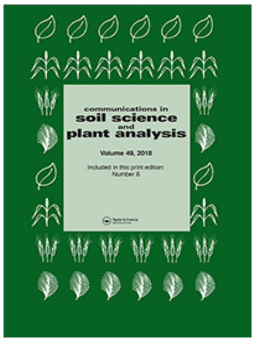

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Common Bean Productivity Following Diverse Boron Applications on Soil

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ABSTRACT
Aspects related to micronutrients management are still poorly studied and need to be clarified to guarantee sustainable production. In this way, the study aimed to evaluate boron (B) fertilization effects on nutrition and production of common beans. The parameters measured were relative chlorophyll index, dry mass, boron transport, utilization and absorption efficiencies, B content, and B accumulation in plant tissues. The study showed that the dry mass production was negatively affected by B application, with linear dry mass decrease following the increase of B doses. Accordingly, it was found that the common bean cultivar Esplendor presented high absorption capacity of boron, and can reach 175 mg kg^{-1}. However, the high absorption caused phytotoxicity and reduced dry matter production by up to 30%, reflecting the efficiency of boron use by the plant, which was reduced in up to 75%.

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Common bean; micronutrients; nutritional efficiency; plant nutrition; soil fertility

Introduction

Brazil is currently the biggest common bean producer in the world (*Phaseolus vulgaris* L.), with 60% of production provided by familiar agriculture (CTSBF 2012). This crop is an important income for small farmers (Posse et al. 2010), and combined technologies have been recommended to improve both crop productivity and sustainability (Farinelli et al. 2006).

One of the most important strategies to increase plants production, with a low input of consumables and without increase in production costs, is to ensure a balanced plant nutrition with macro and micronutrients, once higher productivity requires higher micronutrients doses (Barbosa and Gonzaga 2012). Since these nutrients can be toxic when overdoses are applied, it is necessary to establish convenient doses in different crop systems, i.e., with and without irrigation, conservative vs. traditional systems, and using more than one cultivation per year (Fageria 2000).

Micronutrient applications in common beans are not well established (Barbosa and Gonzaga 2012). Silva et al. (2006) showed that foliar application of boron (B) can improve seed quality, with higher germination rates, observed in seeds of common beans obtained from from plants fertilized with boric acid (Lima et al. 2013). However, B levels of toxicity and deficiency are only slightly different (Lima et al. 2013), being important to determine these levels to guarantee adequate plant production and quality.

Boron has many functions in plants related to cell wall syntheses and integrity, carbohydrate transport, and reproductive growth (Prado 2008). Inducing B deficiency in crops, Malavolta (1980) observed smaller roots development, gnarled new leaflets and yellowish, death of the terminal bud,

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no flowering, and underdeveloped plants. According to Moraes-Dallaqua, Beltran, and Rodrigues (2000), the B omission can disturb cell division, differentiation, and elongation, affecting root growth and as a consequence, causing plant death. In this way, the aim of this work was to evaluate the effect of increasing B doses on nutrition and productivity of common beans.

Material and methods

The experiment was carried out under greenhouse condition at the Federal University of Goiás, State of Goiás, Brazil (coordinates: 16°35” latitude south and 49°21′ longitude west) at approximately 730 m of altitude and 1,600 mm average annual rainfall. The climate is Aw (tropical climate with summer rains) or tropical savanna, with dry winters and rainy summers, according to Köppen classification. The soil analysis showed the following properties as 5.0 for hydrogen potential; 2.0 g kg^{-1} for organic matter; phosphorus (P) = 5.5 mg kg^{-1}; potassium (K) = 60 mg kg^{-1}; calcium (Ca) = 2.7 mmol$_c$ kg^{-1}; magnesium (Mg) = 0.5 mmol$_c$ kg^{-1}; boron (B) = 0.21 mg kg^{-1}; copper (Cu) = 2.8 mg kg^{-1}; iron (Fe) = 82 mg kg^{-1}; manganese (Mn) = 44 mg kg^{-1}; zinc (Zn) = 4.6 mg kg^{-1}; H + Al = 1.8 mmol$_c$ kg^{-1}; cation exchange capacity = 5.2 mmol$_c$ kg^{-1}; base saturation (V%) = 65.1%, with 432 g kg^{-1} of clay.

