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TOLERANCE AND TOXICITY LEVELS OF BORON IN MUNG BEAN (*VIGNA RADIATA* (L.) WILCZEK) CULTIVARS AT EARLY GROWTH STAGES

AMMARAH HASNAIN, SEEMA MAHMOOD, SAEED AKHTAR[2], SAEED A. MALIK[*] AND NAHIDAH BASHIR

Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.
[2]Department of Food Science & Technology, University College of Agriculture, Bahauddin Zakariya University Multan, Pakistan

Abstract

Boron (B) toxicity has been recognized as a serious problem in arid and semi arid regions of the world. This study was aimed to determine critical levels of B by studying phenotypic variation for B-tolerance/ toxicity at the germination and seedling stage in three mung bean (*Vigna radiata*) cultivars; M-6, M-8 & 96009. Boron levels ranging from 0-20 ppm were applied using Boric acid. Germination, growth and photosynthetic attributes were significantly ($p<0.001$) influenced by varying B levels. However, the cultivars were significantly invariable for germination, seedling height and leaf number. B levels (5-10 ppm) appeared to be nutritionally critical whereas, 15-20 ppm induced B toxicity. The toxicity was expressed in terms of reduction in plant's growth as well as by visible symptoms which included chlorosis and necrosis of the foliage. The present study also demonstrated variation in B tolerance at the seedling stage in these cultivars. Among the tested cultivars, M-6 and M-8 exhibited better growth responses as compared with 96009. Fresh biomass and shoot: root ratio appeared to serve as selection criteria for B tolerance. The study further suggested screening of cultivars/ accessions on a large scale to explore more diversity of traits as well as the use of biochemical markers for mechanistic understanding of B tolerance.

Introduction

Boron (B) is found naturally in soils of arid and semi arid environments of the world and also originates through various anthropogenic activities. Irrigation water, wastes from surface mining, fly ash and industrial chemicals are important sources of boron (Nable *et al.*, 1997). Excessive Boron has been reported in soils of South Australia, India, Iraq, Pakistan, Peru and USA (Nable, 1992).

B is an essential micronutrient which influences major cellular functions and metabolic events in plants (El-Hamdaoui *et al.*, 2003). It is critically required by actively growing regions of the plants where cells rapidly divide and differentiate thus plays a crucial role in growth and development (Ali & Jarvis, 1988). However, excessive B can be injurious to plants and produces visible toxicity symptoms which in turn have subsequent impact on crop yields (Bennett, 1993). Since, B translocates in plants mainly through xylem thus accumulates more in the leaf tissues where it causes chlorosis and necrosis. This gives the foliage a scorched appearance and eventually results in complete senescence (Bergmann, 1992).

Mitigation of high boron from soils is extremely difficult through leaching. It can be leached out of the root zone only in higher rainfall areas or under excessive irrigations. However, in dry areas or in soils with impermeable sub-soil layers, B concentrations in the root zone can be high, making amelioration extremely difficult. Therefore, research has been focused on the exploration of variation for B tolerance in crop cultivars to facilitate the exploitation of tolerant germplasm under such soil conditions.

[*]Corresponding author E-mail: drseemapk@gmail.com

Variation in boron concentrations even in a fraction of one part per million can be injurious to plants (Marschner, 1995). Screening of a number of accessions or cultivars of different crop species revealed wide variation in B tolerance or toxicity which varies considerably with different growth stages of plants (Nable *et al.*, 1997). Successful germination and seedling establishment are of fundamental importance for overall crop growth and yield (Loomis & Durst, 1992). Therefore, identification of crop cultivars that are tolerant to excessive B at their early establishment phases may be of great implications because better initial growth can signify greater productive potential of a crop.

Pulse crops are an important component of profitable agriculture in Pakistan because a large population segment has to rely on pulses, being low priced source of protein (Usman *et al.*, 2007). The demand for pulses is increasing day by day because of the population growth. Thus, the maximum utilization of soil resources is inevitable to meet the increasing demand for food.

