Effects of different boron concentrations on the morphology and foliar generalities of mung bean

JIAO Xiao-Yan¹,², WANG Gang¹, CHENG Bin¹, WANG Hong-Ting¹, Quick W. P. ², Jarvis B. C. ²

¹. Institute of Soil & Fertiliser, Shanxi Academy of Agricultural Sciences, Taiyuan 030031, China

Abstract: The effects of boron deficiency and toxicity on the morphology and foliar generalities of mung bean (Phaseolus aureus Roxb) were investigated in hydroponic culture. Reduced biomass production in response to limited supply of boron was accompanied by an increased shoot to root ratio. Foliar generalities were also altered by boron deficiency; leaf expansion was inhibited together with specific leaf area (SLA), implying that there was a high leaf cell density due to restricted cell expansion. An increased leaf weight ratio (LWR) and inter-veinal chlorosis evidenced in boron deficient leaves may indicate excessive carbohydrate accumulation, leading to a decline in chlorophyll content. Compared with shoot to root ratio, when optimum boron was supplied, high levels of boron did not induce significant changes in shoot to root ratio, suggesting that supra-optimum boron has similar effects on shoot and root growth. Boron toxicity

Foundation item: Financially supported by the University of Sheffield Overseas scholarship and Natural Science Foundation of Shanxi Province

Received date: 2002-07-17; Accepted date: 2002-11-28

Biography: JIAO Xiao-Yan, Ph. D. Professor, Major research areas are plant nutrition, environmental sciences and protected cultivation.

Acknowledgements: The first author is grateful to Drs R. Reid and J. Hayes (University of Adelaide, Australia) for very helpful critical comments and correction of the manuscript.
reduced total leaf area by way of abscission of the old leaves, but did not influence SLA or LWR.

**Key words:** boron; plant morphology; foliar generalities; SLA; LWR; mung bean (*Phaseolus aureus* Roxb.)

Boron (B) deficiency in higher plants adversely affects both physiological and morphological features[1·2]. It inhibits the growth of plants. For most plant species, remobilisation of B within the plant is minimal and this may be due to the formation of stable B complexes within the cell wall[3·4]. Consequently, symptoms of B deficiency first appear in new tissue rather than in mature tissues. Removal of B from the growth medium results in a rapid cession of root elongation, e.g. within 3 to 6 h[5], and a thick, coral-like root with a brown discolouration of the root apex[6]. B deficiency also inhibits the rooting of stem cuttings[7·9]. In the shoot, symptoms of B deficiency first appear at the shoot apex and in actively growing leaves[1·2·7]. Symptoms may include inter-veinal chlorosis, inhibited expansion of new leaves, leaf shape deformity and shortened internodes, giving the plants a bushy or rosette appearance.

Notwithstanding a large volume of previous work, the primary role of B remains unclear. There have been few studies on the effects of B deficiency on characteristics of leaf development, which may underlie the role of B for plant growth. The aim of the current study was to quantify the influences of B on the morphology and foliar generalities of mung bean.

1 MATERIALS AND METHODS

1.1 Materials and growth conditions

Seeds of mung bean (*Phaseolus aureus* Roxb.) were surface-sterilised by immersion in 4% bleach (Domestos, containing less than 5% of sodium hypochlorite) for 20 min. After thorough rinsing, the seeds were soaked overnight in running tap water and then transferred to fine granular vermiculite in seed trays that were placed in 2.5 cm of distilled water. Seedlings were initially grown for five days under continuous fluorescent light (photosynthetic active radiation (PAR) of 100 μmol m⁻² s⁻¹) at a temperature of 22°C. Only distilled water was supplied to the seedlings during these five days.