A completely randomized design was used for five B doses (0, 1, 2, 4, and 6 kg ha^{-1}), with four replications. Pots with a 4 dm^3 capacity were filled with 3.5 dm^3 of a Rhodic Hapludox (Soil Taxonomy 2006), drawn from the topsoil layer (0–0.2 m depth). Soil acidity was not corrected, since base saturation was higher than 60%. Macronutrients were applied for the following doses: 110 kg ha^{-1} of P_2O_5, using single superphosphate; 120 mg kg^{-1} of nitrogen (N) as urea, being 20 mg kg^{-1} of N during sowing. Nitrogen after sowing was applied with two separate parts of 50 mg kg^{-1} of N 20 days and 50 mg kg^{-1} of N 40 days. In addition, potassium chloride was applied at a rate of 60 mg kg^{-1} of K_2O applied during sowing (Souza and Lobato 2004). Micronutrients were applied for the following doses: 1.5 mg kg^{-1} of copper (Cu) [copper sulfate ($CuSO_4.5H_2O$ p.a.)], 0.15 mg kg^{-1} of molybdenum (Mo) [sodium molybdate ($NaMoO_4.2H_2O$ p.a.)], 4.0 mg kg^{-1} of iron (Fe) [$Fe_2(SO_4)_3.4H_2O$ p.a.], and 5.0 mg kg^{-1} of zinc (Zn) [zinc sulfate ($ZnSO_4$ p.a.)] (Mesquita et al. 2004). Boron was applied using boric acid as a source (17% of B). The B doses were applied to the soil surface and incorporated into 10 cm depth at seedling emergence.

A cut for standardization was performed at 10 days after plants emergence, living two plants per pot. Soil humidity was maintained at 60% of the maximum soil water retention capacity during the experimental period. Plants were monitored daily to document thenutritional disorder symptoms as they develop. At 20 and 40 days after plants emergence (DAS), the relative chlorophyll index (RCI) was taken from five leaves per pot, with an Falker® device, model ClorofiLOG CFL1030.

The nutritional status for B was determined at the beginning of the flowering stage, at 60 days after garmination (DAG). On the last date was measured as well as the dry mass of aboveground part and roots of common beans.

Plant tissue samples were washed with a 0.1% detergent solution and a 0.3% acid solution and distilled water, and dried in an oven at 65 °C for 48 h for aerial part and root dry mass determinations (second cut, only). The manganese contents from aerial part and root plant tissues were determined following the methodology described by Silva (2009).

Some nutritional indices were calculated by using both dry matter (DM) and nutrient content data of plants as described by Prado (2008). The absorption efficiency (AB_{ef}), transport efficiency (TR_{ef}), and nutrient use efficiency for conversion to DM (UT_{ef}) were calculated as showed in Equation 1, 2 and 3, respectively.

$$AB_{ef} = \frac{\text{total nutrient content in plant}}{\text{root dry matter}} \tag{1}$$

(Swiader, Chyan, and Freiji 1994).

$$TR_{ef} = \frac{\text{nutrient content in partial part}}{\text{total nutrient content in the plant}} \tag{2}$$

(Li, McKe, and Allen 1991).

$$UT_{ef} = \frac{(\text{total dry matter produced})^2}{\text{total nutrient content in the plant}} \quad (3)$$

(Siddiqi and Glass 1981).

Results were subjected to the analysis of variance using the software Sisvar Inc., Viçosa, MG, Brazil (Ferreira 2014) and to polynomial regression analysis. The maximum points were calculated by deriving the significant equations. Variables were correlated by the Pearson linear correlation test (SigmaPlot Inc., San Jose, CA, USA), considering the correlation significances ($p \leq 0.01$ and 0.05).