A number of pant species such as barley, wheat, lentil, medics and rice have been screened for phenotypic variability to B-toxicity or tolerance at their early growth stages (Eraslan *et al.*, 2007) but *Vigna radiata* (L.) Wilczek (mung bean) has not yet been addressed. Mung bean has a great value as food, owing to its quick cooking, digestibility and its anti- flatulent properties. It is also important as fodder and green manure and can considerably improve soil fertility through biological nitrogen fixation. Moreover, two growing seasons for these species in Pakistan and optimum temperature (25-32^{o}C) during spring and autumn make it a feasible experimental material.

The present study was conducted to assess variation in morphological and photosynthetic parameters at germination and initial growth of mung bean cultivars to determine their B tolerance/ toxicity.

Materials and Methods

Seeds of three cultivars of mung bean 96009, M-6 and M-8 were obtained from Pulse Crop Division, Ayub Agriculture Research Institute Faisalabad, Pakistan. Seeds of the test cultivars were surface sterilized in 5% solution of sodium hypochlorite for 5 minutes before the experiment to avoid fungal contamination. Seeds were then washed thoroughly with deionized water.1000 ppm B solution was prepared using H_3BO_3 (Merck, Germany).

Treatment solutions comprised of control (deionized water), 5, 10, 15 and 20 ppm boron and were made by carrying out appropriate dilutions of 1000 ppm solution. Forty-five glass Petri dishes (9 cm internal diameter) were washed, oven dried, labeled and filled with 50 g of thoroughly washed quartz sand.

Twenty seeds of each cultivar were sown into each of these Petri dishes. Each treatment was replicated three times for each cultivar and 20 ml of appropriate treatment solution was applied to each Petri dish. The experiment was arranged in a Complete Randomized manner in a growth chamber at 25^{o}C with 12 hours light /12 hours dark period, (illumination of 2500 lux, Philips T2 40W/33 lamp, U.K). The seeds were observed daily for germination. Fresh solutions were added after rinsing out the old ones. Data records were made for germination percentage and for embryonic root length.

Seedling growth was carried out in 45 earthen pots (20 cm in height and 15 cm internal diameter) filled with 1.5 kg of air-dried crushed and sieved (2mm sieve) sandy loam soil. The holes of pots were blocked completely with polythene to avoid leaching of the treatment solutions. Five seeds of each cultivar were sown equidistant into each of these pots. The experiment was arranged in a Complete Randomized manner and in order to simulate field conditions, it was conducted in a wire netting green house (temperature 25°C ± 5 and light 12 hours day length) at the Botanic Garden, Bahauddin Zakariya Universty, Multan, Pakistan during March, 2008. The seedlings established for two weeks were treated with solutions containing various boron concentrations. The plants were exposed to different concentrations of boron for 4 weeks. The plants were carefully removed after taking out whole soil from the pot. During the course of experiment, the water loss was compensated by gentle sprinkling using a spray gun and measurements were made for various growth attributes just after harvesting. Plant material was oven dried at 70°C for 48 hours for dry weights. The leaf samples were scanned using DESKSCAN software and the images obtained were analyzed using Delta-T-SCAN leaf image analysis software. Chlorophyll *a* and *b* was quantified using a spectrophotometer (U-2000 HITACHI, Japan) following Arnon (1949).

Statistical analysis: Data were subjected to two-way analysis of variance using MINITAB version 11.21. Multiple comparisons between means of boron levels and cultivars were carried out by Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

Data presented in Table 1 depicted a marked influence ($p<0.001$) of B on germination percentage but the responses of cultivars were found to be significantly invariable. Likewise, radicle length was also considerably ($p<0.001$) affected by various boron levels however, the cultivars showed no significant variability for this attribute (Table 1). It is evident from Fig. 1A that application of upto10 ppm boron had a stimulatory effect on germination but higher levels (15 and 20 ppm) had caused germination inhibition except in cultivar M-6. However, the extent of germination inhibition was greater in cultivar 96009 compared with M-8 at 20 ppm. The cultivars exhibited the maximum emergence of radicles at 10 ppm followed by a profound decline at 20 ppm B (Fig. 1B). Among the tested cultivars, M-6 depicted better embryonic root growth at all B levels except at 20 ppm where poor root elongation was observed.

