

## Boron sources and rates on soybean seed physiological quality and root system volume

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**Abstract:** Soybean has shown increasing performance in terms of yield in recent years. However, despite this growth, the lack of quality control and knowledge about the effects of the nutritional status of plants has compromised the germination and vigor of a significant proportion of seeds. The aim of this study was to evaluate the physiological performance of seeds and root growth of soybean cultivated with boron (B) sources with different solubilities incorporated into the soil. The experimental design was completely randomized in a 2x5 factorial arrangement, with two B sources (boric acid and ulexite) and five B rates (0, 2, 4, 8, and 16 mg.kg<sup>-1</sup>). 100-seed weight (100SW), germination, seedling shoot length (SSL), seedling root length (SRL), accelerated aging (AA), root protrusion (RP), lignin content in the seed coat (LCSC), and root volume (RV) were evaluated. The B sources and rates showed a significant interaction with increases in RV, RP, SSL, SRL, and 100SW, while the LCSC decreased with increasing rates with no interaction effect, indicating a reduction of seed quality with rates up to 8.0 mg.kg<sup>-1</sup>, regardless of the B source used.

**Index terms:** *Glycine max* L., lignin content in the seed coat, root system, seed quality.

**Resumo:** A soja tem apresentado desempenho crescente em termos de produtividade nos últimos anos. Todavia, mesmo com esse crescimento, a falta de controle de qualidade e a carência de estudos sobre os efeitos do estado nutricional das plantas tem comprometido a germinação e o vigor de parte significativa das sementes. O objetivo deste trabalho foi o de avaliar o desempenho fisiológico de sementes e crescimento radicular da soja cultivadas com fontes e doses de boro (B) com diferentes solubilidades incorporada ao solo. O delineamento experimental utilizado foi o inteiramente casualizado em esquema fatorial 2x5, com duas fontes (H<sub>3</sub>BO<sub>3</sub> e ulexita) e cinco doses de B (0, 2, 4, 8 e 16 mg.kg<sup>-1</sup>). Foram avaliados o peso de 100 sementes (P-100), germinação, comprimento da parte aérea (CPA), radicular de plântulas (CR), envelhecimento acelerado (EA), protrusão radicular (PR), teor de lignina no tegumento (LT) e volume radicular (VR). As fontes e doses de B apresentaram interação significativa, com aumento do VR, PR, CPA, CR e PS a soja, enquanto o teor de LT diminuiu com o incremento das doses sem efeito de interação, indicando efeito deletério para o aumento da longevidade para o período de armazenamento das sementes com doses acima de 8,0 mg.kg<sup>-1</sup> de B, independentemente da fonte de B utilizada.

**Termos para indexação:** *Glycine max* L., lignina no tegumento, sistema radicular, qualidade da semente.

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## INTRODUCTION

Seed lots of selection prior to planting is of fundamental importance in the production chain because the quality of germination and vigor of the initial development of the plants are necessary to obtain high yields (França-Neto et al., 2016). The seed coat protects the seed against climatic, biotic, or abiotic agents, keeps its internal parts together, and maintains its moisture content and gas exchange for an adequate quality in germination (Abati et al., 2022). The physiological quality of seeds can be reduced by deterioration resulting from biochemical and physiological changes, which cause alterations to viability and decreases in germination capacity due to disruption of cell membrane systems, resulting in an increase in cell permeability (Mann et al., 2002; Marcos-Filho, 2015).

The physiological functions and genetic factors that influence the viability and vigor of seeds are poorly understood, and in general, selection is based on varieties that have superior germination and vigor characteristics, without considering the biochemical action (Reis et al., 1989; França-Neto et al., 2016). In this context, the high lignin content of the seed coat gives the seeds less permeability, making them less susceptible to deterioration during the storage period (Abati et al., 2022). Furthermore, during fertilization, it is necessary to apply nutrients in a balanced way, considering the physiological needs of each stage of the plant.