Results and discussion

RCI and B-foliar

The application of increasing B rates into the soil did not affect the chlorophyll index (RCI) at 40 DAS (Table 1), which showed an average of 39,8 μg cm^{-2}; while at 20 DAS, RCI showed a linear adjustment (Figure 1), with the lowest RCI observed following the highest B dose application, it was 8% lower than control application. Since RCI has a strong correlation with N content, a N reduction

Table 1. Relative chlorophyll index (RCI) and B amounts in leaves of *Phaseolus vulgaris* in function of increasing boron doses in soil.

Doses of B	RCI[1]	RCI[2]	B content
kg ha^{-1}	μg cm^{-2}		mg kg^{-1}
0	37.27	39.79	38.00
1	35.24	40.89	44.87
2	34.85	41.69	78.92
4	34.66	39.62	124.85
6	33.76	36.89	177.70
F	*	ns	**
CV	3.54	6.65	5.14

RCI[1] and RCI[2] – Relative chlorophyll index at 20 and 40 days after sowing. –**significant at 5% of probability by the F test; * significant at 1% of probability by the F test; and ns, not significant at 5% probability by the F test. CV: coefficient of variation.

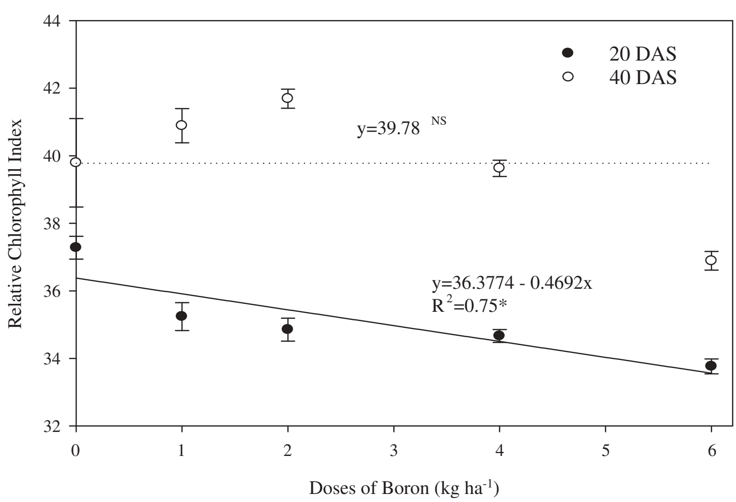

Figure 1. Relative chlorophyll index measured 20 days after sowing (DAS) and 40 DAS of *Phaseolus vulgaris* in function of increasing boron doses in soil.

content in plants with application of increasing B doses is expected . Similar results were observed by Silva et al. (2015), who observed a reduction in N content following increasing B doses application.

The RCI measures the green color intensity, which is related with N content in plants (Godoy et al. 2008), since four N atoms are necessary to produce each chlorophyll molecule (Prado 2008). In this sense, the chlorophyll meter use is a viable, fast, nondestructive, and practical alternative for monitoring the N status in plant tissues (Maia et al. 2013). Studies carried out by Flores et al. (2017b), with application of B doses up to 8 kg ha^{-1} in Common beans BRS Estilo cultivar, found that there were no increases in RCI at 40 DAS. It was attributed to the low nutrient mobility in the phloem, since it may reduce photosynthesis. In another study reported by Flores et al. (2017a) with BRS Estilo cultivar, the RCI was affected by B application only at 40 DAS, with increments up to the dose of 4.39 kg ha^{-1} of B, which reached 45.97 µg cm^{-2}.

In the literature, there are several researches reporting the N effects in B concentration in foliar tissues, since nitrate concentration can be reduced in leaves and roots when plants are under B deficiency conditions, interfering to some plant physiological processes (Cristóbal and Fontes 2007; Papadakis et al. 2004; Silva 2007; Silva et al. 2016; Simón et al. 2013). Among the processes, boron is related to RNA metabolism, participating in uracil synthesis, which is a RNA component (Epstein 1975; Prado 2008). In this way, in B-deficient plants, there is an accumulation of N in amino acids form compared to N in protein form, since there is an increase in RNAase activity, which hydrolyzes RNA, and also a lower uracil synthesis from the orotic acid (Prado 2008).