A decline in seed germination percentage and embryonic root length at the elevated levels of boron as indicated in the present study are in lines with the findings of Yau & Saxena, 1997 who reported that permissible levels of boron are crucial for cell division but the cessation of cell growth is associated with exceeding levels of boron.

Various boron levels considerably influenced the seedling height ($p<0.05$) but the cultivars had shown consistent responses (Table 1). Though, seedlings grown for the control exhibited shorter height but lower levels of B (5 and 10 ppm) promoted seedling growth. Conversely, higher concentrations had caused an inhibitory effect as a drastic decline in seedling height occurred at the elevated B levels (Fig. 1C). It is now well established that plants grown under excessive boron sustain growth but do not differentiate into various organs such as roots, shoot, leaves and flowers (Ali & Jarvis, 1988). Thus, shorter seedlings at elevated B levels may imply an undifferentiated young stem.

Table 1. Summary of analysis of variance (Mean squares, significance and LSD) for various attributes in three mung bean cultivars after exposure to increasing levels of boron.

Attributes	Cultivars			Levels			Interaction	
	MS	Significance	LSD	MS	Significance	LSD	MS	Significance
Germination								
Germination percentage	274.79	n.s.	-	577.814	***	16.4	111.21	n.s.
Radilce length (mm)	22.480	n.s.	-	89.939	***	5.04	13.393	n.s.
Growth								
Seedling height (cm)	5.904	n.s.	-	79.877	*	8.5	34.889	n.s.
Fresh biomass/plant (g)	0.012	**	0.05	0.052	***	0.05	0.067	***
Dry biomass/plant (g)	0.008	*	0.006	0.007	*	0.08	0.007	*
Shoot/root biomass	22.121	***	2.006	3.327	n.s.	-	1.436	n.s.
Photosynthetic attributes								
Number of leaves	0.870	n.s	-	39.074	***	3.19	3.966	n.s.
Leaf area (cm^2)	0.213	*	0.62	1.979	*	0.91	0.403	n.s.
Chloropyhll *a* (mg g$^{-1)}$	0.148	*	0.83	0.081	*	0.67	-	-
Chlorophyll *b* (mg g^{-1})	0.293	*	1.02	0.764	**	0.24	-	-

MS = Mean Square, n.s. = Non-significant, *, **, *** = Significant at p = 0.05, 0.01 and respectively. Least Significance Difference (LSD) by Duncan's Multiple Range Test at 5% level of probability.*(df*, levels = 4, cultivars = 2, interaction= 8, residual = 30