The lack of nutrients, especially of those with low mobility in the phloem vessels, leads to the need for continuous flow so that adequate conditions exist until full seed or fruit formation (Malavolta, 2006; Camacho-Cristóbal et al., 2018). Boron (B) is one of the micronutrients that most affects yield, and its deficiency is observed in many areas. Since it is immobile in the phloem vessels and is preferentially taken up by mass flow (Barber, 1984), it is not redistributed in plants; therefore, B deficiency is more prevalent in younger or growing organs (Malavolta, 2006; Marschner, 2012).

The main functions of B are the translocation of sugars and the formation of the cell wall (Moraes et al., 2002). In seed formation, after fertilization, nutritional disturbance affects embryogenesis, resulting in seed deterioration or the formation of incomplete or damaged embryos (Brdar-Jokanović, 2020). Boric acid ( $H_3BO_3$ ) and ulexite ( $Na_2Ca_2B_{10}O_{18} \cdot 16H_2O$ ) are some of the main products used as B sources (Dameto et al., 2022), and in soybean (*Glycine max* (L.) Merrill), B is the most limiting factor for yield, and boric acid is the preferred source of this nutrient. However, the use of less-soluble sources, such as ulexite or colemanite ( $Ca_2B_6O_{11} \cdot 5H_2O$ ), can more efficiently supply sufficient quantities for the entire crop cycle (Havlin et al., 2013). Moreira et al. (2016) when evaluating the bioavailability of nutrients in seeds of 24 soybean cultivars, observed a variation from 15.8 to 36.2 mg kg<sup>-1</sup> of B, indicating different efficiency between the materials.

The aim of this study was to evaluate the effects of the rates application of two B sources with different solubilities (boric acid and ulexite) on the root volume and physiological quality of seeds of soybean grown in a Typical Red Oxisol.

## MATERIAL AND METHODS

### Site and treatments

The experiment was conducted under greenhouse conditions at Embrapa Soja located in Londrina, Paraná State (23°11'37" S, 51°11'03" W; average altitude 630 m asl). The soil used was a Typical Red Oxisol collected in Ponta Grossa, Paraná State, Brazil, in an area of native forest with the following chemical attributes at the 0–20 cm depth: pH (CaCl<sub>2</sub>) = 4.2, soil organic matter (SOM) = 38.1 g.kg<sup>-1</sup>, phosphorus (P) = 7.1 mg.kg<sup>-1</sup> (Mehlich 1 extractant), potassium (K<sup>+</sup>) = 0.3 cmol<sub>c</sub>.kg<sup>-1</sup>, calcium (Ca<sup>2+</sup>) = 2.8 cmol<sub>c</sub>.kg<sup>-1</sup>, magnesium (Mg<sup>2+</sup>) = 1.2 cmol<sub>c</sub>.kg<sup>-1</sup>, aluminum (Al<sup>3+</sup>) = 0.9 cmol<sub>c</sub>.kg<sup>-1</sup>, potential acidity (H+Al) = 8.3 cmol<sub>c</sub>.kg<sup>-1</sup>, cation exchange capacity (CEC) = 12.7 cmol<sub>c</sub>.kg<sup>-1</sup>, sulfur (S-SO<sub>4</sub><sup>2-</sup>) = 4.0 mg.kg<sup>-1</sup>, boron (B) = 0.3 mg.kg<sup>-1</sup>, copper (Cu) = 1.4 mg.kg<sup>-1</sup>, iron (Fe) = 221.6 mg.kg<sup>-1</sup>, manganese (Mn) = 29.2 mg.kg<sup>-1</sup>, zinc (Zn) = 1.1 mg.kg<sup>-1</sup>, sand = 38 g.kg<sup>-1</sup>, and clay = 510 g.kg<sup>-1</sup>. The analyses were performed according to the procedures described by EMBRAPA (1997).