There was an increase in the B content in leaves and roots with increasing B rates application; it showed a linear behavior (Figure 2), reaching 175.1 mg kg^{-1} of B in leaves. Comparing the control with the highest dose, 466% enhance in B content was observed.

This result can be indicative of the onset of toxicity caused by excess B in the initial development phase of bean. At 20 DAS with the plant still at the beginning of its development, the doses of B applied may have contributed to cause a moderate toxicity that disrupts the plant development.

In common beans cultivated in flooded soil, Mariano et al. (2000) observed that the lower B levels ranged from 44 to 68 mg kg^{-1} while the higher levels ranged from 144 to 199 mg kg^{-1}. Malavolta, Vitti, and Oliveira (1997) found that the adequate B content in leaves ranged from 30 to 60 mg kg^{-1} and Raij, Quaggio, and Furlani (1996) found that the B content ranged from 15 to 26 mg kg^{-1}.

According to Marschner (2012), the adequate B content differs among plant species, with B content ranging from 5 to 10 mg kg^{-1} in grass, 20– to 70 mg kg^{-1} B in the most dicot, and from 80 to 100 mg kg^{-1} in the species with a higher demand of B, as poppy. In common beans as dicot, B complex in cell walls can reduce B availability, resulting in higher demand of B in these plant species (Loomis and Durst 1992; Prado 2008).

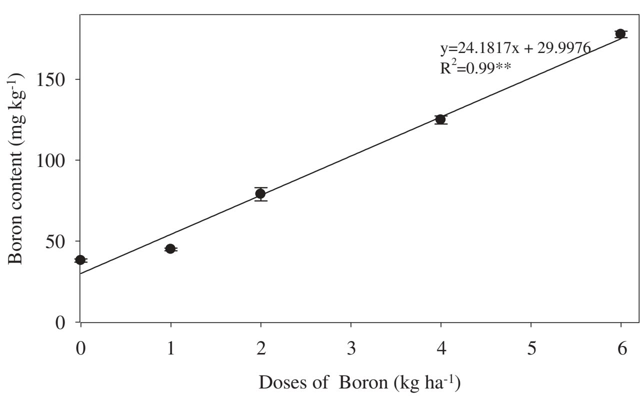

Figure 2. Boron content in leaves of *Phaseolus vulgaris* in function of increasing boron doses in soil.

In recent studies, Flores et al. (2017b) evaluated the B application on leaves of common bean BRS Estilo cultivar, using soluble sources in an irrigated system, and observed high B levels (100 mg kg^{-1}) in plants, mainly when boric acid was applied. These authors attributed the high B content in leaves to the high boric acid (H_3BO_3) solubility B absorption through the leaf cuticle. In another study, Flores et al. (2017a) obtained increases that reached 288 mg kg^{-1} of B in the leaves with the use of 6 kg ha^{-1} of B, under greenhouse conditions with BRS Estilo cultivar. However, this dose promoted a significant reduction in dry mass production.

Dry mass production

Boron application in soil affected roots, shoots, and entire plant dry mass production (Table 2). In all evaluated compartments, significant reductions were observed with linear adjustments, which reached 4.87, 8.16, and 13.03 g $plant^{-1}$ with the highest dose (6 kg ha^{-1}), a reduction of 31%, 19%, and 44% compared to control (Figure 3).

Boron supply favors the permeability of the plasmatic membrane and the water flux, changing nutrients absorption (Mattiello et al. 2009), which may have stimulated the meristematic activity of cells such as cell elongation and differentiation (Silva et al. 2015). However, if B is applied in high doses, a inhibition of these process and a dry mass reduction can be expected, as observed in this experiment.

