RESEARCH ARTICLE

Grain yield of *Phaseolus vulgaris* in a function of application of boron in soil

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Abstract

The objective of this study is to evaluate the effects of sources and doses of boron on the physiological quality and production yield of *Phaseolus vulgaris* grown using a central pivot irrigated system on a tropical climate region. The experiment was a 4x5 factorial design representing four sources of boron (boric acid, borax, borogran and FTE BR 12) and five boron doses (0, 1, 2, 3 and 4 kg ha^{-1}), with four replications. The following parameters were measured: relative chlorophyll index, transpiration, CO_2 internal concentration, stomatal conductance, net photosynthesis, B content, grain yield and economic analysis. The relative chlorophyll index (RCI) of plants showed no significant effects at both 28 and 46 days after emergence (DAE). The extraction order of boron was boric acid > borax > FTE BR12 > Borogran. The application of boron did not affect the physiological quality (stomatal conductance, transpiration and internal concentration of CO_2). However, the net photosynthetic rate was lower when boric acid was applied in relation to other sources. The maximum production (4,446 kg ha^{-1}) was obtained using 1.35 kg ha^{-1} of B with the use of boric acid. However, according to an evaluation of economic viability, it is observed that there are economic differences among sources of boron and evaluated doses. The application of 4 kg ha^{-1} of borax provides the highest economic return.

Keywords: Plant nutrition, micronutrients, economic viability, physiological quality, photosynthesis

1. Introduction

Common beans (*Phaseolus vulgaris* L.) is a basic food, with a high socio-economic importance to Brazil (Richetti *et al*., 2013). Brazil is second largest world producer of bean grains, following India (FAO, 2012). The importance of bean grain production is because this food is part of several food security programs of developing countries (Embrapa, 2012).

However, in production systems aiming high productivities, a proper management of mineral fertilization programs required to maintain the nutritional balance of plants is essential. Regarding fertilization, although required in smaller quantities, micronutrients are very important for the growth, development and production of dry beans. However, with regard to their fertilization recommendation, they are still incipient (Couto Júnior *et al*., 2013). Among micronutrients, boron (B) has a narrow range of suitable contents and toxic contents in the soil solution. This evidences the need for further calibration of its supply via fertilization management (Trautmann *et al*., 2014). Among micronutrients, the low natural boron content in the soil has been a limiting factor for the production of common bean grains in Brazilian tropical regions (Mattiello *et al*., 2009; Euba Neto *et al*., 2014). Its availability is mainly influenced by soil pH values (Sá and Ernani, 2016), soil organic matter (Lima *et al*., 2007), mobility in the soil profile (leaching) due to the high intensity of rainfalls (Prado, 2008), soil parental material, topography and soil type and use (Zhu *et al*., 2016).

B is essential to many plant physiological processes such as transport of sugars, cell wall synthesis, lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, IAA metabolism, phenolic metabolism, ascorbate metabolism, germination and pollen tube growth. It is also related to the integrity of the plasma membrane (Malavolta, 2006; Dechen and Nachtigall, 2006; Prado, 2008; Marschner, 2012). Moreover, B is related with physiological activity in crops, which may block the chlorophyll synthesis and, as a consequence, the photosynthetic activity in plants with B deficiency (Yadegari. 2016). According with Wasaya *et al*. (2017), plants with adequate B levels are more tolerant to abiotic stress.

The cultivation of common beans using irrigated systems is the subject of great attention because of potential losses of B by leaching in Brazilian tropical soils, especially where there are textures from medium to sandy (<35% clay) (Prado, 2008). Flores *et al*. (2017) observed that B application rates up to 4 kg ha^{-1}, using borax as a B source, can increase grain yield with a positive economical budget. Thus, studies on these environments involving boron and bean crops are important for a better understanding of fertilization management. In this sense, studies on the use of sources and doses containing boron in such regions may contribute to a sustainable grain production, avoiding losses of B and preserving environmental resources. Thus, the objective of this study was to evaluate the effects of sources and doses of boron on the physiological quality and production yield of common beans grown in a central pivot irrigated system on a tropical climate region.

