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Boron fertilization affects the physiological quality of soybean seeds, conventional and transgenic

Ivana da Silva Gomes[1], Cleiton Gredson Sabin Benett[1*], Ricardo Caldas Xavier[1], Katiane Santiago Silva Benett[1], Érica Fernandes Leão-Araújo[2], Anderson Rodrigo da Silva[2] and Ademilson Coneglian[1]

[1]State University of Goias, Ipameri Campus, Ipameri, Goias, Brazil (Universidade Estadual de Goiás, Campus Ipameri, Goiás, Brasil)
[2]Goiano Federal Institute, Urutai Campus, Urutai, Goias, Brazil (Instituto Federal Goiano, Campus Urutaí, Goiás, Brasil)

***Corresponding author: cleiton.benett@gmail.com**

Abstract

High physiological quality soybean seeds are essential for a successful production, because they promote optimal plant density in the field. The objective of this work was to evaluate the effect of boron doses applied at different phenological stages of two soybean varieties on the physiological quality of the seeds. Two field experiments were carried out, one with each variety of soybean, conventional and transgenic. Five doses for boron were applied to the soil as boric acid (17%): 0, 1.0, 2.0, 3.0 and 4.0 kg ha^{-1}, at three phenological stages (V6, V9 e R1). In R8 stage the seeds are harvested and then tested for physiological quality, through the tests of Germination, germination first count, germination speed index, accelerated aging and electrical conductivity. For the conventional variety, the boron doses promoted a quadratic effect on variables when the boron was applied near the reproductive stages. For the transgenic variety, the first-degree polynomial model presented the best fit, meaning that the higher the boron dose the better the physiological quality. The optimal boron application stage for producing high vigor seeds was R1 for both varieties. Four (4.0) kg ha^{-1} of boric acid is recommended for both varieties to produce high quality seeds.

Keywords: *Glycine max* L., boron dose, seeds vigor.
Abbreviations: AA_acelerated aging; CO_conventional soybean; EC_electrical conductivity; FC_germination first count; G_germination; GMO_genetically modified organism**;** GSI_germination speed index.

Introduction

The first requirement for producing a successful crop is using seeds of high physiological quality. Seeds are responsible for providing optimal plant stands in field, given that the minimum recommended number of plants per area is one of the main requirements for achieving satisfactory yields.

According to Oliveira et al. (2015), the processes used to produce, sell and distribute seeds are extremely important and their success depends on the perfect interaction between the seed breeding process and the professionals responsible for seed production, contributing significantly to the development of seeds and even more resistant and productive cultivars.

The use of high vigor seeds favors the emergence and establishment of the seedling stand, as well as the dry mass production of seedling shoot (Abati et al., 2017). Thus, adequate nutrient availability leads to satisfactory seed vigor and physiological quality, because it influences the development of the embryonic axis and reserve material needed for plant growth (Oliveira et al., 2015). The boron (B) influence the cell differentiation, cell division, carbohydrate transport and metabolism and participates in the synthesis of cell wall compounds and in the reproductive process by affecting pollination, growth of the pollen tube and production of fruits and seeds (Coetzer et al., 1990).

The use of cultivars and varieties that are highly productive and resistant to hard-to-control diseases continues to grow and has contributed significantly to production increases. However, the productive potential of those genetic materials in the Brazilian cerrado (savanna) has not always been reached, mainly because of the nutritional instability of soils (Reis et al., 2008). Low levels of organic material and high levels of sand contribute the lack of boron that is common in Brazilian soils and that has led to significant productivity losses (Oliveira et al., 1996). Gomes et al. (2017) working with boron application in different soybean cultivars indicate 3.51 kg ha^{-1} of B for the maximum soybean production without occurrence of toxicity. The occurrence of toxicity is also dependent on the type of soil, organic matter and soil moisture.

According to Furlani et al. (2001), soybean is one of the crops with the highest boron demands and frequently lack of sufficient quantities. In general, the nutritional demands of crops are highest at the beginning of the reproductive stage because this nutrient is essential for forming and developing new reserve organs (Carvalho and Nakagawa, 2012). More commonly used agronomic practices accompanied by improved varieties may favor nutrient uptake during different growth stages (Bender et al., 2015).