### *Statistical design, fertilization, and sowing*

The experimental design used was completely randomized in a  $2 \times 5$  factorial arrangement, with two B sources [boric acid (18% of B) and ulexite (12% of B)] applied at five B concentrations (0, 2, 4, 8, and 16 mg.kg<sup>-1</sup>), with four replications. Thirty days before sowing, an amount equivalent to 5.0 Mg ha<sup>-1</sup> of dolomitic limestone (MgO > 12%, neutralizing power of 95%) was applied. Nitrogen was provided by inoculating the seeds with a cocktail of *Bradyrhizobium elkanii* + *Bradyrhizobium japonicum* and B at the concentrations within each experiment, while fertilization with P, K, S, cobalt (Co), Cu, Fe, Mn, molybdenum (Mo), nickel (Ni), and Zn was performed according to Moreira et al. (2011) as adapted from Allen et al. (1976). Those authors indicated that, for experiments conducted under greenhouse conditions, fertilization should be performed with: 150 mg.kg<sup>-1</sup> of P-monoammonium phosphate (MAP), 1.5 mg.kg<sup>-1</sup> of Cu (CuSO<sub>4</sub>·7H<sub>2</sub>O), 0.1 mg.kg<sup>-1</sup> of Mo (Na<sub>2</sub>Mo<sub>4</sub>·2H<sub>2</sub>O), 2.5 mg.kg<sup>-1</sup> of Fe (FeSO<sub>4</sub>·2H<sub>2</sub>O), 0.01 mg.kg<sup>-1</sup> of Co (CoCl<sub>2</sub>), 0.01 mg.kg<sup>-1</sup> of Ni (NiSO<sub>4</sub>·6H<sub>2</sub>O), 5.0 mg.kg<sup>-1</sup> of Mn (MnSO<sub>4</sub>·3H<sub>2</sub>O), and 5.0 mg.kg<sup>-1</sup> of Zn (ZnSO<sub>4</sub>·7H<sub>2</sub>O). At the V2 and V4 growth stages, the topdressing fertilizers were split and applied twice with 50 mg.kg<sup>-1</sup> of K (K<sub>2</sub>SO<sub>4</sub>), totaling 100 mg.kg<sup>-1</sup> in the cycle.

Ten soybean seeds of indeterminate cycle (BRS 1003 IPRO) were sown. After thinning, two uniform plants were left per 3 L clay pot. The pots were irrigated daily with deionized water to maintain the soil close to 70% of the total pore volume (TPV).

### *Seed physiological analysis*

At the end of the soybean cycle (R8 growth stage) (Fehr et al., 1971), the pods were threshed, and the samples were collected to determine the 100-seed weight (100SW) and for the subsequent testing of physiological quality of soybean seeds according to Brasil (2009). Subsequently, the seeds were weighed to determine the seed yield (SY) per pot.

Due to seed limitations, the germination test was performed with twenty-five seeds and five replications, according to the standards for analysis of soybean seeds (Brasil, 2009). For the average germination percentage, the values of normal seedlings of the four subsamples were added and divided by two.

For seedling shoot length (SSL) and root length (SRL), ten seeds with five replications were arranged with the micropyle facing the bottom of the paper, thus directing the rectilinear growth of the seedlings. After three days, the seedlings were removed from the germinator, scanned, and measured using the VIGORS® program (Leite et al., 2018).

For the accelerated aging test (AA), the seeds were divided into four replications and the procedures described by Krzyzanowski et al. (2020) were used. After 24 and 48 h of exposure to 41 °C, the samples were removed to perform the germination test and their degree of deterioration was analyzed.

For root protrusion (RP), 10 seeds per replication were placed in plastic boxes sterilized at 25 °C in a germinator. After 48 h, the seedlings' roots were measured with a ruler to determine which produced primary roots of at least 2.0 cm (Krzyzanowski et al., 2020).

The lignin content in the seed coat (LCSC) was analyzed in duplicate with 10 seeds using the method of Moreira-Vilar et al. (2014), in which the seeds remained submerged in water for approximately 12 h, the seed coat was then separated from the cotyledon, and, after this procedure, the seed coats were dried in a laboratory oven, at 60 °C, for 24 hours. The dry cotyledons with 60 mesh granulations were transferred to a 250 mL flask, 80% ethanol (w/v) was added, and the suspension was heated at 100 °C under reflux for 10 min. The dry matter obtained was ground and homogenized. After that, 0.3 g was weighed for the extraction of proteins bound to the cell wall. After the material free of proteins was obtained, the amount of lignin was quantified by the acetyl bromide method.