2. Material and Methods

2.1. Growing conditions

An experiment was carried out at Federal University of Goiás, Goiania, Brazil (16° 35" latitude south and 49° 21' longitude west, at approximately 730 m of altitude and 1,600 mm average annual rainfall), in an area with central pivot irrigation system. The climate is Aw (mega thermal) or tropical savannah, with dry winters and rainy summers, according to Köppen classification. The soil analysis showed the following properties: pH (CaCl$_2$) = 5.1; organic matter = 29 g dm^{-3}; P = 6.7 mg dm^{-3}; K = 0.52 cmol$_c$ dm^{-3}; Ca = 1.7 mmol$_c$ dm^{-3}; Mg = 0.6 mmol$_c$ dm^{-3}; S= 1.2 mg dm^{-3}; B = 0.19 mg dm^{-3}; Cu = 2.8 mg dm^{-3}; Fe = 82 mg dm^{-3}; Mn = 44 mg dm^{-3}; Zn = 4.6 mg dm^{-3}; H+Al = 2.4 mmol$_c$ dm^{-3}; cation exchange capacity (CEC) = 52 .2 mmol$_c$ dm^{-3}; base saturation (V%) = 54%, with 420 g kg^{-1} of clay.

2.2. Treatments and experimental design

The experimental units were five rows containing bean plants spaced 0.45 m apart, 5 m long, forming a total area of 11.25 m^2. The two central rows were considered as floor area, and 0.5 m in length were discarded, totaling 3.6 m^2. The experiment was randomized blocks (RBD) in a 4 x 5 factorial design representing four sources containing boron applied to the soil at planting (borax - 10%, boric acid - 17%, borogran - 10%, and FTE (fritted trace elements) BR 12 - 1.8%) at five doses: 0 (control), 1, 2, 3 and 4 kg ha^{-1}, with four replications. Soil was classified as Rhodic Hapludox (Soil Taxonomy, 2006).

2.3. Treatments application and analysis

The implementation of the research took place on June 23, 2015, using a central pivot irrigation system. On this occasion, acidity correction was performed using 310 kg ha^{-1} of limestone (92% Relative Total Neutralization Power (RTNP)) to increase soil base saturation to 65%. After that, the soil was prepared with a plow and a corrective grid for the incorporation of acidity correction.

The sowing of common beans, cultivar BRS Estilo, was carried out in a spacing of 0.45 m between rows, 17 seeds per meter. The fertilization in furrows was 20 kg ha^{-1} of N, 120 kg of P_2O_5 and 60 kg ha^{-1} of K_2O in the form of urea, simple superphosphate and potassium chloride, respectively. Cover fertilization was performed at 20 and 40 days after germination with 80 and 40 kg ha^{-1} of N as urea, respectively (Sousa and Lobato, 2004).

2.4. Physiological parameters

The physiological parameters measured were: transpiration rate (T) (mmol m^2 s^{-1}), stomatal conductance (gs) (mmol m^2 s^{-1}), CO_2 internal concentration (Ci) (ppm) and net photosynthesis (μmol m^2 s^{-1}). These measurements were taken at R5 stage (between pre-flowering and pods formation), using an Infrared Gas Analyzer (IRGA, Li-COR, Lincoln, USA). Inside the plot, plants were chosen in a randomized pattern, and the first completely expanded leaf from the apex of the main stem was used. The measurements were performed between 9:00 h and 14:00 h.

The relative chlorophyll index (RCI) was determined at 28 and 46 DAE (days after emergence), using a chlorophyll meter, model ClorofiLOG. In each plot, plants were taken in a randomized pattern, and the first completely expanded leaf from the apex of the main stem was used.

The foliar B level was evaluated in the pre-flowering stage. Samples were taken from 20 plants in each plot, using the diagnostic leaves (first leaf completely expanded), as recommended by Souza *et al.* (2011). Boron analyses were performed as described by Bataglia *et al.* (1983).

In each plot, 2 m of the three central lines were harvested in 5th of October 2015. The plant´s material was weighed and sub-sampled to determine grain yield (kg ha^{-1}).

2.5. Economic analysis

The economic analysis was done using the partial budget technique (Noronha, 1987). The differential profit was calculated using the budget costs and the differential income, using the control treatment as a reference. Grain productivity was used to calculate the productivity gain in B treatments related to the control (PG). The production value (PV) was obtained by the following Equation 1:

PV= PG*P   Equation 1

Were:

P= product price in Brazil. The average price deflated of the US$ kg^{-1} 0.81 (Agrolink 2016) was used, this value was calculated using data of price from the last 15 years (2001 up to 2016).

The differential profit was obtained by the following Equation 2:

Pd = Id – Cd   Equation 2

Were:

Pd = differential profit

Id = differential Income = Iti – It$_0$

Cd = differential cost = Cti – Ct$_0$

ti = Treatment i

t$_0$ = Control

2.6. Statistical analysis

The statistical analyses were performed in a factorial scheme with two factors: B sources and B doses. When was observed significant interaction between the factors, the interactions were partitioned and the separated effect of each factor was not considered. Using the Sisvar programme, Brazil (Ferreira, 2014) the B source effect was evaluated using the Tukey´s test ($p \leq 0.05$) and the B doses effect was described using a regression analysis. The parameters used to choose the regression model were the F test significance, predict and adjusted R^2 and the residual plots independence (including Durbin-Watson test for verify correlation between adjacent residuals).

3. Results

3.1. Relative chlorophyll index (RCI) and boron content

Regardless of the sources and doses of boron (B) applied to the soil, the relative chlorophyll index (RCI) of plants showed no significant effects ($p>0.05$) both at 28 and 46 days after emergence (DAE) (Table 1). The average values for RCI were 36.46 and 38.18 µg cm^{-2} at 28 and 46 DAE, respectively, regardless of the boron source and dose applied (Table 1).

Table 1. Relative chlorophyll index (RCI), B amounts in leaves, and grain yield of *Phaseolus vulgaris* in function of sources and increasing boron rates in soil.

Treatments	RCI[1]	RCI[2]	B-leaf	Grain yield
Sources (S)	----------µg cm^{-2}---------		mg kg^{-1}	kg ha^{-1}
Borax	35.84 a	37.40 a	53.61ab	4181b
Boric acid	37.43 a	38.84 a	55.62a	4319a
Borogran	36.68 a	38.62 a	48.45b	3934c
FTE BR 12	35.89 a	37.88 a	52.61ab	4151b
F	1.06^{ns}	0.93^{ns}	3.34*	32.32**
Rates (R) (kg ha^{-1})				
0	35.56	36.73	38.57	4027
1	36.06	39.77	44.70	4169
2	35.96	37.43	53.55	4231
3	36.91	38.98	60.48	4170
4	37.80	38.01	65.57	4140
F	1.20^{ns}	2.45^{ns}	35.95**	5.65**
S x R	0.70^{ns}	0.86^{ns}	1.50^{ns}	32.43**
C.V.	7.78	6.99	12.17	2.62

Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. C.V. - Coefficient of variation. [1] and [2] - at 28 and 46 days after germination, respectively. **, * and ^{ns} significant at 1 and 5% and not significant at 5% of probability by the F test, respectively.

Boron absorption by bean crops was affected in function of the source applied to the soil. The extraction order was boric acid > borax > FTE BR12 > Borogran, with average contents of 55.62, 53.61, 52.61 and 48.45 mg kg^{-1}, respectively (Table 1). The highest absorption of boron, when boric acid was applied, reflected in an increased productivity of the culture regardless of the dose applied (Table 1).

When performing polynomial regression analysis for the effects of doses on boron contents in leaves, it was noted that the absorption of B by the culture showed a similar behavior: all had a significant linear adjustment, i.e., leaf boron content increased with a greater supply thereof in the soil (Figure 1). The increases in leaf B contents were 118, 49, 76 and 56% using borax, boric acid, borogran and FTE BR12, respectively, with application of the maximum dose (4 kg ha^{-1}) in relation to the control treatment (no application of B).
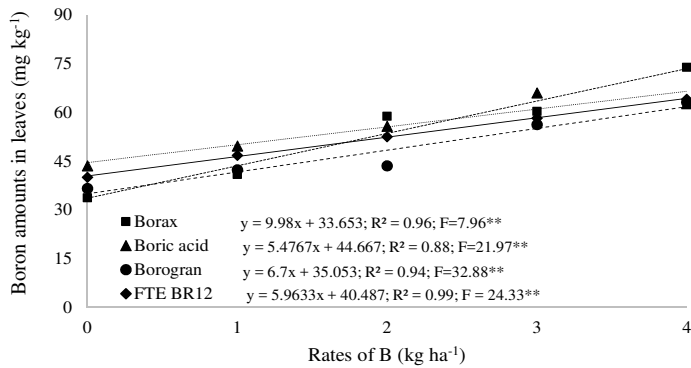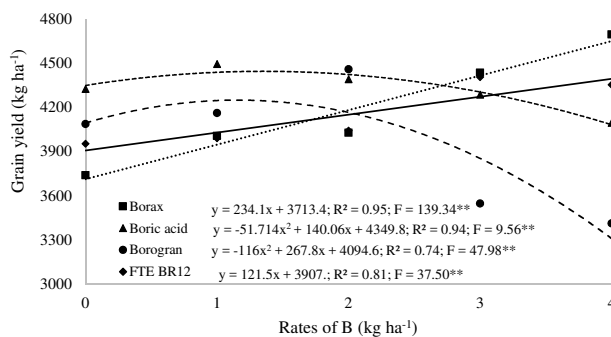

Figure 1. Boron amounts in leaves of *Phaseolus vulgaris* in function of sources and increasing boron doses in soil. ** - significant at 1% of probability by the F test.

3.2. Grain yield

The application of boron as boric acid promoted the highest grain yield when compared to the other sources regardless of the dose applied to the soil (Table 1). The highest yields were obtained using boric acid. The maximum grain yield (4,444 kg ha^{-1}) was obtained using 1.35 kg ha^{-1} of B (Figure 2).

Similarly, grain yield, when borogran was applied, presented a quadratic behavior. The maximum production (4,249 kg ha^{-1}) was obtained by using 1.15 kg ha^{-1} of B. However, for borax and FTE 12 BR, the significant adjustment was linear, i.e., the highest yields (4,649 and 4,393 kg ha^{-1}) were obtained using the highest dose, 4 kg ha^{-1} (Figure 2).

Figure 2. Grain yield of *Phaseolus vulgaris* in function of sources and increasing boron doses in soil. ** - significant at 1% of probability by the F test.

3.3. Physiological parameters and economic analyses

The application of boron did not affect the physiological quality (stomatal conductance, transpiration and internal concentration of CO_2) of bean plants in function of the source applied regardless of the dose applied to the soil (Table 2). However, the net photosynthetic rate was lower when boric acid was applied in relation to other sources (Table 2). The physiological quality (net photosynthesis rate and stomatal conductance) was influenced by the increase in the supply of boron into the soil independent from the source applied (Table 2). There was an increase in stomatal conductance amounting to 560, 588 and 493 mmol m^2 s^{-1} using 2.33, 1.96 and 2.34 kg ha^{-1}, respectively (Figure 3).

Table 2. Physiological variables of *Phaseolus vulgaris* in function of sources and increasing boron rates in soil.

| Treatments	Stomatal conductance	Liquid photosynthesis	Transpiration	Internal concentration
Sources (S)	mmol m^2 s^{-1}	μmol m^2 s^{-1}	mmol m^2 s^{-1}	ppm
Borax	423.00a	5.34ab	0.79a	396.23a
Boric acid	449.40a	4.96b	0.79a	398.04a
Borogran	445.03a	5.56a	0.81a	394.61a
FTE BR 12	429.41a	5.53a	0.80a	396.24a
F	2.77^{ns}	5.54**	0.14^{ns}	0.17^{ns}
Rates (R) (kg ha^{-1})				
0	294.63	4.17	0.78	404.80
2	451.65	5.49	0.81	397.85
4	511.15	5.68	0.80	390.23
6	526.58	6.04	0.86	395.59
8	399.54	5.31	0.76	392.92
F	125.04**	29.98**	2.56^{ns}	2.21^{ns}
S x R	27.40**	28.78**	2.25^{ns}	2.02*
C.V.	6.67	8.38	10.42	3.27

Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. C.V. - Coefficient of variation. **, * and ^{ns} significant at 1 and 5% and not significant at 5% of probability by the F test, respectively.
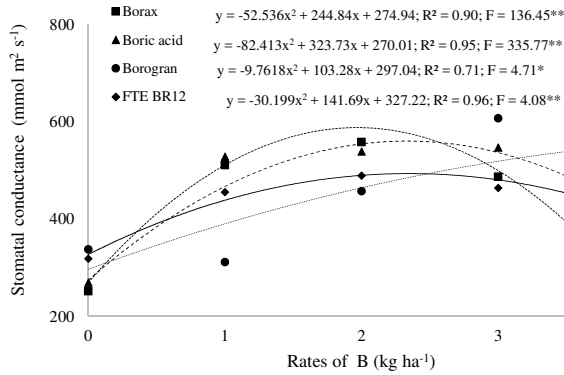
Figure 3. Stomatal conductance of *Phaseolus vulgaris* in function of sources and increasing boron doses in soil. ** and * - significant at 1 and 5% of probability by the F test, respectively.

Net photosynthesis rate had the same behavior with respect to stomatal conductance, i.e., quadratic increases in function of B doses applied to the soil (Figure 4), with the exception of borogran, which was not significant. Thus, the photosynthetic rate increased up to doses of 1.97, 4 and 1.95 kg ha^{-1}, reaching 6.2, 7.1 and 6.1 µmol m^2 s^{-1}, respectively (Figure 4).
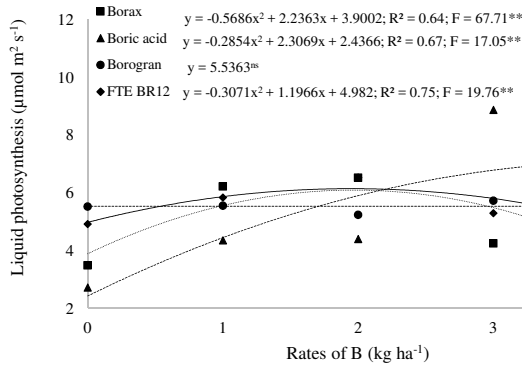
Figure 4. Liquid photosynthesis of *Phaseolus vulgaris* in function of sources and increasing boron doses in soil. ** and ^{ns} - significant at 1% and not significant at 5% of probability by the F test, respectively.

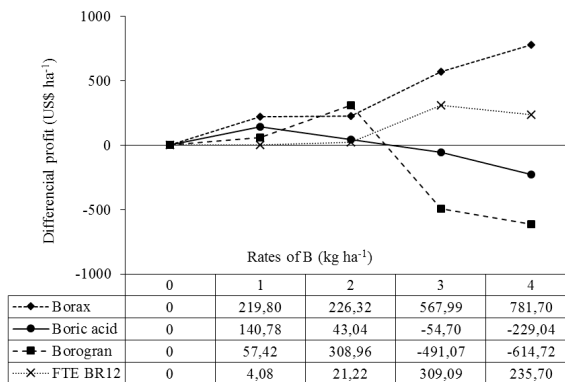When evaluating the internal concentration of CO_2, there was a significant adjustment only for boric acid, which decreased by using a high supply of boron, reaching 375.71 ppm, i.e., 10% lower when compared to the control treatment (Figure 5).
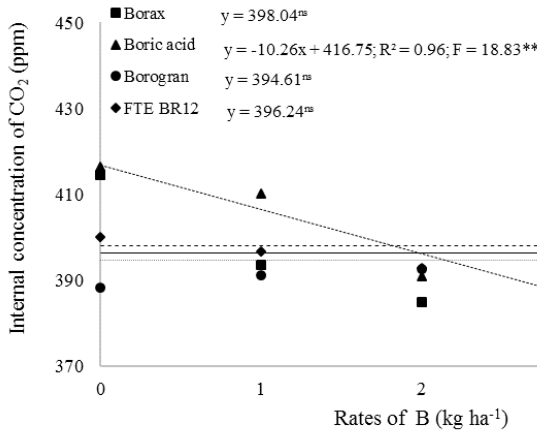
Figure 5. Internal concentration of CO_2 of *Phaseolus vulgaris* in function of sources and increasing boron doses in soil. ** and * - significant at 1 and 5% of probability by the F test, respectively.

When performing the analysis of economic viability in function of applied treatments, it was observed that all doses provided positive differential yields for the sources FTE BR12 and borax. The latter was economically more efficient than the other sources at all doses, except for the dose 2 kg ha^{-1} of boron in relation to borogran. This can be explained because, at that dose, borogran provided the maximum technical yield (Figure 6). The dose of borax was still at increasing marginal yields.

Rates of B (kg ha^{-1})	0	1	2	3	4
---◆--- Borax	0	219,80	226,32	567,99	781,70
——●—— Boric acid	0	140,78	43,04	-54,70	-229,04
- -■- - Borogran	0	57,42	308,96	-491,07	-614,72
·····✕····· FTE BR12	0	4,08	21,22	309,09	235,70

Figure 6. Differential profit of *Phaseolus vulgaris* in function of sources and increasing boron doses in soil.

The doses 1 and 2 kg ha^{-1} of boron provided positive differential yields for all sources at the price level established in this study, indicating that the application of boron, under such conditions, is economically advantageous for the producer regardless of the source.

The sources boric acid, borogran and FTE BR12 showed decreasing differential yields from the doses 1, 2 and 3 kg ha^{-1}, respectively, indicating that they are the most economically efficient doses for each source. The source borax had the highest positive differential gain: US$ 781.70 per hectare at a dose of 4 kg ha^{-1} of boron among all sources (Figure 6). However, it is noteworthy that the experiment was conducted under controlled conditions and uncontrolled factors such as drought or attacks by pests and diseases may affect the results.

4. Discussion

4.1. Relative chlorophyll index (RCI) and B content

The evaluation of RCI in function of soil boron application is justified by the fact that boron is related to the nitrogen assimilation process (N) by plants (Ouzounidou *et al*., 2013). This is because boron can optimize the synthesis of nucleic acids and proteins, increasing photosynthetic activity and carbohydrate metabolism (Malavolta, 2006). The relative chlorophyll index (RCI) is based on the intensity of the green color in leaves, which is indirectly related to the chlorophyll content and, consequently, to the N content of the plant tissue (Godoy *et al*., 2008). Thus, boron deficiency in plants causes accumulation of N-amino acids in relation to N-proteins mainly due to the action of boron in the synthesis of nucleic acids, thus affecting plant nitrogen metabolism (Prado, 2008). Wasaya etal. (2017) also observed increases in chlorophyll content following B application, which

increases in grain yield. However, Flores *et al*. (2017) studing the B effect in commun beans did not observe increases in chlorophyll content.

It is important to note that, upon the application of larger doses of boron, B contents in common bean leaves were above those considered suitable (30 to 50 mg kg^{-1}) for cultivation in the region (Dechen and Nachtigall, 2006).

The boron mobility in the soil is high. The ion-root contact process is made through mass flow, following the transpiration stream until achieving plant shoots (Dechen and Nachtigall, 2006), factors that favor the nutrient absorption process by the culture (Mattiello *et al*., 2009). However, after the absorption and transport process via xylem, boron has a limited redistribution (via phloem), which favors a greater accumulation of complexed B on the cell wall (Prado, 2008; Marschner, 2012), providing high levels of B in diagnostic leaves suitable for the assessment of nutritional status.

During the cycle, the highest boron doses applied favored the appearance of some typical symptoms of toxicity such as shriveling and burning of old leaves. This symptom is typical when there is an excess of boron in plants, which may advance to chlorosis and necrosis at the edges and tips of older leaves (Trautmann *et al*., 2014), coinciding with the region where there is a greater transpiration and nutrient accumulation (Lemiska *et al*., 2014). This behavior was also observed by Flores *et al*. (2017) with foliar doses higher than 4 kg ha^{-1} of B.

4.2. Grain yield

This may be related to the high solubility of this source, since boric acid (H$_3$BO$_3$) is in the form of crystals with a B content ranging between 17 and 18% (Trautmann *et al*., 2014). In this case, the high solubility by this source promotes a fast release of boron into the soil solution, thus favoring its absorption by the root

system via mass flow (Dechen and Nachtigall, 2006; Mattiello *et al*., 2009), especially in irrigated systems. The production of bean grains decreased when doses greater than 1.5 kg ha^{-1} were applied using boric acid and borogran as source. This behavior may have occurred mainly due to the phytotoxic effect of the soil along the furrow, thereby reducing the potential for seed germination (Lima *et al*., 2013) and the growth of the root system (Trautmann *et al*., 2014). Still, its excess in the cell groove may decrease the photosynthetic rate (Chen *et al*., 2012), interfering with enzyme activity and affecting the synthesis of chloroplasts and electron transport capacity (Simon *et al*., 2013). Flores *et al*. (2017) also observed some phytotoxic effects of B when it was applied in doses higher than 4 kg ha^{-1}, using boric acid as a source. These authors evaluated B doses up to 8 kg ha^{-1}.

Some boron fertilizers have a high solubility, which may favor their mobility in the soil profile and thus promote greater leaching rates, especially in sandy soils (Trautmann *et al*., 2014). However, the application of boric acid (a preferable form absorbed by the culture) and clayey soils favor their absorption by plants and may thus reduce nutrient leaching rates (Dechen and Nachtigall, 2006; Prado, 2008). Thus, fertilization with boron aiming a high productivity, under the study conditions and using boric acid, may reduce the recommended dose by 25% without causing injuries to grain yield.

The increase in the grain yield of common beans in function of boron fertilization is due to the functions performed by the nutrient in the plant such as germination of pollen, pollen tube growth and seed formation (Dechen and Nachtigall, 2006; Prado, 2008; Marschner, 2012; Fageria *et al*., 2015). Thus, it becomes evident that an adequate supply of B via soil, both by the source and the dose at fertilization, may improve the nutritional balance of B in common bean plants and positively influence grain yield.

4.3. Physiological parameters and economic analyses

It can be observed that the increase in leaf B, when boric acid (Figure 1) was applied, caused a decrease in net photosynthetic rates (Table 2) mainly by reducing the synthesis of sugars (Simón *et al*., 2013). It caused damages to the structures of thylakoids (Papadakis *et al*., 2004), affecting chloroplasts and electron transport capacity (Simon *et al*., 2013). The same behaviour was observed by Flores *et al*. (2017), since foliar application of B, using B doses up to 4 kg ha^{-1} (borax) also affected the photosynthetic rates in common beans. However, using boric acid as a source, a linear increase in photosynthetic rates was observed using B doses up to 8 kg ha^{-1}.

According to Silva (2007), an excessive fertilization using B may promote increases in the concentration of B in the cytoplasm. It increases the formation of complexes with NAD$^+$, directly affecting the photosynthetic process. Still, it may interfere with the absorption and metabolism of cations, especially Ca cations, and water absorption by plants (Dechen and Nachtigall, 2007).

This reduction in the internal concentration of CO_2 is directly related to the low photosynthetic rate of the plant when boric acid is applied. This is because the decrease in photosynthesis promotes the reduction of the intake of atmospheric CO_2, thereby reducing the plant internal CO_2 rate (Salisbury and Ross, 1992).

This study showed that B application on soil using doses up to 2 kg ha^{-1} is economically viable, regarless B source. However, in doses higher than that, borax showed a higher economic gain. Theses results are in accordance with Flores *et al*. (2017), who observed higher economic gain applying 4 kg ha^{-1} of B in leaves, using only one application of borax applied when flowering started.

5. Conclusions

Regarding the productive yield of common beans, the more soluble source (boric acid) promoted the greatest absorption of B by the culture, resulting in the highest yield of common beans. In this case, the results suggest that the fertilization recommendation for common bean crops grown in an irrigated system under the same soil and climatic conditions as of this study can be reduced by 25% without losses in yield. Regarding economic viability in function of differential gain, it was possible to identify differences among the economic efficiencies of sources and boron doses evaluated, suggesting the application of 4 kg ha^{-1} of borax as the most efficient source. The historical price series, used as a basis to calculate average bean sack prices, has no record of the price that provided a negative differential profit, indicating that boron application would be economically efficient over the past 15 years for the source with the greatest efficiency detected by the data of this study.

Acknowledgement

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