The boron reserve in seeds is extremely important because seed lots that are deficient in this nutrient have low germination rates caused by developmental defects that may produce weak normal or abnormal seedlings (Dordas, 2006). Studies on the relationship between boron and the physiological quality of seeds show contradictory results. Thus, the topic deserves further research. Kappes et al. (2008) showed that the quality of soybean seeds was not influenced by foliar applications of boron. Lima et al. (2013) observed increases in physiological seed quality after boron applications to the planting groove intercropped with common beans and castor beans.

Seed vigor plays a relevant role in agricultural production since more vigorous seeds provide a rapid and uniform germination, favoring shoot and root system growth, giving the plant better development conditions, as well as a greater ability to withstand adverse environmental conditions during germination process (Carvalho and Nakagawa, 2012).Thus, management practices, such as the timing and doses of micronutrient applications, especially boron, may contribute to increase soybean seed quality.

We analyzed the effect of boron doses at different phenological stages of soybean on the phenological quality of conventional and genetically modified plants.

Results and Discussion

Physiological quality tests of seed

Table 1 shows a summary of the joint analysis of variance for the conventional (CO) and genetically modified (GMO) plants regarding their physiological seed quality. The experimental factors Varieties, Doses and Stages presented, alone or through interactions, significative ($p < 0.05$) effect on all variables of physiological quality of seeds.

The GMO variety produced generally better seed quality (Table 2). This result may be related to genetic characteristics of the variety, providing tolerance to efficient weed-control herbicide and tolerance to some insect-pests, which could ultimately benefit the quality of the seeds produced.

Carvalho et al. (2012) found conflicting results when evaluating the physiological quality of conventional and GMO soybean seeds in the state of Paraná, Brazil. The study showed that the germination rate of the conventional variety (CD 206) was higher than that the GM (CD 206 RR). However, the GMO seed that Carvalho et al. (2012) evaluated was derived from a conventional cultivar and had only one genetic modification (herbicide tolerance).

Germination first count - FC (Table 1) showed triple (V x D x S). A quadratic effect of seed quality is observed for each stage and variety. The values of the CO soybean fit a quadratic model with minimum estimated values of 2.06 and 1.73 kg ha^{-1} B at V9 and R1 stages, respectively (Fig 1A). For the V6 stage no model fitted FC data. The percentage of normal seedlings in the FC started increasing at doses of 2.0 kg ha^{-1} B applied during the reproductive stage (R1). The FC also tended to increase when boron was applied during V9 at doses equal to and higher than 2.0 kg ha^{-1}. However, the number of normal seedlings obtained with 4.0 kg ha^{-1} was similar to the number without boron application. The values of the GMO soybean only fit a positive linear model for the R1 stage. The number of normal seedlings increased continuously during the five days with higher Boron doses along this reproductive stage. None of the models fit the data from the V6 and V9 stages (Fig 1B). The effect of boron when applied during the reproductive period may be related to a direct relationship between the supply of this element and pollen production capacity, since boron affects microsporogenesis, germination and particularly the development of the pollen tube (Agarwala et al., 1981). Boron affects pollen germination and growth of the pollen tube, which is important for producing quality seeds (Leite et al., 2011). High numbers of normal seedlings in the FC indicate high seed vigor because one of the first effects of low vigor seeds is a reduction in germination speed (Marcos Filho, 2015; Mondo et al., 2013). Our data confirm the role of boron in seed formation, as described by Teixeira et al. (2005) that nutrient availability influences the formation of the embryo and cotyledons, and positively affects the physiological quality of the seeds. Triple interaction was also observed for the germination (G) (Table 1). For CO soybean, a quadratic relationship was found for the three stages (Fig 1C), with minimum values of 1.73, 2.02 and 1.73 kg ha^{-1} B and the V6, V9 and R1 stages, respectively. For G, as similar to FC, seed quality increased from 2.0 kg ha^{-1} B for applications at all three stages. The G for the GMO variety, as a function of boron and application stage, fit to a quadratic model with a minimum of 4.0 kg ha^{-1} B for the R1 stage (Fig 1D). However, none of the other stages fit regression models. The highest germination rates were obtained with the dose 4.0 kg ha^{-1}. These results confirm that the physiological quality of the seeds continuously increased under boron applications during the reproductive stage of soybean plants. Table 2 shows that the germination rate of the GMO variety was 14.1% higher than that of the conventional variety.