The roots were removed from the pots, and the root volume (RV) was quantified with a volumetric beaker as a function of each treatment.

### *Statistical analysis*

The data were subjected to the normality and homogeneity of variance tests, followed by the joint analysis of variance (ANOVA) and F-test. In the presence of significant interaction of treatments (B sources and application rates),

the necessary splits were performed ( $p \leq 0.05$ ). Regression and correlation (seed yield [SY] and root volume [RV]) tests were conducted at 5% significance level.

## RESULTS AND DISCUSSION

The results for accelerated aging (AA), germination, seedling shoot length (SSL), seedling root length (SRL), root protrusion (RP), and 100-seed weight (100SW) are shown in Table 1. The percentages of AA and germination were not influenced by the B sources and rates or the interaction between sources and rates, whereas the SSL, SRL, RP, and 100SW showed highly significant interactions. For SSL, the significance was isolated for the studied factors, and for SRL, RP, and 100SW there was a significant double interaction, and, except for SRL, which had no response to the B sources,

Table 1. Means of accelerated aging (AA), germination, seedling shoot length (SSL), seedling root length (SRL), root protrusion (RP), and 100-seed weight (100SW) in response to boron (B) sources and application rates.

B rates (mg.kg <sup>-1</sup> )	AA (%)	Germination (%)	SSL (cm)	SRL (cm)	RP (%)	100SW (g)
<b>H<sub>3</sub>BO<sub>3</sub></b>						
0	87	96	7.0	12.6	19	10.9
2	86	99	7.1	13.0	38	15.0
4	86	99	7.7	13.9	39	14.5
8	87	98	7.6	15.8	39	15.7
16	97	100	6.1	14.6	46	16.8
Mean	89	98	7.1	14.0	36	14.6
<b>Ulexite</b>						
0	92	98	7.5	13.7	17	13.4
2	80	99	8.0	14.5	28	16.1
4	96	98	8.0	14.9	42	16.7
8	96	99	7.2	14.3	32	17.1
16	96	100	7.0	14.1	36	14.7
Mean	92	99	7.6	14.3	31	15.6
<b>Mean</b>						
0	89	97	7.3	13.2	18	12.2
2	83	99	7.6	13.8	33	15.6
4	91	99	7.9	14.4	41	15.6
8	91	99	7.4	15.1	36	16.4
16	97	100	6.6	14.3	41	15.7
Mean	90	97	7.3	14.1	34	15.1
<b>F-Test</b>						
Sources (S)	0.460 <sup>NS</sup>	0.250 <sup>NS</sup>	6.387*	1.171 <sup>NS</sup>	7.439*	9.624*
Rates (R)	2.496 <sup>NS</sup>	1.469 <sup>NS</sup>	5.872*	3.549*	4.738*	9.751*
S × R	0.707 <sup>NS</sup>	0.406 <sup>NS</sup>	2.229 <sup>NS</sup>	2.803*	14.880*	15.170*
CV (%)	10.54	2.57	7.41	7.76	18.63	7.24

\* and <sup>NS</sup> indicate significant and non-significant, respectively ( $p \leq 0.05$ ). CV: coefficient of variation.

there was also an effect of B sources and rates. This result supports those of Hanson (1991) and Marschner (2012), who described that B availability influences the formation of the embryo and cotyledons and has a positive effect on the vigor and physiological quality of seeds. However, the lack of effect on AA and germination percentage demonstrates that, regardless of nutritional quality, the absence of seed coat damage and adequate moisture and temperature during the short storage time of the seeds may be the main factors for these two variables.

SSL and SRL ranged from 7.0 to 7.7 cm and 12.6 to 15.8 cm with boric acid and from 7.0 to 8.0 and 13.7 to 14.9 cm with ulexite, respectively, and the highest values occurred at 8.0 and 4.0 mg.kg<sup>-1</sup> of B, respectively (Table 1). SSL and SRL under the boric acid source showed a significant correlation with seed yield ( $r = +0.64$  and  $-0.65$ , respectively,  $p \leq 0.05$ ), whereas with ulexite, only SRL was significant ( $r = +0.73$ ,  $p \leq 0.05$ ), indicating different responses between the higher- and lower-solubility B sources. The increase in SSL and SRL is important for rapid initial development of the plants and a greater capacity to absorb nutrients from the soil at the formation and end of seed reserve. In addition, during the germination phase, the seeds of more vigorous plants are more likely to transfer the dry weight from the reserve tissues to the embryonic axis, resulting in seedlings with greater accumulation of dry weight (Nakagawa, 1999).