Application of boron affects the root system growth and development (Martin et al. 2014), since it is a component of polysaccharides from cell walls, stimulating the division and expansion of cells (Malavolta 2006; Marschner 2012; Trautmann et al. 2014). Further, in soils with low B levels, boron application promotes roots growth, being particularly important in condition with water deficit (Silva et al. 2015). However, boron excess can reduce photosynthesis (Chen et al. 2012), enzymatic activity, and electrons transportation (Simón et al. 2013), reducing roots growth, as observed in this study. Other researches evaluating B doses in tropical soils also verified DM reduction after application of high B doses (Trautmann et al. 2014).

Flores et al. (2017a) observed a significant increase in roots, shoots, and entire plant dry mass following B application on common beans, with the highest dry mass production observed at a dose of

Table 2. Dry mass of aboveground part, roots and entire plant of *Phaseolus vulgaris* in function of increasing boron doses in soil.

Doses	Aboveground part	Roots	Entire plant
kg ha^{-1}		g $plant^{-1}$	
0	10.26	9.08	19.34
1	9.78	8.45	18.23
2	9.02	6.38	15.40
4	8.98	6.20	15.18
6	8.18	5.11	13.29
F	**	**	**
CV	7.46	8.77	6.53

**Significant at 1% of probability by the *F* test. CV: coefficient of variation.

Table 3. Boron accumulation in aboveground part, roots and entire plant of *Phaseolus vulgaris* in function of increasing boron doses in soil.

Doses	Aboveground part	Roots	Entire plant
kg ha^{-1}		g $plant^{-1}$	
0	0.37	0.30	0.67
1	0.44	0.42	0.86
2	0.42	0.29	0.71
4	0.51	0.43	0.94
6	0.61	0.54	1.15
F	**	**	**
CV	9.49	16.53	10.83

**Significant at 1% of probability by the*F* test. CV: coefficient of variation.

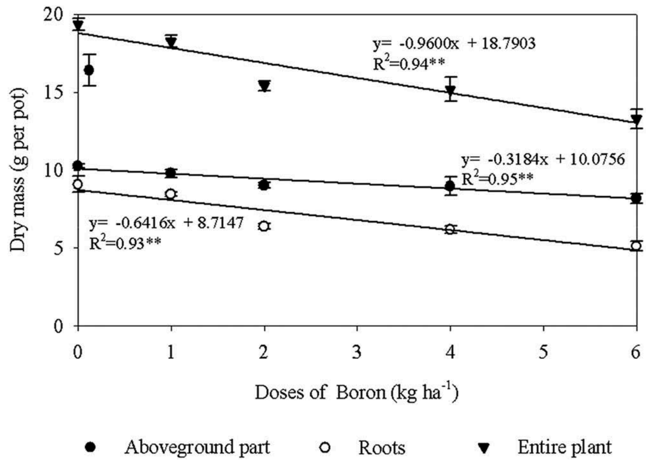

Figure 3. Dry mass of aboveground part, roots, and entire plant of *Phaseolus vulgaris* in function of increasing boron doses in soil.

4.0–4.5 kg ha^{-1} of B in soil. Since the environmental conditions and boron doses were the same in this study and in Flores et al. (2017a), the differences in the results are related with common beans cultivar.

Reis et al. (2008) observed a linear increase in common beans DM with foliar application of increasing B rates, ranging from 0 up to 2 kg ha^{-1}. When the same B doses were applied to soil, these authors found a quadratic adjustment for DM, with the highest DM production observed at a dose of 1.2 kg ha^{-1}. Côrrea et al. (2006) suggested that the DM decrease after B application can be attributed to the high absorption of B, since the high concentration of B in the soil can increase the differential of the chemical potential between B content in soil and plant, allowing the direct passage of the element through the cell wall, caused by the absence of charge. The preferential passage of the nutrient, considering the applied doses in the soil, can explain the excess in the absorption of B by the crop, damaging its root growth and DM of the aerial part, as observed in this work.