Similar results were obtained by Lima et al. (2013), evaluating castor bean seeds intercropped with common beans and used germination first count and electrical conductivity to show that boron applications positively influenced the physiological quality of the seeds. On the other hand, Ambrosano et al. (1999) evaluated the effect of micronutrients on the physiological quality of bean seeds and observed that the treatments did not affect the percentage of normal seedlings, concluding that the micronutrients did not affect seed quality, as determined by germination test.

Furthermore, Bevilaqua et al. (2002) evaluated the physiological quality of soybean seeds and found that foliar applications with Ca and B did not increase seed quality at any application stage. Kappes et al. (2008) showed that different foliar application rates of boron at V5, V9 and R3 did not increase soybean seed quality as determined by germination test.

A study on foliar applications of Ca and B on beans demonstrated no effect on germination percentages (Silva et al., 2006). Other authors have also reported increases on physiological seed quality resulting from fertilizer applications with the addition of boron and other nutrients such as calcium (Farinelli et al., 2006).

The germination speed index showed triple interaction (V x

Table 1. Summary of the analysis of variance of germination first count (FC), germination (G), germination speed index (GSI), accelerated aging (AA) and electrical conductivity (EC) of soybean seeds as a function of variety, stage and boron doses.

Source	P-value					
	DF	FC	G	GSI	AA	EC
Variety (V)	1	<0.01	<0.01	<0.01	< 0.01	0.0002
Stage (S)	2	0.02	0.01	0.01	0.11	0.05
Dose (D)	4	<0.01	<0.01	<0.01	<0.01	0.10
Block/Variety	6	0.25	0.15	0.15	0.008	<0.01
S x D	8	0.004	0.01	0.01	0.0002	0.06
V x S	2	0.0006	0.0008	0.0008	0.06	0.01
V x D	4	0.0001	0.004	0.0004	0.006	0.14
V x D x S	8	0.006	0.02	0.02	0.003	0.23
				Mean Squares		
Residuals	84	84.8	93.7	1.46	112.4	36394
Overall mean	---	39.4	10.49	2.07	34.81	1405.84
CV (%)	---	16.50	16.55	16.55	20.58	18.05