The values of RP and 100SW showed an interaction of B sources  $\times$  rates and significant increases with the linear increment of B rates under the boric acid source (RP,  $\hat{y} = -27.271 + 2.388x$ ,  $R^2 = 0.53$  and 100SW,  $\hat{y} = -19.373 + 1.747x$ ,  $R^2 = 0.69$ ,  $p \leq 0.05$ ), while with ulexite, there was a quadratic effect for RP ( $\hat{y} = 20.785 + 3.710x - 0.177x^2$ ,  $R^2 = 0.57$ ) and 100SW ( $\hat{y} = 13.911 + 0.865x - 0.051x^2$ ,  $R^2 = 0.91$ ) (Table 1). The mean of the B sources had a quadratic effect, with the highest values of RP and 100SW obtained at the maximum estimated rates (MER) of 16.0 and 10.0 mg kg<sup>-1</sup>, respectively. Boron aids in the transfer of sugars and other nutrients from the leaves to the reproductive organs, increasing the quality and quantity of pollination and improving seed development (Blevins and Lukaszewski, 1998; Cirak et al., 2006).

In addition, on the average of the two sources, B application resulted in a significant increase in seed yield - SY ( $\hat{y} = 18.997 + 0.690x - 0.053x^2$ ,  $R^2 = +0.61$ ,  $p \leq 0.05$ ), with maximum yield obtained with the estimated application of 6.4 mg kg<sup>-1</sup> of B, and the correlation analysis indicated that one of the factors involved was the greater 100SW ( $\hat{y} = 0.6475 + 0.705x$ ,  $r = +0.77$ ,  $p \leq 0.05$ ). Regarding RP, despite the positive effect of B rates on the mean of the two B sources, it showed no correlation with SY ( $r = +0.04$ ,  $p > 0.05$ ).

There was an effect of B sources (S) and rates (R) on the lignin content in the seed coat (LCSC) with no S  $\times$  R interaction, indicating that regardless of the B source used, the LCSC decreased with increasing B rates (Figure 1). Therefore, excessive B fertilization must be observed carefully with constant monitoring, since although high levels of LCSC are promising for maintaining the physiological quality of soybean seeds for a longer period, low concentrations can be harmful when a harvest delay is necessary (Braccini, 1993; Krzyzanowski et al., 2023). Zhou et al. (2021) observed an inverse relationship between tissue B concentration and LCSC, with greater lignin accumulation in plants with B deficiency. When analyzing the main enzymes involved in lignin biosynthesis, those authors found that B deficiency increased the activity of phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C<sub>4</sub>H), hydroxycinnamoyl, CoA reductase (CCR), and cinnamyl alcohol dehydrogenase (CAD). Similarly, the expression levels of key genes for lignin biosynthesis increased under stress caused by B deficiency, which are factors that decrease the quality and quantity of lignin in the plant. An accumulation of large amounts of lignin in the cell walls and vascular bundles of the leaf veins alters the structure and restricts the function of the vascular bundles, hindering the transportation of the sap through both the phloem and the xylem. In addition, many small, new, and structurally incomplete vascular bundles around the original are compressed, rupturing the epidermis, and altering the tissue quality (Zhou et al., 2021). Due to greater availability, the LCSC in treatments with H<sub>3</sub>BO<sub>3</sub> rates was higher than that with ulexite and ranged from 45.0 to 41.4  $\mu\text{g.g}^{-1}$  and 44.8 to 40.2  $\mu\text{g.g}^{-1}$ , with mean values of 43.1 and 42.3  $\mu\text{g.g}^{-1}$  under the H<sub>3</sub>BO<sub>3</sub> and ulexite sources, respectively. Bellaloui et al. (2012) also verified that the increase of B in the plant tissue decreases the concentration of LCSC.