Boron accumulation

The B application on soil affected B accumulation in plants tissue (Table 3). Boron accumulation in all plant parts after increasing B doses had a linear increase pattern. The highest B accumulation was observed after application of 6 kg ha^{-1} of B in soil, resulting in a B level of 0.51, 0.60, and 1.12 g plant^{-1}, in root, shoot, and entire plant, respectively. Boron accumulation with 71%, 61%, and 66% was observed to be higher than that in control plants (Figure 4).

Flores et al. (2017a) also observed a higher B accumulation after B application on common beans (BRS Estilo cultivar); however, these authors found a different behavior, with a quadratic model, with the highest B accumulation observed at a rate of 4 kg ha^{-1} of B. According to Prado (2008), boron moves in soil by mass flow, so the absorption, and consequently the accumulation of B, is dependent on the transpiration and the concentration of this element in the soil solution. Studies developed by Souza et al. (2011) and Mariano et al. (2000) also observed that B increase in soil favored higher absorption and accumulation of B by the crop, in agreement with the results observed in this work. Thus, with increasing doses of B applied in the soil, the concentration of this element in the soil solution was increased, resulting in greater absorption and accumulation by the bean crop. Similar results were found by Souza et al. (2011) and Mariano et al. (2000).

The highest B accumulation in common beans' shoots compared to roots shows the high efficiency of these plants to transport B in xylem when it was applied in soil. Moreover, the high accumulation of B in roots can be related to the low mobility of this nutrient in phloem, being the highest B accumulation observed in the old leaves (Dechen and Nachtigall 2007; Malavolta 2006; Marschner 2012).

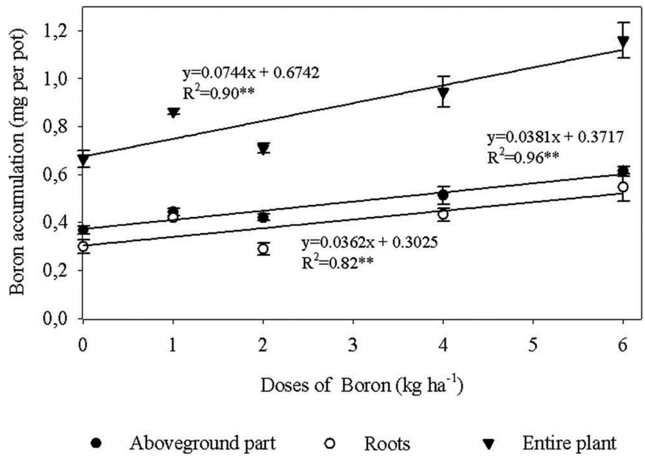

Figure 4. Boron accumulation in aboveground part, roots, and entire plant of *Phaseolus vulgaris* in function of increasing boron doses in soil.

Absorption, transport, and utilization efficiencies

Boron application on soil did not affect B transport efficiency in common beans, with an average value of 54.7% (Table 4). However, the B absorption (Ef_{abs}) and utilization (Ef_{uti}) efficiencies by crop were altered. Boron Ef_{abs} increased linearly (205% higher than control, Figure 5) and B Ef_{uti} was linearly reduced (74.5% lower than control) after increasing B doses application (Figure 6).

Absorption efficiency indicates the ability of plants to absorb the nutrient present in the soil solution. The first step of the absorption consists of the B-root contact, which occurs through the movement of this element by mass flow being positively affected by the concentration of the element in the soil solution (Prado 2008). In this study, since increases in B absorption efficiency were not followed by dry mass production increases, it may suggest that the common beans BRS Estilo cultivar has no mechanism to avoid B phytotoxicity.

On the other hand, the efficiency of use indicates the ability of the plant to convert the absorbed nutrient to total DM (Prado 2008). In this context, the increase in B concentration in the soil tends to decrease the utilization efficiency, since with greater amount of nutrient there is a slight increase or even reduction in the production of dry mass, as observed in this experiment.

The optimization of nutritional efficiency, whether of absorption, transport, and/or use, is of significant importance for the production of annual crops, such as common bean, due to the cost of fertilizers, essential for increasing productivity (Fageria 1998). However, these efficiencies may be influenced by several factors, such as genetic factors that may provide differences in nutrient leaf

Table 4. Efficiency of absorption, transport, and utilization of boron in *Phaseolus vulgaris* in function of increasing boron doses in soil.

Doses	Efficiency of absorption	Efficiency of transport	Efficiency of utilization
kg ha^{-1}	mg g^{-1}	%	mg g^{-1}
0	0.07	55.43	569.19
1	0.10	51.34	387.03
2	0.11	59.33	334.75
4	0.15	54.17	244.94
6	0.23	53.22	152.91
F	**	ns	**
CV	7.54	6.87	13.33

**Significant at 1% of probability by the *F* test; ns, not significant at 5% of probability by the *F* test. CV: coefficient of variation.

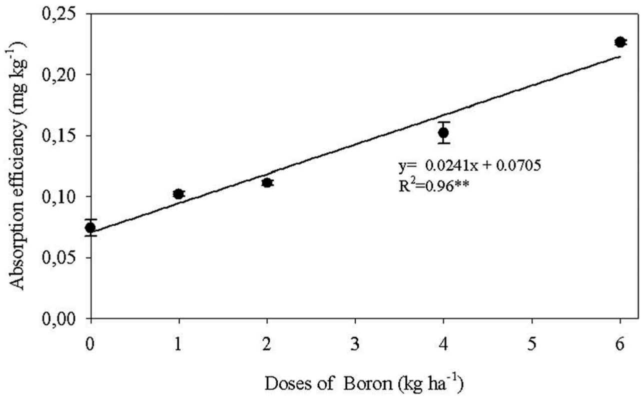

Figure 5. Adsorption efficiency of boron in *Phaseolus vulgaris* in function of increasing boron doses in soil.

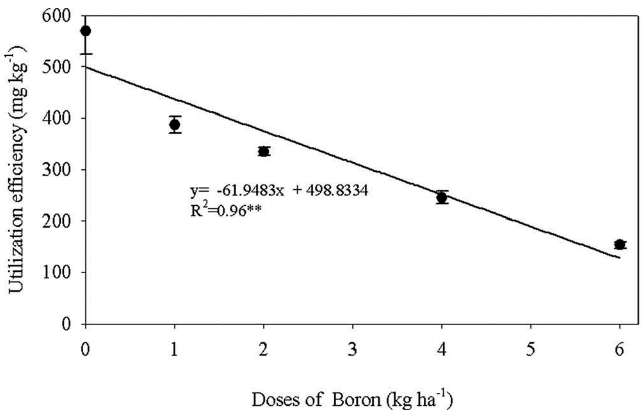

Figure 6. Utilization efficiency of boron in *Phaseolus vulgaris* in function of increasing boron doses in soil.

content, indicating cultivars with greater or lesser efficiency in the absorption, translocation, or nutrient utilization by plants (Cury et al. 2013). Genotypic differences are related to nutritional requirement at the cellular level, compartmentalization, shoot utilization, short- and long-distance transport, affinity with absorption sites, minimum concentration (km), and rhizosphere modifications (Tomaz et al. 2011).

Flores et al. (2017a) observed higher B absorption and transport efficiencies after application of increasing B doses on soil, being the highest values observed at a rate of 4 kg ha^{-1} of B, while B utilization efficiency was reduced with the B doses increase. These results are in accordance with the present study, which indicates that bean plants have high B absorption and transportation capacity; however, dry mass production did not show the same pattern, since it is reduced after application of increasing B doses. These results indicate that more studies related to micronutrient-containing fertilization management are necessary, especially B, since it has a narrow range between the critical level of deficiency and excess.

Conclusions

The common bean (BRS Esplendor cultivar) presents high boron absorption capacity, accumulating up to 175 mg kg^{-1}. However, the high absorption causes phytotoxicity and reduces DM production up to 30%, reducing up to 75% the B efficiency of use by the plant.

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