.020	0.144	0.030	0.034	0.011	0.030

TABLE 4. Effect of Boron (B) on Number of Flowers Dropped Plant^{-1} in French bean (*Phaseolus vulgaris* L.) at Different Growth Stages

Treatments B (mg kg^{-1} soil)	First Year Age of plants (DAS)				Second Year Age of plants (DAS)			
	60	75	90	105	60	75	90	105
0.0	27.0	24.0	23.0	19.0	29.0	21.0	20.0	17.0
2.0	15.0	12.0	4.0	2.0	16.0	13.0	4.0	2.0
3.0	14.0	11.0	3.0	2.0	13.0	10.0	2.0	2.0
LSD (P=0.05)	1.22	1.31	1.11	1.45	1.30	1.29	1.16	1.37

TABLE 5. Effect of Boron (B) on Number of Pods Dropped Plant^{-1} in French bean (*Phaseolus vulgaris* L.) at Different Growth Stages

Treatments B (mg kg^{-1} soil)	First Year Age of plants (DAS)				Second Year Age of plants (DAS)			
	75	90	105	At harvest	75	90	105	At harvest
0.0	9.00	7.00	4.50	3.00	9.50	7.50	5.00	3.00
2.0	4.00	1.25	0.50	0.50	4.50	1.00	0.50	0.50
3.0	3.00	1.00	0.50	0.50	3.00	1.00	0.50	0.50
LSD (P=0.05)	0.34	0.44	0.36	0.33	0.33	0.44	0.34	0.33

TABLE 6. Effect of Boron (B) on Yield and Yield Attributes of French bean (*Phaseolus vulgaris* L.)

Treatments		First Year				Second Year			
B ($mg\ kg^{-1}$ soil)		Length of Pods (cm)	Number of Seeds Pod^{-1}	Seed Weight $Plant^{-1}$ (g)	Test Weight of 100 Seeds (g)	Length of Pods (cm)	Number of Seeds Pod^{-1}	Seed Weight $Plant^{-1}$ (g)	Test Weight of 100 Seeds (g)
0.0	0.0	7.30	5.80	9.90	14.71	7.33	5.68	10.35	14.50
2.0	0.0	7.00	5.00	12.10	14.90	7.44	7.00	13.05	14.80
3.0	0.0	7.90	5.00	11.70	15.10	7.64	7.50	12.15	15.00
LSD (P=0.05)		0.29	0.42	0.16	0.11	0.28	0.44	0.21	0.12

Relatively high rates of flower and pod abortion are common phenomena in French bean. Plants treated with B showed greater pod setting in plants in comparison to B. In the present experiment also there was a significant decrease in flower and pod drop (Table 4 and 5) by application of B. In *Larix decidua* Mill, suspensor development was found to be dependent on boron concentration, below 35 mM boron, suspensor formation was blocked completely, whereas with an increase upto 1 mM B there is progressively faster development of suspensor bearing embryos (Ulrike and Zoglauer, 1996). In this regard, Jacob *et al.* (1972) reported preponderance of female flowers reduced fruit setting and parathenocrapy in B deficient *Cola nitida*. The pod and seed development is more sensitive to deficiency of B than the development of flowers (Bell *et al.*, 1990). In recent years, inductively coupled plasma-mass spectrometry was used to show that foliar applied B, including ^{10}B, is translocated from leaves of fruit and nut trees (Hanson, 1991; Brown *et al.*, 1992; Shu *et al.*, 1994), and of broccoli (Shelp *et al.*, 1995). As reported by Shelp *et al.* (1995) in this regard, after 14 days, the contribution of B acquired after inflorescence emergence was always higher (66-99%) than B acquired before inflorescence emergence. Boron acquired after inflorescence emergence was transiently accumulated in the stem petioles and middle and upper portions of the plant canopy. The healthy development of pods through B application improved yield attributes (Table 6). Lopez- Andreu *et al.* (1988) reported that B deficiency reduced the number of fruits per plant in tomato. Application of B increased the fruit weight, sugar accumulation, seed setting and vitamin content in fruits (Badawi *et al.*, 1981; Zaki *et al.*, 1981). The foliar spray of B at the end of flowering increases the 100 seed weight per plant in French bean

(*Phaseolus vulgaris* L.). Similar evidences were valid in case of soybean (Buzetti *et al.*, 1990) and *Onobrychis arenaria* (Radeva *et al.*, 1991). Its application produced significantly more grain yield per hectare as compared to other micronutrients (Dhillon *et al.*, 1969). Evidences support the present research and elucidates the significance of foliar applied B, as B is involved in nitrogen fixation (Balanos *et al.,* 1996), nodules, nodulin protein (ENOD2) in nodule parenchyma cells and malfunction of oxygen diffusion barrier (Bonilla *et al.,* 1994), B in carbohydrates metabolism (Marschner, 1995) especially with sugar alcohols (Bellaloui *et al.,* 1999), phenolic metabolism (Marschner, 1995), ion uptake (Marschner, 1995), plasma membrane-bound H^+ATPase (Camacho-Cristóbal, 2008) and cell wall structure and membrane integrity (Marschner, 1995), seed protein, oil, fatty acids, and sugars (Bellaloui *et al.,* 2013). Foliar B application to soybean has been previously reported (Ross *et al.,* 2006) but there is no clear evidence that B directly affects nitrogen metabolism (Bonilla *et al.,* 1997) or seed protein, oil, fatty acids, and sugars (Bellaloui *et al.,* 2013).On the basis of overall findings, as discussed in the preceding pages, it may also be assumed that B helps better growth of bacteroids in root nodules along with other micronutrients in soil indispensable for nodulation, nitrogenise constitution and nitrogen fixation. Through the chemical analyses of experimental soil presented in Table 1, it is apparent that B contents are below the required level for the growth of pulse crop, in general. The same may be limiting to provide a congenial environment to the N_2-fixing microbial partner inside the root nodules, which requires the supply of micronutrients, including B for their optimal activities of N_2-fixation and release of requisite growth promoting substances. This micronutrient helps a higher rate of N_2-fixation not especially because of their direct role in N_2-fixation but because of their possible role in producing a sufficient bacteroid mass. Consequently, the total fixed N output expected to be more by the increased bacterial population and this may be one of the good reasons why the total N content of plant is significantly enhanced. Similarly requisite supply of micronutrients including B has a positive impact on the activity of nitrate reductase enzyme system, which collectively adds to the assimilable N-status of the plant.To confirm and to understand the intricacies, the same investigations were also done through pot experiments not only for two but for the three consecutive years. However, depending more on field data, the much improved pot experimental data under the controlled conditions have been not included. The related work is under progress.

CONCLUSIONS

Physiological significance of boron appears to be obvious and there are no doubts about their role in the stimulation of better growth, nutrient uptake, flowering, pod setting and yield. The flower and pod drop in French bean (*Phaseolus vulgaris* L.) might be due to nutrient imbalance particularly inadequate supply of B because application of B significantly reduced the flower and pod drop, which is a bottleneck in pulse production particularly in this legume crop.

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