Despite the negative effects of the B rates, the values were well above the 35.9  $\mu\text{g.g}^{-1}$  obtained by Gris et al. (2010) in the evaluation of physiological quality and LCSC in soybean cultivars grown under field conditions; therefore, different cultivation conditions (greenhouse and field) may have influenced these differences in LCSC levels.

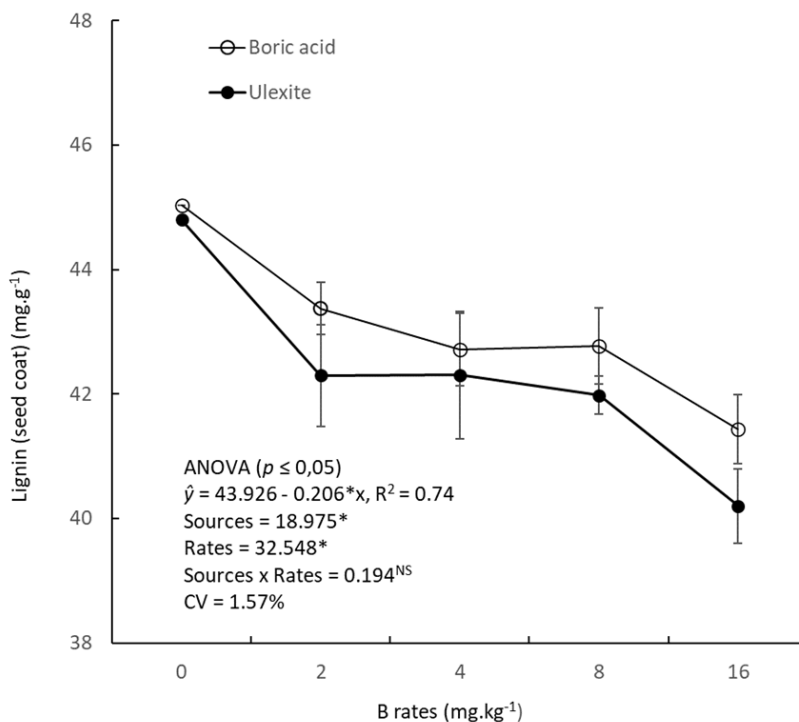


Figure 1. Lignin content of seed coat (LCSC) of soybean seeds in response to boron (B) sources (boric acid and ulexite) and application rates. \* and <sup>NS</sup> indicate significant and non-significant, respectively ( $p \leq 0.05$ ). CV: coefficient variation.