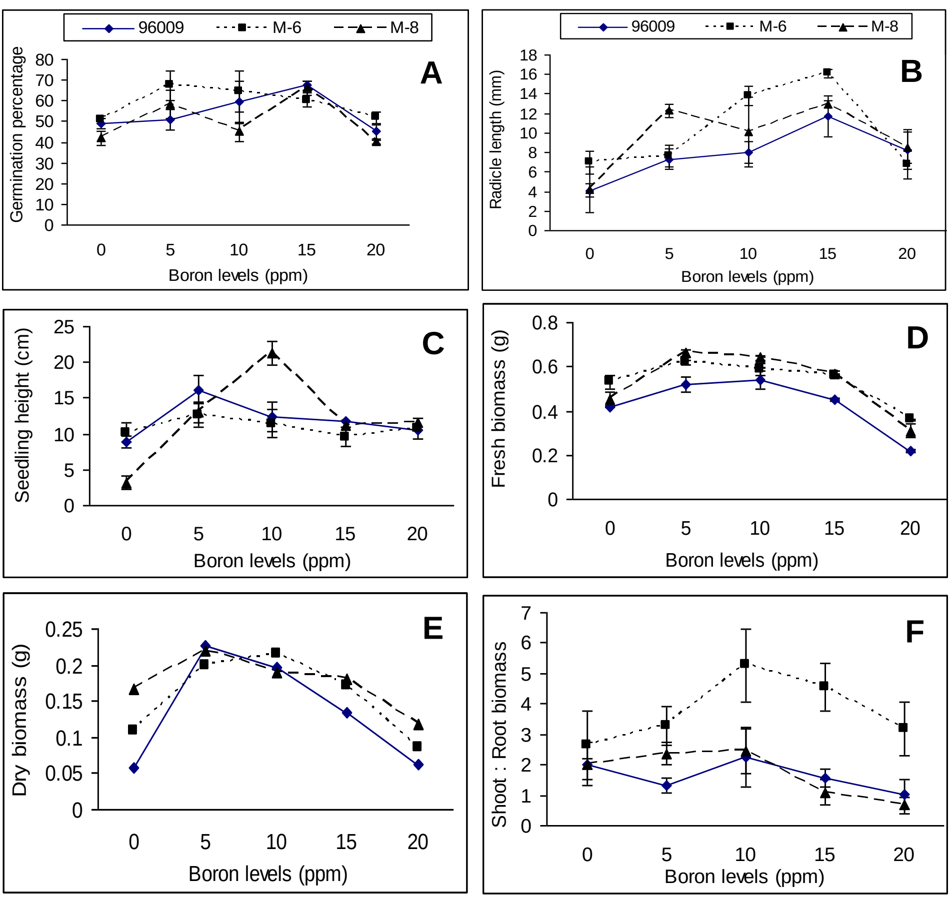

Fig. 1. Effect of increasing levels of boron on germination (A), radicle length (B), seedling height (C), fresh biomass (D), dry biomass (E) and shoot: root biomass (F) in three cultivars of mung bean.

Seedling biomass responses presented in Fig. 1D & E and ANOVA in Table 1 depicted a marked influence ($p<0.001$) of B and significantly ($p<0.05$) variable responses of the cultivars. The cultivar 96009 had exhibited a greater reduction in fresh biomass at the applied B levels as compared to the cultivars M-6 and M-8. The dry biomass followed a similar trend as shown by fresh biomass and there was a considerable decline in dry biomass with increasing B concentrations. Reduction in biomass yield can be attributed to decrease in assimilates under limited water and nutrient supply to the photosynthetic organs in the presence of excessive trace elements. The negative impact of high B has also been reported by Alpaslan & Gunes (2001) and Sotiropoulos *et al.*, (2002).

Shoot: Root regulation has been regarded as a prerequisite for predicting and understanding biomass allocation strategies in plants in relation to various essential nutrients (Canham *et al.*, 1996). In general, shoot: root ratio is affected particularly by the exceeding amount of micronutrients in the growth medium as they influence water and nutrients uptake as well as their distribution within the plants. Under restricted supply of water and nutrients to the shoot, a decline in shoot:root ratio occurs while the reverse is true if the root retains more water and nutrients. Among the tested cultivars, a considerable decline in shoot: root biomass was observed at elevated boron levels (Fig. 1F) which clearly suggests restricted transfer of water and minerals to the above ground tissues. However, cultivar M-6 consistently showed greater shoot: root ratio, indicating a better threshold for water and mineral uptake to the above ground tissues thus a greater amount of assimilates. Nevertheless, differential biomass partitioning strategies of the cultivars became evident in the current study.

The process of photosynthesis is directly correlated with the quantitative and qualitative photosynthetic attributes. The number of leaves was not adversely affected by varying levels of B since, greater number of leaves was observed at 10-20 ppm B (Fig. 2A). However, by contrast, Fig. 2B indicated a gradual decline in leaf area with increasing levels of B but the cultivars had shown invariable responses for this trait. Though, excessive concentrations of various essential micro nutrients had been reported to affect initiation of leaf primordial (Cramer & Quarrie, 2002; Huh, 2002) but the results of this study did not depict any decline in leaf number in all three cultivars. However, a significant ($p<0.05$) reduction in the photosynthetic area can be ascribed to B toxicity which is normally expressed as marginal and tip necrosis of the older leaves. Moderate to severe B toxicity induces a progressive leaf necrosis, beginning at the tip or margins and gradually covering the whole leaf was also observed by Apostol & Zwiazek, 2004. We also observed that necrosis progressed from leaf tips and margins towards the midrib and at the base of leaflets. The leaves showed parched appearance and ultimately abscission. Similar findings were also reported by Riley *et al.*, (1994;) and Reid *et al.*, (2004).) Moreover, the negative impact of high B on photosynthetic area is also in close conformity to Bennett, (1993) and Bergmann, (1992).