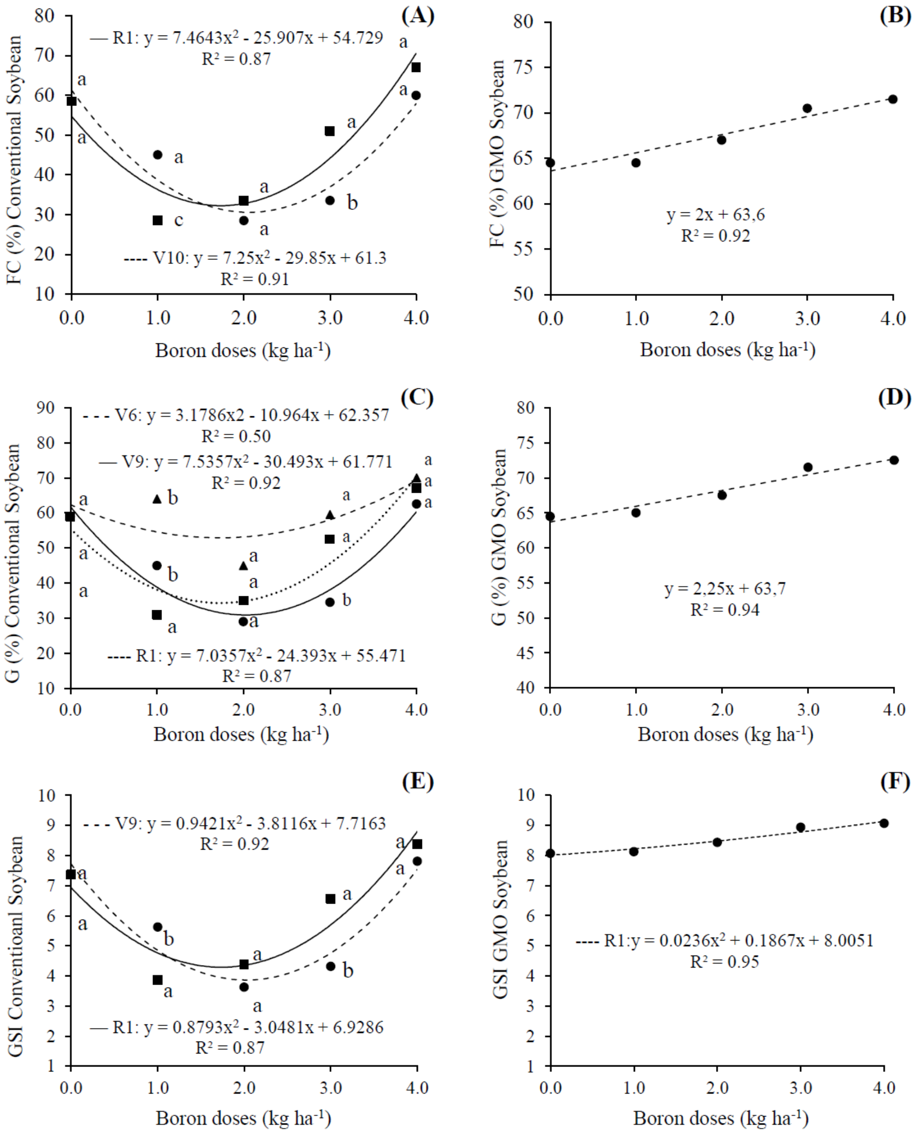

Fig 1. (A-B) Germination first count (FC), (C-D) germination (G) and (E-F) germination speed index (GSI) of a conventional soybean variety **(**on left**)** and GM variety **(**on right**)** as function of boron doses and phenological stages.

Table 2. Overall mean of germination first count (FC), germination (G), germination speed index (GSI), accelerated aging (AA) and electrical conductivity (EC) for conventional and GM soybean seeds, and mean values of electrical conductivity (EC) for conventional and GM varieties at each phenological stage.

Variety	FC	G	GSI	AA	EC
	---------- % ----------		------	---- % ----	µS cm^{-1} g^{-1}
Conventional	50.2 b*	51.4 b	6.43 b	46.23 b	77.3 a
GM	64.9 a	65.5 a	8.18 a	56.79 a	62.6 b
CV (%)	16.50	16.55	16.55	20.58	18.05

Variety	V6	V9	R1
	------------------------- EC (µS cm^{-1} g^{-1}) -------------------------		
Conventional	68.45 a*	67.21 a	64.46 a
GM	63.46 b	57.60 b	51.81 b
CV%		18.05	

*Means followed by the same letter within a column do not differ by the Tukey test at 5% probability.

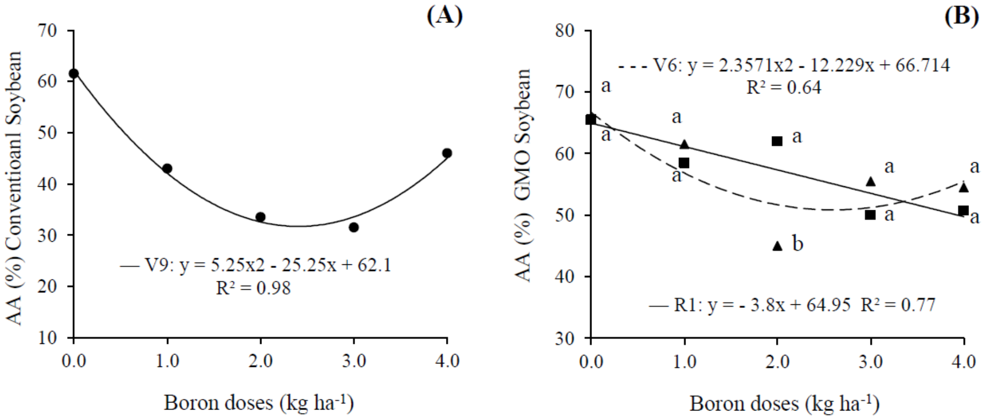

Fig 2. Accelerated aging (AA) of a conventional soybean variety (A) and a GM variety (B) as a function of boron doses and phenological stages.