Five-day-old seedlings were transplanted into acid-washed black plastic pots (800 cm³) which were filled with Rorison solution[10] without aeration. The nutrient solution consisted of: 2 mM Ca(NO₃)₂, 1 mM MgSO₄·H₂O, 1 mM K₂HPO₄, 68 μM Fe-EDTA, 9 μM MnSO₄·4H₂O, 0.15 μM (NH₄)₂MoO₄·2H₂O·4H₂O, 1.5 μM ZnSO₄·7H₂O, 1.5 μM CuSO₄·5H₂O. Variable concentrations of B were supplied as H₃BO₃. Seedlings were cultured under controlled conditions (light/dark regime of 16h/8h, temperature 25°C/18°C) with a PAR of 350±20 μmol m⁻² s⁻¹ supplied from metal-halide lamps. For the first experiment, B was supplied at 0, 5, 50, 250 or 500 μM. Two harvests were made, on the 19th and 33rd days after transplanting. On each occasion, one seedling was harvested from each of five replicate pots. For the second experiment (investigating individual leaf development), B was supplied at 0, 0.05 μM to all treatments until the second trifoliate leaf emerged. One day after the second trifoliate leaf emerged, the roots were washed with distilled water and the plants transferred to nutrient medium with 0, 0.05, 0.5 or 50 μM B. Two harvests were made, on the 12th and 20th days after the B treatments were first supplied. The nutrient solution was replenished every two days for the duration of all experiments.

2.2 Data collection and statistical analysis

At all harvests, plants were divided into leaves, stem and root. Leaf area was determined with a leaf area meter (LI-3000 Devices Ltd., Cambridge, UK). Harvested plant material was then dried at 80°C for 48 h. Growth parameters were calculated according to Hunt[11]. The leaf area ratio (LAR) is the ratio of
total leaf area to whole plant biomass. The leaf weight ratio (LWR) is the ratio of total leaf biomass per plant to the total biomass per plant. The specific leaf area (SLA) is the ratio of total leaf area per plant to total leaf biomass per plant. Relative growth rate (RGR) is calculated from the following formula:

\[ RGR = \frac{\log_e W_2 - \log_e W_1}{t_{2} - t_{1}} \]

Where, \( W_2 = \) total biomass per plant from the final harvest; \( W_1 = \) total biomass per plant from the initial harvest. \( t_2 - t_1 = \) time between harvest (days).

The data were subjected to 2-sample t-tests or one-way analyses of variance (ANOVARs) using the statistical package Minitab 11. Where required, Tukey multiple comparison tests were used following ANOVAs to identify significant differences between individual treatments.

2 RESULTS

2.1 Effects of boron on plant morphology and growth parameters

Symptoms of B deficiency were visible in roots after only two days of growth in the absence of B. Root tips were brown and there was a thickening of the apex. Root elongation stopped after two days of B deficiency. After ten days, expansion of the first trifoliate leaf was markedly reduced and growth of the second trifoliate leaf had ceased. No new leaf initials developed thereafter. At the first harvest the primary leaves were dark green, but after twenty days without B the first trifoliate leaves showed inter-veinal chlorosis and at the second harvest, the primary leaves also showed inter-veinal chlorosis.

Visible signs of B toxicity first appeared in the primary leaves of plants grown in solution containing 500 \( \mu \)M B after eight days’ treatment, by way of marginal chlorosis. Inhibition of root growth with high concentrations of B was seen after a further two days. After fourteen days of treatment the second trifoliate leaf also showed marginal chlorosis. Primary leaves had abscised by the first harvest. After a similar length of treatment the first trifoliate leaves of plants raised in 250 \( \mu \)M B showed similar toxicity symptoms. At the second harvest, marginal chlorosis had spread to all but the newest leaf when 500 \( \mu \)M B was supplied. Plants grown in 50 \( \mu \)M B solution showed no symptoms of deficiency or toxicity during the experiment.

At the first harvest, all B-containing treatments had significantly greater biomass production compared to the no B treatment (Fig. 1a). An absence of B led to diminished RGRs (Fig. 1b) and at the second harvest, the biomass of seedlings grown in the absence of B was only 13% of that when B was supplied at 50 \( \mu \)M. Even when B supply was supra-optimum for growth, at 250 and 500 \( \mu \)M, the biomass of seedlings was significantly higher than that of seedlings which lacked B. Compared with that when B was present, biomass allocation between shoot and root was also altered by the absence of B at both harvests (Fig. 1c). B-deficient seedlings had significantly higher shoot to root ratios; however, at both harvests 500 and 250 \( \mu \)M B did not induce significant changes in shoot to root ratio compared with plants raised in 50 \( \mu \)M B. This may reflect that supra-optimum B concentrations exert similar effects on both shoot and root growth.