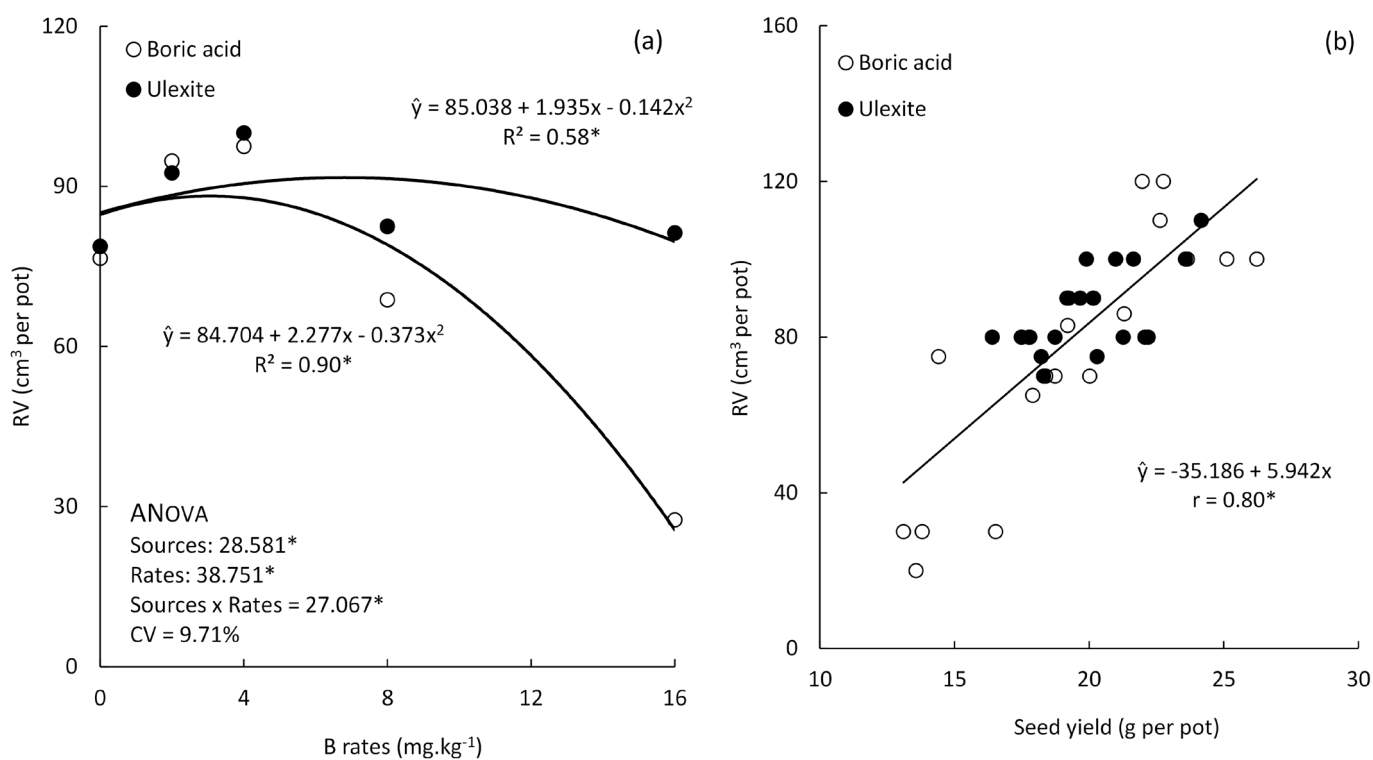


Figure 2. Effect of boron (B) sources (boric acid and ulexite) and rates on root volume (RV) in pots (a) and their correlation with seed yield (SY) (b). \*significant ( $p \leq 0.05$ ). CV: coefficient of variation.

There was a significant effect of the interaction between B rates and sources on RV (Figure 2a) and a linear response between SY and RV (Figure 2a and 2b). The sources ensured the availability of nutrients at adequate levels during the development of the plant without causing phytotoxicity up to the maximum estimated rate (MER) of 4.6 mg.kg<sup>-1</sup>. The lower RV under H<sub>3</sub>BO<sub>3</sub> at the highest B concentrations is related to the high-water solubility of this source, which allows for greater contact with the plant root system and a consequent increase in nutrient uptake (Moreira et al., 2006). The increase in RV up to the MER corroborates the arguments of Marschner (2012), who reported that the lack of B causes inhibition of root elongation due to problems observed during cell division and cell elongation, resulting in thick roots with low volumes and necrotic tips. Boron deficiency also affects the complexation of organic compounds with cis-diol function, which inhibits root growth (Römheld, 2001). Similar results of the positive effect of B on the root system were obtained by Moreira et al. (2006), who reported an increase in the number of roots and the presence of phytotoxicity of B with the high boric acid application rates.

Based on the results of the present study, the application of B in the soil is a viable alternative, with positive results for increasing the quality of soybean seeds. However, the choice of application rate must be determined with discretion depending on the solubility of the B source. Despite these advances, Cirak et al. (2006) reported that there are still contradictory results, and further studies are needed to clarify the effects of B on the physiological quality of soybean seeds.

## CONCLUSIONS

Accelerated aging (AA) and germination percentage are not influenced by B sources and rates.

100-seed weight, seedling root length (SRL), and root protrusion (RP) showed effects of B sources × rates and were closely related to the availability of nutrients in the soil, which was not observed for seedling shoot length (SSL).

Root volume (RV) was directly correlated with seed yield (SY).

The lignin content of seed coat (LCSC) has inverse relationship with the rates up to 8 mg.kg<sup>-1</sup>, regardless of the B source.

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