D x S) (Table 1). As similar to the data found for FC for CO soybean, a quadratic fit was found for the V9 and R1 stages with minimum values of 2.03 and 1.74 kg ha^{-1} B, respectively (Fig 1E). A dose of 4.0 kg ha^{-1} B during the reproductive stage (R1) produced seeds with higher germination speed, relating greater seed vigor.

A higher GSI for the GMO soybean variety followed the results obtained for the FC and G only in the R1 stage, in which the data fitted a quadratic model with critical point at 3.95 kg ha^{-1} B (Figure 1F).

Farinelli et al. (2006) evaluated the physiological quality of seeds of common bean cultivars fertilized with foliar applications of calcium and boron. Quadratic effects were found for the Perola cultivar, with maximum vigor achieved with a commercial application of 3.2 L ha^{-1} (32.3 g ha^{-1} Ca + 1.61 g ha^{-1} B), whereas for the cultivar Campeão 2, increases in doses resulted in linear increases in vigor.

The GSI results were similar to the FC, emphasizing increases in physiological quality of both varieties with boron applications close to the reproductive period of this species. GSI and FC help to indicate the vigor of a seed lot – the higher the vigor the faster the germination process, for It is known that seed quality is the sum of several factors that contribute to stronger seedlings that can germinate and emerge faster and more uniformly (Carvalho et al., 2012).

For AA, the joint analysis of variance also showed triple interaction (V x D x S) (Table 1). The data fit a quadratic regression model with a minimum obtained with 2.40 kg ha^{-1} B (Fig 2A) in V9 stage. For the GMO variety, the quadratic model provided the best fit to the data from the V6 stage with a minimum obtained with 2.59 kg ha^{-1} B and linear fit for the R1 stage (Fig 2B). The results for AA test have shown the negative effect of boron for the quality of seed.

When seed vigor was determined by a stress-resistance test, as in the case of the AA test, the application doses of boron at different stages did not effectively improve the ability to tolerate adverse conditions before and during seedling formation. Boron is important for sugar translocation and cell-wall formation (Bevilaqua et al., 2002). Thus, understanding how boron applications affect these processes after accelerated aging merits further study. One of the first effects of seed deterioration is the disruption of cell membranes (Marcos Filho, 2015). Thus, given that boron acts in cell-wall formation, new studies should be carried out to understand the relationship between cell-wall formation and membrane conformation. The EC variable had a significant ($p < 0.05$) effect of interaction between variety and stage (V x S) (Table 1). Electrical conductivity was higher for the CO variety than for the GMO at all stages (Table 2). High electrical conductivity levels are caused by greater quantities of ions that are released due to impaired membrane integrity, a characteristic of low seed quality (Krzyzanowski et al., 1999).

Isolating the variety factor, the GMO soybean had EC values that were 18.93% smaller than those of the CO soybean (Table 2). The EC values recorded in this study were less than 80 µS.cm^{-1} g^{-1}, which indicates seeds of low vigor (Krzyzanowski et al., 1999). This may be explained by weather conditions that were unfavorable for seed production during the experiment, especially regarding excessive rains at harvest time and strong pressure from insect pests.

Material and Methods

Location

The experiments were carried out in the 2014/2015 crop grown in the agricultural experiment fields at the State University of Goiás – Ipameri Campus, located in Ipameri, Goiás, Brazil (17º43'04'' South, 48º08'43'' West, altitude = 794 m). The climate of the region is classified as Humid Tropical (AW, Köppen) with high temperatures, rainy summers and dry winters. The soil within the experimental area was classified as a Red-Yellow Latosol (Haplustox) (Santos et al., 2013).

Chemical and particle size analyses were carried out before setting up the experiments and using the methodology proposed by Ribeiro et al. (1999). The following chemical attributes were found in the 0.0-0.20 m layer and classified as average: 6.4 mg dm^{-3} P (Melich), 20 g dm^{-3} O.M., 4.7 pH ($CaCl_2$), K, Ca, Mg and H+Al = 2.5, 10.0, 4.0 and 35.0 mmol$_c$ dm^{-3}, respectively, 32% base saturation and B = 0.4 mg dm^{-3}. The particle size analysis of the soil showed: 475, 75 and 450 g kg^{-1} of clay, silt and sand, respectively. The B content presented in the soil analysis is considered average for the Cerrado soils, according to Raij et al. (1996).