B-deprived plants grew the lowest number of leaves at both harvests. At the first harvest, 250 and 500 \( \mu \)M B produced similar numbers of leaves per plant to plants grown in 5 and 50 \( \mu \)M B concentrations. Due to abscission of the old leaves of plants supplied with 500 \( \mu \)M B there were fewer leaves at the second harvest than at the first harvest (Fig. 2a). Total leaf area per plant was also influenced by the supply of B (Fig. 2b). At both harvests plants grown in the absence of B had a significantly smaller total leaf area than those plants supplied with B at 5 and 50 \( \mu \)M. Although 250 and 500 \( \mu \)M concentrations of B were supra-optimum, these plants also had a significantly higher leaf area compared to plants which received no B. At the first harvest, B treatment had no significant effect on the mean value of area per leaf (derived
from total leaf area and number of leaves per plant). However, at the second harvest, an absence of B resulted in a significantly lower mean value of area per leaf compared to B-supplied treatments. This probably reflects a B deficiency-related inhibition of leaf expansion of individual leaves. There were no significant differences in mean value of area per leaf between the different B concentrations (Fig. 2c).

![Bar chart showing effects of boron on total biomass, relative growth rate, and shoot to root ratio](chart1.png)

**Fig. 1** Effects of boron on total biomass (a), relative growth rate (b) and shoot to root ratio (c)

Data presented are means ± SE of five replicates (except Fig. 1b). For each harvest, means with the same letter are not significantly different ($p < 0.05$). First harvest (■), second harvest (□)

Similar LARs were measured at the first harvest for all B treatments (Fig. 3a). At the second harvest, however, plants grown in the absence of B and at 500 μM B had significantly smaller LARs than for the other treatments B. An absence of B resulted in an increase in LWR (Fig. 3b). B deficiency also had a marked influence on SLA (Fig. 3c). Although there were no significant differences in SLA between any of the B concentrations for either harvest, the absence of B reduced SLA by about 30% at the first harvest and 50% at the second harvest.

2.2 Effects of boron on foliar generalities

The influence of B deprivation on leaf development was manifest in different ways, depending on the particular leaf in question. Leaf area of the primary leaves was not affected by B treatment at first harvest (Fig. 4b). However, biomass of the primary leaves was significantly greater when supply of B was limited.
to 0.05 μM or withheld completely, compared to when B was supplied at 0.5 or 50 μM (Fig. 4a). SLA was therefore lower when B supply was either very limited or absent (Fig. 4c).

Other leaves did not show the same responses to B. The first trifoliate leaf developed most rapidly when B was supplied at 0.05 μM, whereas 50 μM B was optimum for growth of the second, third, fourth and fifth true leaves. The second and subsequent trifoliate leaves did not develop at all in the absence of B, and only two trifoliate leaves developed when B was limited to 0.05 μM. The biomass of the first trifoliate leaf was greatest when B was supplied at 0.05 μM (Fig. 4d). However, leaf area was significantly greater when B was supplied than when it was not, and all concentrations of supplied B produced about the same expansion (Fig. 4e). These trends were observed for both harvests. SLA was, therefore, least at 0.05 μM and greatest for the highest concentration of B employed, 50 μM (Fig. 4f). The second trifoliate leaf responded differently to B (Figs. 4g, h & i). Leaf biomass and area both increased with increasing concentrations of B, but such that SLA was again greatest at 50 μM B. When B was supplied at 0.05 μM, development of the second trifoliate leaf was severely limited compared with the same leaf of plants supplied with 50 μM B (Figs. 4g & h). Foliar development beyond the second trifoliate leaf was limited to plants that were supplied with B at 0.5 and 50 μM B. By the first harvest, the third true leaf had developed in both those treatments while the fourth leaf was only evident at 50 μM B. The fifth leaf was only evident at the time of the second harvest (Figs. 4p & q). Comparisons between the third, fourth and fifth trifoliate leaves at the second harvest showed that 50 μM B resulted in greater leaf biomass and leaf area than did 0.5 μM (Figs. 4r~t).