The cultivars exhibited a gradual decline in the amount of both chlorophyll *a* and *b* after exposure to increasing boron levels (Fig. 2C & D). Again, a remarkable decline in the chlorophyll was observed at the highest B level. Several studies have demonstrated that under high B, decrease in chlorophyll content was partly due to impairment of aminolevulinic (ALA), a precursor of chlorophyll (Tewari & Tripathy, 1998). However, other studies have indicated that excessive B can disrupt membranous structures including grana by inducing lipid peroxidation (Somashekaraiah *et al.*, 1992; Luna *et al.*, 1994). Although, the current investigation does not encompass biochemical attributes however, it is imperative to understand insights into the mechanistic explanation of B tolerance of these cultivars.

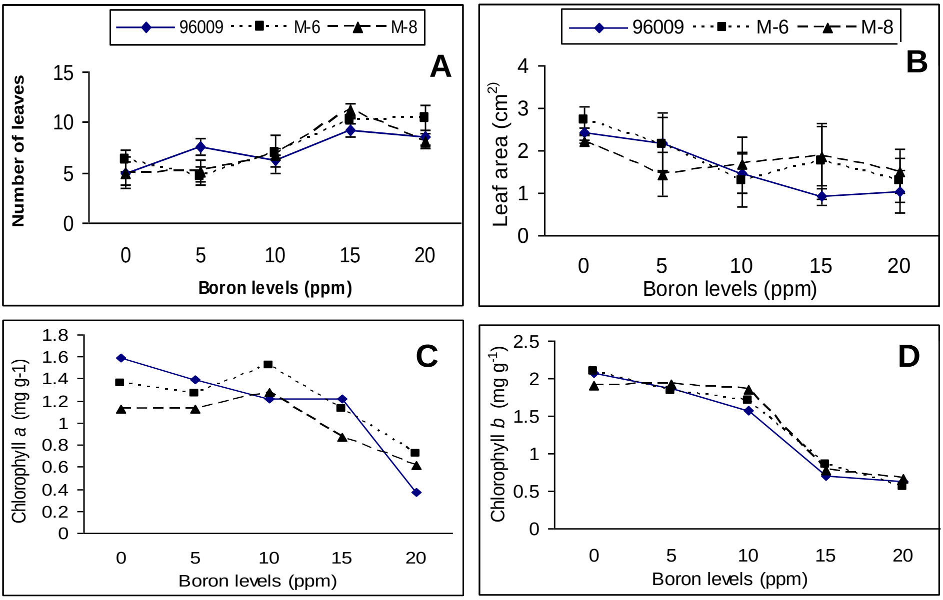

Fig. 2. Effect of increasing levels of boron on number of leaves (A), leaf area (B), chlorophyll *a* (C), and chlorophyll *b* (D) in three cultivars of mung bean.

In conclusion, this study demonstrated variation in B tolerance in *V. radiata* cultivars at the early growth stages. Based on the performance of the cultivars, M-6 and M-8 exhibited better growth responses as compared with 96009. Fresh biomass and shoot: root ratio appeared to serve as good predictors for B tolerance. Likewise, screening of cultivars/ accessions on a large scale can explore more diversity of traits for B tolerance. Application of lower levels of B (5-10 ppm) seemed to have a nutritive role as they promoted growth but thereafter, a drastic decline was observed for various attributes investigated. Boron 15 and 20 ppm seemed to be associated with B toxicity thus appeared to be stressful and accelerated adverse growth. Moreover, consistently low values for all growth attributes in the absence of B (control) clearly suggested that B is essentially required by the plants for their normal growth. However, toxicity of B can be attributed to its excessive amount which brings about nutritional disorders, water deficit conditions or induces oxidative stress. Therefore, future research using biochemical markers can provide better understanding of B tolerance or toxicity mechanism at both inter and intra-specific levels.

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