Plant material and experiments installation

Two varieties of soybean were used for the experiments. A conventional soybean (CO) variety - BRS 7980 and a transgenic soybean (GMO) variety - M 7739 IPRO, both with average cycle. The technology in the GMO variety is that which gives control to the main insect-pests of soybean crop and tolerance to the use of glyphosate. Two experiments were carried out, one for each variety. Plots consisted of six rows (5 meters long), spaced 0.45 m apart with 15 plants per linear meter. The useful area consisted of the three central rows, disregarding one meter from each side of the plot length.

The varieties were planted on November 19, 2014 using the no-till method on *Brachiaria decumbens* straw, 2014. Fertilizer applications were carried out in accordance with the soil analysis and the recommendations of Ribeiro et al. (1999) and consisted of 450 kg of 02-20-18 (N-P_2O_5-K_2O). Sowing was done using a seeder-fertilizer with eight lines and a seed distribution mechanism consisting of horizontal perforated discs and fertilizer row applicators.

Boron applications

The treatments consisted of soil applications of boron at different stages of development of plants. The stages were: V6 (sixth node, fifth open trifoliolate leaf), V9 (ninth node, eighth open trifolium) and R1 (beginning of flowering, up to 50% of plants with a flower), according to the phenological scale of Ritchie et al. (1994). Five doses for boron were applied to the soil using boric acid (17%): 0, 1.0, 2.0, 3.0 and 4.0 kg ha^{-1}. Boron was applied manually during the pre-defined stages.

Physiological quality tests

Seed harvesting and threshing were carried out by hand at the R8 stage, when seed moisture content was approximately 13% (wet basis). The seeds were then stored in Kraft paper bags for 90 days at a relative humidity of 60% and temperature of 16 ± 2 °C. Evaluations of physiological quality of seeds were conducted at the Seed Laboratory of the Goiano Federal Institute - Urutaí, Goiás, Brazil. The details of the physiological quality analysis are described below.

Germination (G) – four replications of 50 seeds sown in germination paper moistened with deionized water equal to 2.5 times the mass of the dry paper. Rolls were made and then placed in a germination chamber at a constant temperature of 25 ºC. Normal seedlings were counted after eight days from sowing and expressed as percentage (Brasil, 2009).

Germination first count (FC) – conducted in along with the germination test. Seedlings were evaluated after five days from sowing and the results were expressed as percentage of normal seedlings (Brasil, 2009). Germination speed index (GSI) – conducted along with the germination test. Seedlings were counted after 24 hours from sowing and then, every 24 hours until the end of the germination test. GSI was calculated using the formula presented by Maguire (1962). Accelerated aging (AA) – determined using the gerbox method. The gerboxes, with distilled water in the bottom and the seeds under the stainless-steel screen, were kept inside the germination chamber for 72 hours at a constant temperature of 42 ºC. After this time, the seeds were sowed as described in the germination test (Brasil, 2009).

Electrical conductivity (EC) – four replications of 50 seeds were used. Each replication was weighed on a precision (0.01 g) scale. The seeds were then soaked in plastic cups (200 mL) with 75 mL deionized water. The seeds were submerged for 24 h at a constant temperature of 25 ºC (AOSA, 2002). After soaking, conductivity was determined using a conductivity meter and the results expressed as µS cm^{-1} g^{-1}.

Experimental design and statistical analysis

Was carried out a joint analysis of the two experiments (varieties). The experimental design was randomized blocks arranged in a factorial 3 x 5 (stages x doses) with four replications. The data were subjected to analysis of variance and the means compared by the Tukey test at 5% probability for varieties and stages. The boron doses effect was evaluated by regression analyses. All statistical analyses were performed using the software R version 3.1.2 (R Core Team, 2015).

Conclusion

The optimal boron application stage for producing high vigor seeds was R1 for both conventional and transgenic soybean. Four (4.0) kg ha^{-1} of boric acid is recommended for both varieties to produce high quality seeds.

Conflict of interests

The authors have not declared any conflict of interests.

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