It was generally noticed that when B was deficient, the leaves were more fragile and had developed inter-veinal chlorosis. At the first harvest, for example, the first trifoliate leaves of plants raised in the absence of B showed chlorosis. So did the second trifoliate leaves of plants grown in 0.05 μM B at the second harvest.

3 DISCUSSION

The experiments reported above have demonstrated symptoms of B deficiency and toxicity in plants where a wide range of concentrations of B has been employed. Since the entire focus of this project is concerned with the role of B in plant development, the comments that follow are almost exclusively restricted to deficiency and deficiency of B. Toxicity effects of B will not be discussed here.

The current work has demonstrated that biomass production is depressed when B supply is absent or
limited. Such an effect might be predicted on the basis of much previous work which has established and confirmed that B is an essential micronutrient\textsuperscript{[14-16]}. Reduced biomass production by mung bean in response to limited B was accompanied by an increased shoot to root ratio (Figs. 1a & c). While both root and shoot growth were diminished when B was in limited supply, the former was more severely restricted than the latter, at least for the duration of the experiments. The increased shoot to root ratio resulting from B deficiency is consistent with the view of Marschner \textit{et al.} (1996)\textsuperscript{[12]} that only those nutrients which are mobile in the phloem show favoured partitioning to the roots.

Notwithstanding an early influence on root growth, B deficiency had significant influences on leaf development. These have been the focus of the current study. In overall terms, when there was no supply of B to the seedlings only one trifoliate leaf developed (Fig. 4d & e). This could imply that B is necessary for the early stages of leaf development and particularly leaf initiation. Such a proposition would be consistent with the general observation that B is necessary for cell division as in the growth of roots\textsuperscript{[7]}. Not only were leaf number and area per plant increased when B was supplied at 5 or 50 µM, other leaf characteristics, such as the mean area per leaf, also differed from those of B-deficient plants. Further study of specific or individual leaves in the current report has highlighted several salient points, representing novel observations.

Primary leaves were influenced by limited B supply in that leaf biomass was enhanced, whereas subsequent trifoliate leaves were dependent on B for their expansion as well as their biomass accumulation. Expansion of the primary leaves was not influenced by a lack of exogenously supplied B (Fig. 4b). The development of the first trifoliate leaf of mung bean was enhanced by supply of B at 0.05 µM that, relative to the same leaf on plants deprived of B, enhanced both biomass (Fig. 4d) and expansion (Fig. 4e). At 0.5 µM, however, B was supra-optimal for biomass production but not expansion at the second harvest. Leaf expansion and biomass production of subsequent trifoliate leaves, however, increased with increasing concentrations of supplied B. Collectively, these observations indicate that seed B can sustain primary leaves and only limited development of the first trifoliate leaves. Further leaf development necessitates a supply of B even during the early stages of development of subsequent trifoliate leaves. The requirement of B for leaf expansion as presented here, is consistent with previous reports\textsuperscript{[14-16]}. 

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![Graph showing effects of boron on foliar generaties](image-url)
Leaf biomass and leaf area did not change in the same proportions with B deficiency. Correspondingly, SLA was decreased with B deficiency (Figs. 3c & 4) and LWR increased with B deficiency (Fig. 3b). The decrease in SLA reflects either increased leaf density or thickness, or both, of leaves when plants were raised under conditions of B deficiency, since SLA is a reflection of both leaf density and thickness as indicated by Witkowski and Lamont[17].

The low SLA and greater biomass of primary leaves when B supply was absent may relate to source-sink relationships. The limited growth evidenced in both roots and new leaves under deficient conditions would, presumably, place a lesser demand on the resources of the primary leaves. The decrease in SLA in primary leaves may correspond with the accumulation of total non-structural carbohydrate. This could reflect a direct influence of B within the functional leaves themselves or an indirect influence of B exerted elsewhere and influencing source-sink relationships within the plant. Therefore, further investigation of the effect of B on photosynthesis and the carbohydrate status of leaves is necessary.

References: