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ORIGINAL ARTICLE

Boron in the phytotechnical, reproductive, and seed production components of hybrid Brachiaria (*Brachiaria brizantha* x *Brachiaria ruziziensis*) BRS RB331 Ipyporã

Boro nos componentes fitotécnicos, reprodutivos e de produção de sementes da braquiária híbrida (Brachiaria brizantha x Brachiaria ruziziensis) BRS RB331 Ipyporã

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ABSTRACT: The objective of this study was to evaluate the effects of boron at different doses and times of application on BRS RB331 Ipyporã. Two outdoor pot tests were conducted at the 2016/17 crop. In both tests, the treatments were represented by four doses of boron and control and two times of application in a randomized block design in a factorial scheme with three replications, and each plot represented by a pot with one plant. The substrate was composed of sand:soil (3:1). Fertilization was performed according to the need, considering the treatments. Substrate samples were analyzed. This experiment evaluated the number of vegetative tillers and plant height in two different times, chlorophyll and leaf analysis before the beginning of floral differentiation; flowering onset, number of reproductive tillers, and number of fully expanded inflorescences; quantification, viability, and pollen germination in full flowering. Seeds were evaluated for percentage of number and weight of full and empty seeds, germination, germination speed index, first germination count, and viability. Fresh and dry biomass and dry mass of the aerial part and roots were evaluated at the end of the experiment. The variables were analyzed using Assistat 7.7 software. Boron, applied at different doses and dates, did not affect the production (yield and quality) of BRS RB331 Ipyporã seeds. The phytotechnical components of the BRS RB331 Ipyporã hybrid were not influenced by the addition of boron. Boron had a positive effect on root production of the hybrid BRS RB331 Ipyporã.

RESUMO: *Objetivou-se avaliar os efeitos do boro em diferentes doses e épocas de aplicação na BRS RB331 Ipyporã. Foram conduzidos dois ensaios em vasos, a céu aberto, safra 2016/17. Em ambos os ensaios os tratamentos foram representados por quatro doses de boro e duas épocas de aplicação mais um tratamento controle (4x2+1), delineados em blocos casualizados, em esquema fatorial, com três repetições, e cada parcela representada por um vaso com uma planta. O substrato foi composto por areia:solo (3:1). A adubação foi efetuada de acordo com a necessidade, considerando os tratamentos. Amostras do substrato foram analisadas. Foram avaliados número de perfilhos vegetativos e altura da planta em duas épocas, clorofila e análise foliar antes do início da diferenciação floral; início do florescimento, número de perfilhos reprodutivos e número de inflorescências totalmente expandidas; quantificação, viabilidade e germinação de pólen no pleno florescimento. As sementes foram avaliadas quanto à porcentagem do número e do peso de sementes cheias e vazias, germinação, índice de velocidade de germinação, primeira contagem de germinação e viabilidade. A biomassa verde e seca e matéria seca da parte aérea e das raízes foram avaliadas ao final. As variáveis foram analisadas com programa Assistat 7.7. O boro, aplicado em diferentes doses e épocas, não afetou a produção (produtividade e qualidade) de sementes de BRS RB331 Ipyporã. Os componentes fitotécnicos do híbrido BRS RB331 Ipyporã não foram influenciados pela adição de boro. O boro foi benéfico para a produção de raízes do híbrido BRS RB331 Ipyporã.*

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1 Introduction

Launched by Embrapa Gado de Corte, in 2017, in partnership with UNIPASTO, BRS RB331 Ipyporã entered the market to meet the demand for new cultivars with good productivity, relatively easy handling, and a high degree of resistance to the pasture leafhopper (Valle *et al.*, 2017).

Similar to other forage genotypes, BRS RB331 Ipyporã shows high productivity of empty spikelets, which is always a challenge to be overcome in tropical forage breeding programs and which may be caused by several factors (Valle *et al.*, 2017).

This low production may result from irregularities in meiosis, however, environmental interferences may occur in the genetic control of this cell division, as differences in seed productivity have already been observed in some forage hybrids when cultivated in different regions (França, 2011). Araújo *et al.* (2008) pointed out that the behavior of a forage plant results from the interaction of its genetic potential with the environment and, according to França (2011), this interaction may affect pollen viability, making it male sterile or that the hybrid genotype may have a gametophytic or sporophytic incompatibility allele, preventing the formation of the endosperm.

It is known that boron (B) is a micronutrient that in the reproduction phase plays an active role in pollen tube growth, seed retention, protein synthesis, and carbohydrate translocation (Almeida *et al.*, 2015). Taiz & Zeiger (2017) also state that boron is an essential element for plant growth and takes part in several processes such as cell elongation, lignification, cell wall structure, respiration, and nucleic acid metabolism.

Javorski *et al.* (2015) found that the application of calcium and boron through the leaves in the V6 stage of the maize crop influenced the seed yield at the dose of 6 $L.ha^{-1}$. Galindo *et al.*, (2018) when evaluating the effect of forms of application and doses of boron in the wheat crop, found that the application of B in the soil at sowing at an approximate dose of 2 kg ha^{-1} provided a greater number of spikelets per ear and wheat grain yield.

Almeida *et al.* (2015) observed that in *Panicum maximum* Jacq. cv. Mombasa, the production of apparent and pure seeds per panicle and per unit of area, as well as the mass of a thousand pure seeds were increased with the foliar application of boron in early March and the same did not occur when applied at the beginning of anthesis, which demonstrates the importance of the time of application of the nutrient on seed production. In addition, they found that boron application did not have consistent effects on the physical purity and germination of seeds.

In this context, the objective of this work was to evaluate the effects of boron at different doses and times of application on the phytotechnical, reproductive, and seed production components of Brachiaria BRS RB331 Ipyporã.

2 Materials and Methods

Two tests were carried out outdoor using 15-L pots in Campo Grande, State of Mato Grosso do Sul in the 2016/17 harvest.

In the first trial, the treatments were represented by four doses of boron (0.5; 1.0; 2.0 and 4.0 $kg.ha^{-1}$), in the form of borax (10%) and carried out in two different times of application (base and topdressing, at 41 days after sowing - DAS) in addition to a control treatment (boron zero) constituting a randomized block design in a 4x2+1 factorial arrangement, totaling nine treatments with three replications each, and each plot represented by a pot with a plant.

For the second trial, the treatments were four doses of boron (1.0; 2.0; 4.0; and 8.0 $kg.ha^{-1}$), in the form of borax sulfate (10%), split into two combinations of time of application, namely: 1) base (0.5; 1.0; 2.0 and 4.0 $kg.ha^{-1}$) + topdressing at the beginning of floral differentiation at 125 DAS (0.5; 1.0, 2.0 and 4.0 $kg.ha^{-1}$); 2) topdressing at 41 DAS (0.5; 1.0; 2.0 and 4.0 $kg.ha^{-1}$) + topdressing at 125 DAS (0.5; 1.0; 2.0 and 4.0 kg $.ha^{-1}$) in addition to a control treatment (boron zero). Similar to the previous test, a randomized block design was adopted, in a 4x2+1 factorial scheme, (four doses, two combinations of time of application and one control treatment), totaling nine treatments with three replications each, and each plot represented by a pot with a plant.

The substrate used in the experiment was 3:1, three parts of washed sand and one part of medium-textured soil (25% clay). Substrate density was evaluated in both components and in the substrate, mixture using the test tube method (Embrapa, 1979), resulting in 1.69 $g.dm^3$, a value used as a basis for correction and fertilization. The soil was amended and fertilized with 2.5 $t.ha^{-1}$ of limestone (90.2% PRNT), 133 $kg.ha^{-1}$ of KCl; 227 $kg.ha^{-1}$ of triple superphosphate; 43 $kg.ha^{-1}$ of urea; 333 $kg.ha^{-1}$ of gypsum; 18.6 $kg.ha^{-1}$ of manganese sulfate; 15 $kg.ha^{-1}$ of iron chelate; 15 $kg.ha^{-1}$ of zinc sulfate heptahydrate; 513 $g.ha^{-1}$ of sodium molybdate and 12.5 $kg.ha^{-1}$ of copper sulfate. The borax decahydrate was added according to the treatments described in the epigraphs. Each pot was filled with 22 kg of the substrate and composite samples from all treatments were collected and analyzed in the laboratory. It should be stressed that all fertilization carried out in topdressing fertilization was carried out in the form of a solution (dissolved in 250 mL per pot) and carried out after the interruption of manual irrigation for two days.

The seeds were treated with 0.07 kg of carboxin + 0.07 kg of thiram.100 kg^{-1} seeds and 0.0625 L of fipronil.100 kg^{-1} seeds on the day before sowing, which was carried out on November 16, 2016, using four to five seeds per pot, with subsequent thinning (December 21, 2016) leaving only one plant per pot.

Pest control was carried out by applying 0.71 gL^{-1} thiamethoxam + 0.53 $g.L^{-1}$ lambda-cyhalothrin, on two occasions, at a dose of 5 $mL.L^{-1}$ and manual weed control was recurrently performed.

Irrigation was performed manually as needed. For this, the water saturation in the soil of the pots was initially calculated and, based on that, by measuring the average weight of ten pots every two days, the soil was maintained at 60% of its field capacity.

On February 7, 2017, and March 15, 2017, topdressing fertilization was performed with 0.44g of urea per pot and 0.44g of potassium chloride per pot diluted in 250 mL of water.

The beginning of floral differentiation was determined by a visual evaluation of the vegetative tillers every two days. For all treatments, it started on March 21, 2017. The number of vegetative tillers and plant height at 69 DAS (February 24, 2017) and 111 DAS (April 04, 2017) were evaluated. Chlorophyll (SPAD unit) was measured at 111DAS (April 04, 2017) at the pre-flowering using a portable chlorophyll meter "Minolta SPAD-502" in 10 tillers per pot, on the first or second fully expanded leaf.

Leaf analysis was performed in the pre-flowering of the plants and, for that, samples composed of the third fully expanded leaves from the apex to the base of the plant were collected.

Flowering onset was also evaluated when no more than one inflorescence was fully expanded in at least one pot per treatment, which occurred on March 27, 2017, corresponding to 131 DAS. From then on, the following evaluations were carried out, three times a week: number of reproductive tillers and number of fully expanded inflorescences. The evaluation was completed at seed maturation and beginning of seed shattering, immediately before harvest.

For pollen quantification, an inflorescence was collected in each pot at pre-anthesis. Pre-anthesis corresponds to the closed spikelet next to the spikelet in anthesis, that is, open flower. Under a stereoscopic microscope, a spikelet was opened to remove an anther from the hermaphrodite flocculus. The anther was transversally cut in the medial region and macerated with a needle inside a 2 mL microtube, containing 180 µL of distilled water and 20 µL of carmine propionic. Then, the microtube was shaken (inverted 10 times) to homogenize the pollen grains. For quantification, the volume of 20 µL of this suspension was placed in a Neubauer chamber and all pollen grains contained in the four quadrants were counted, which corresponded to 0.1 mm³ per quadrant in the two rulers (total of eight quadrants), with the aid of a microscope with a 20x-objective lens and a final magnification of 200x. Subsequently, the quantification was extrapolated to 200 µL and the result was expressed as the amount of pollen grain per anther, regardless of its morphology and viability.

Pollen viability was evaluated using an anther taken from the same spikelet used for pollen quantification, which was macerated with the aid of a needle on a microscopic slide. A drop of propionic carmine dye (1%) was placed on the slide and then covered with a glass slide and observed under a microscope with a 20x-objective. The stained pollen grains were considered fertile and the colorless ones were infertile.

For the evaluation of pollen germination, the inflorescences were collected in the anthesis phase (around 10:00 a.m.), taken to the laboratory, and kept in a container with water and, when determined, were "beaten" on the culture. The medium used was M4, composed of 100 ml of distilled water, 1.0 g of agar, 10 g of sucrose, 0.03 g of boric acid, 0.15 g of calcium chloride, and 17 µl of streptomycin. The medium containing the pollen was taken to a BOD at a temperature of 27.5°C and a photoperiod of 8 hours of light and 16 hours of dark and, after 24 hours, it was evaluated under a microscope (100x), in two fields, for the total amount of grains and the number of germinated ones, obtaining the average percentage of germinated grains.

Seeds were harvested per pot three times a week, starting on May 2, 2017, and ending at the end of June. The seeds were evaluated for viability through the tetrazolium test, for which 120 seeds were used per plot. The seeds were pre-conditioned for 24h in water and then longitudinally sectioned to expose the embryo. Afterward, they were soaked in a tetrazolium solution (0.5%) for four hours at a temperature of 30ºC and then analyzed following RAS standards (Brasil, 2009).

For the standard germination test, gerboxes-type boxes were used. Two sheets of blotting paper were placed in each box, moistened with distilled water with 2.5 times the weight of the dry substrate, according to RAS (Brasil, 2009). The boxes were placed under an alternating regime of temperature and light (15°C for 16 hours and 35°C for 8 hours) and distilled water was used to maintain humidity. Germination was evaluated for the percentage of normal seedlings. Counts were performed daily until 21 days, according to RAS (Brasil, 2009).

To calculate the GSI, the formula of Maguire (1962) was used, when the first germination count (FGC) consisted of the percentage of normal seedlings of the first count of the germination test, carried out on the seventh day after installation, according to the RAS recommendations (Brazil, 2009).

For the fresh biomass, at the end of the seed harvest, the fresh material of the leaves and roots was weighed and subjected to drying in a forced ventilation oven at 60°C, until reaching constant weight for the determination of dry biomass and dry matter.

The assessed variables were submitted to analysis of variance and the means by Tukey's test, using the Assistat software version 7.7 (Silva & Azevedo, 2009).

3 Results and Discussion

In the chemical analysis of the substrate of the first and second tests, the base saturation value was approximately 32.47%, which is lower than expected as liming was performed to raise the saturation to a value of 60%. This fact may have occurred because of the short time for the limestone reaction in the substrate in the interval from fertilization to soil analysis. The pH in water the average values were 5.43 and 4.80 for the pH in $CaCl_2$, while the organic matter was 10.67 g.dm^{-3}, a reasonably good value considering the proportion of sand used to form the substrate. Regarding the other values, the means were 4.3 mg.dm^{-3} of P; 0.14 cmol.dm^{-3} of K; 0.62 cmol.dm^{-3} of Ca; 0.3 cmol.dm^{-3} of Mg; 1.1 cmol.dm^{-3} of Ca+Mg; 0.07 cmol.dm^{-3} of Al; 2.5 cmol.dm^{-3} of H; 2.57 cmol.dm^{-3} of Al+H; 1.24 cmol.dm^{-3} of S and 3.81 cmol.dm^{-3} of T.

In relation to micronutrients, all of them presented very similar values, with means of 146.0 mg.dm^{-3} of Fe, 42.6 mg.dm^{-3} of Mn, 1.72 mg.dm^{-3} of Zn and 3.53 mg.dm^{-3} for Cu. As for boron, the values differed among treatments, which was already expected, with 0.12 mg.dm^{-3} in the control treatment, 0.15 mg.dm^{-3} for 0.5 kg.ha^{-1} of boron; 0.4 mg.dm^{-3} in the dose 1.0 kg.ha^{-1} of boron; 0.87 mg.dm^{-3} for 2.0 kg.ha^{-1} of boron and 2.28 mg.dm^{-3} at a dose of 4.0 kg.ha^{-1} of boron.

For the foliar analysis of the first and second tests, all treatments showed similar amounts of absorbed nutrients with averages 8.4 g.kg^{-1} N; 0.34 g.kg^{-1} P; 17.0 g.kg^{-1} K; 3.9 g.kg^{-1} Ca; 3.2 g.kg^{-1} Mg and 0.8 g.kg^{-1} S. As for the micronutrients, the means were 185.0 mg.kg^{-1} Fe; 25.0 mg.kg^{-1} Mn; 10.0 mg.kg^{-1} Zn and 2.1 mg.kg^{-1} Cu. In relation to boron, the means of treatments in the first trial was 19.8 mg.kg^{-1}, and the mean of the control treatment (zero boron) was 12.9 mg.kg^{-1} of B, which shows a lower absorption concerning the treatments, as expected. As for the second trial, the treatment at a dose of 8 kg.ha^{-1}, split into two times of application (topdressing fertilization at 41 DAS plus topdressing at 125 DAS), resulted in the highest absorption (59.26 mg.kg^{-1}) and the lowest absorption (12.97 mg.kg^{-1}) in the control treatment. The overall mean of boron absorption in the other treatments was 27.5 mg.kg^{-1}.

Regarding the phytotechnical components (Table 1), fresh biomass (BV) and dry matter (%DM) of leaves were not influenced by boron application at different times. As for the dry biomass (DS) of leaves, a difference was observed for the time factor, in which the boron applied to the substrate resulted in the highest DB production (64g).

Fresh root biomass was influenced by the dose and time of application factors but without interaction between these factors. For the dose, the treatment that showed the highest root production was 2 kg.ha-1 of boron (529g), and the lowest one occurred at the dose of 0.5 kg.ha-1 of boron (356g). When considering the time of application, the most appropriate was the operation performed on the substrate, 532g per plant.

For dry biomass, dry matter and root density, only the time of application provided a difference in the management result, with the treatment on the substrate and the best results were 180g, 32.9%, and 0.13 respectively.

Such results for the phytotechnical components showed that boron does not influence the production of biomass of the aerial part and has positive effects on the production of roots of BRS Ipyporã, with the best dose of 2 kg.ha-1 of boron applied to the substrate. Trautmann *et al*. (2014), in a study with soybean, concluded that the application of increasing doses of B in Eutrofferric Red Latosol with a sandy texture negatively interferes with the development of the root system and does not influence the development of the aerial part.

Table 1. Fresh biomass (FB), dry biomass (DB), and dry matter (DM) of the aerial part and roots from the first BRS Ipyporã test, under different doses of boron and times of application (Base-B, Topdressing 41 DAS-TD1). Campo Grande-MS, 2016.

Tabela 1. Biomassa verde (BV), biomassa seca (BS) e matéria seca (MS) da parte aérea (Folhas) e das raízes do primeiro ensaio BRS Ipyporã, sob diferentes doses de boro e épocas de aplicação (Base-B, Cobertura 41 DAS-C1). Campo Grande-MS, 2016.

Leaves						
	FB (g)		DB (g)		**DM (%)**	
D/E	**B**	**TD1**	B	TD1	**B**	**TD1**
0.0	**115aA[1]**	**115aA**	59abA	59aA	**51.0aA**	**51.0aA**
0.5	**120aA**	**96aA**	62abA	52aA	**52.1aA**	**54.8aA**
1.0	**106aA**	**111aA**	56bA	56aA	**53.3aA**	**50.7aA**
2.0	**128aA**	**108aA**	64abA	56aA	**50.8aA**	**52.3aA**
4.0	**162aA**	**121aA**	75aA	55aB	**52.4aA**	**46.7aA**
Mean	**119**		60		**51.8**	
Control treatment	**115**		59		**51.4**	
CV	**16.94**		**12.31**		**6.56**	

Root								
	FB (g)		**DB (g)**		DM (%)		**D**	
D/E	B	TD1	**B**	**TD1**	B	TD1	**B**	**TD1**
0.0	520abA[1]	520aA	**183aA**	**183aA**	34.4aA	34.4aA	**0.13aA**	**0.13aA**
0.5	433bA	278bB	**148aA**	**63aA**	33.6aA	22.7aB	**0.11aA**	**0.04aA**
1.0	514abA	323abB	**152aA**	**79aA**	29.6aA	24.4aA	**0.11aA**	**0.06aA**
2.0	654aA	405abB	**254aA**	**130aB**	37.4aA	31.6aA	**0.18aA**	**0.09aB**
4.0	628abA	343abB	**167aA**	**97aA**	31.1aA	27.9aA	**0.12aA**	**0.07aA**
Mean	447		**136**		29.8		**0.10**	
Control treatment	520		**183**		34.4		**0.13**	
CV	19.46		**36.27**		19.24		**36.27**	

[1] Means followed by the same uppercase letter in the rows and lowercase letters in the columns do not differ by the test of Tukey at 5% probability.

[1] Médias seguidas pela mesma letra maiúsculas nas linhas e minúscula nas colunas não diferem entre si pelo teste de Tukey, a 5% de probabilidade.

In the second test, for the fresh biomass (FB) and dry biomass (DB) of the leaves, there was no influence of the micronutrient boron (Table 2). As for the dry matter (%DM), it was observed that application of boron in the substrate in addition to the topdressing at 125 DAS (B+TD2) resulted in the best production (54.2%).

For all phytotechnical components of the root, whose results were obtained from the development of the second test, there was a difference in the time of application factor, in which the treatment B+TD2 showed the best results in relation to the application TD1+TD2, with values of 512g for fresh biomass, 185g for dry biomass, 35.3% for dry matter and 0.13 for root density. However, in none of the analyses, a difference was observed between the boron doses or between the treatments and the control treatment, so it can be said that boron did not influence the biomass production of the BRS Ipyporã plant in the second test.

In the first trial, plant height at 69 DAS (A69) was influenced by the combination of application times and doses (Table 3). The dose of 0.5 kg.ha^{-1} in the topdressing at 41 DAS (31 cm) provided the highest plant height in relation to the other doses (29 cm), as well as 2.0 kg.ha^{-1} of boron in the base. However, it is noteworthy that the means of the treatments did not differ from the average of the control treatment.

Table 2. Fresh biomass (FB), dry biomass (DB) and dry matter (DM) of the aerial part (leaves) and roots of the second BRS Ipyporã test using different doses of boron and time of application (Base+Topdressing at 125 DAS-B+TD2, Topdressing at 41 DAS+Topdressing at 125 DAS-TD1+TD2). Campo Grande-MS, 2016.

Tabela 2. Biomassa verde (BV), biomassa seca (BS) e matéria seca (MS) da parte aérea (Folhas) e raízes do segundo ensaio BRS Ipyporã, sob diferentes doses de boro e épocas de aplicação (Base+Cobertura aos 125 DAS-B+C2, Cobertura aos 41 DAS+Cobertura aos 125 DAS-C1+C2). Campo Grande-MS, 2016.

leaves						
	FB (g)		DB (g)		**DM (%)**	
D/E	**B+TD2**	**TD1+TD2**	B+TD2	TD1+TD2	**B+TD2**	**TD1+TD2**
0.0	**115aA[1]**	**115aA**	59aA	59Aa	**51.0aA**	**51.0aA**
1.0	**90aA**	**131aA**	47aB	66aA	**52.4aA**	**50.7aA**
2.0	**109aB**	**154aA**	56aA	75aA	**52.0aA**	**49.0aA**
4.0	**103aA**	**119aA**	57aA	57aA	**55.8aA**	**49.0aB**
8.0	**105aA**	**95aA**	59aA	49aA	**56.5aA**	**51.4aA**
Means	**113**		58		**52.1**	
Control treatment	**115**		59		**51.0**	
CV	**22.23**		**19.38**		**7.02**	

Root								
	FB (g)		**DB (g)**		DM (%)		**D**	
D/E	B+TD2	TD1+TD2	**B+TD2**	**TD1+TD2**	B+TD2	TD1+TD2	**B+TD2**	**TD1+TD2**
0.0	520aA[1]	520aA	**183aA**	**183aA**	34.4aA	34.4aA	**0.13aA**	**0.13aA**
1.0	478aA	453aA	**163aA**	**129aA**	33.3aA	29.3aA	**0.11aA**	**0.09aA**
2.0	574aA	405aA	**207aA**	**114aA**	34.8aA	26.9aA	**0.15aA**	**0.08aA**
4.0	495aA	342aA	**167aA**	**90aA**	33.6aA	26.6aA	**0.12aA**	**0.07aA**
8.0	502aA	286aB	**202aA**	**83aB**	39.5aA	29.6aB	**0.15aA**	**0.06aB**
Means	442		**144**		31.7		**0.10**	
Control treatment	520		**183**		34.4		**0.13**	
CV	25.21		**37.13**		17.97		**37.13**	

[1] Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ by the test of Tukey, at 5% probability.

[1] Médias seguidas pela mesma letra maiúsculas nas linhas e minúscula nas colunas não diferem entre si pelo teste de Tukey, a 5% de probabilidade.

Table 3. Height at 69 DAS (February 24, 2017) (A69) and number of vegetative tillers at 69 DAS (VTN69) in BRS Ipyporã in the first test using different boron doses and times of application (Base-B, Topdressing 41 DAS-TD1) and in the second test using different doses of boron and times of application (Base+Topdressing at 125 DAS-B+TD2, Topdressing at 41 DAS+Topdressing at 125 DAS-TD1+TD2). Campo Grande-MS, 2016.

Tabela 3. Altura aos 69 DAS (24/02/17) (A69) e número de perfilhos vegetativos aos 69 DAS (NPV69) em BRS Ipyporã no primeiro ensaio, sob diferentes doses de boro e épocas de aplicação (Base-B, Cobertura 41 DAS-C1) e no segundo ensaio, sob diferentes doses de boro e épocas de aplicação (Base+Cobertura aos 125 DAS-B+C2, Cobertura aos 41 DAS+Cobertura aos 125 DAS-C1+C2). Campo Grande-MS, 2016.

First test					**Second test**				
	A69 (cm)		**VTN69**			**A69 (cm)**		VTN69	
D/E	B	TD1	**B**	**TD1**	D/E	**B+TD2**	**TD1+TD2**	B+TD2	TD1+TD2
0.0	26abA[1]	26abA	**24aA**	**24aA**	0.0	**26aA**	**26aA**	24aA	24aA
0.5	23bB	31aA	**24aA**	**22aA**	1.0	**28aA**	**30aA**	22aA	23aA
1.0	26abA	24bA	**24aA**	**22aA**	2.0	**26aA**	**28aA**	17aA	22aA
2.0	29aA	27abA	**24aA**	**24aA**	4.0	**25aA**	**27aA**	23aA	22aA
4.0	23abA	25bA	**26aA**	**25aA**	8.0	**27aA**	**28aA**	24aA	25aA
Means	26		**23**		Means	**27**		22	
Control treatment	26		**24**		Test.	**26**		24	
CV	9.06		9.93		CV	**9.35**		15.73	

[1] Means followed by the same uppercase letter in the rows and lowercase letter in the columns do not differ by the test of Tukey at 5% probability.

[1] Médias seguidas pela mesma letra maiúsculas nas linhas e minúscula nas colunas não diferem entre si pelo teste de Tukey, a 5% de probabilidade.

The number of vegetative tillers at 69 DAS (VTN69) of the first test as well as A69 and VTN69 in the second trial did not respond to treatments. These results corroborate those found in the evaluation of the biomass of the aerial part of the plants, where it was observed that boron did not influence the vegetative growth of the aerial part of the BRS Ipyporã plants.

When the variables present in Table 3 were evaluated at 111 DAS, the same results were observed again for the first test, in which the height (A111) was not influenced by the treatments (Table 4). On the same occasion, the number of vegetative tillers (VTN111) showed a difference in relation to the time of application of the micronutrient, in which the best treatment resulted from the topdressing at 41 DAS (27). As for chlorophyll, a variable evaluated in SPAD units, it was not affected by boron application.

In the second test, A111 was also not affected by boron application at different times. VTN111 and SPAD values were influenced by the time of application and the best results were found in the TD1+TD2 treatment, with values of 28 and 31, respectively.

The results differed from those found by Almeida *et al.* (2015) in which they did not observe the influence of boron doses and time of application on the number of vegetative tillers, the number of total tillers, and the amount of $DM.ha^{-1}$ in Mombasa grass (*Panicum maximum* Jacq. cv. Mombasa).

Regarding the production components, no response was found for the treatments on the number of fully expanded inflorescences (IN), number of reproductive tillers (RTN), and number of inflorescences per reproductive tiller (NI/RTN) in the first and the second trials (Table 5). It can also be said that BRS Ipyporã, a characteristic of these variables is an average production of 23 NI, 11 RTN, and 2.0 inflorescences per reproductive tiller.

In a similar study of boric fertilization for seed production in common bean, Reis *et al.* (2008) observed that the components of crop production, namely: pod per plant, seeds per pod, weight of 100 seeds, and seed production, were not affected so they found that the micronutrient, when used at a dose of 3kg B ha-1 in wheat tillering, responded with an increase in the number of ears produced.

Flowering started on April 19, 2017, at 120 DAS, and lasted until June 12, 2017, when the seeds began to mature and were later harvested. Over time, for both trials, the behavior regarding the emission of fully expanded inflorescences was similar, with complete flowering (minimum of 20 fully expanded inflorescences per plant) occurring between May 17 to 24, 2017, that is, 30 days after the beginning of flowering. It is stressed that the beginning of shattering occurred 60 days after the beginning of flowering and approximately 30 days after full flowering.

Table 4. Height at 111DAS (April 4, 2017) (A111), number of vegetative tillers at 111 DAS (VTN111) and chlorophyll (SPAD unit) at 111 DAS in BRS Ipyporã in the first test using different doses of boron and times of application (Base-B, Coverage 41 DAS-TD1) and in the second test using different doses of boron and time of application (Base+Topdressing at 125 DAS-B+TD2, Topdressing at 41 DAS+Topdressing at 125 DAS-TD1 +TD2). Campo Grande-MS, 2017.

Tabela 4. Altura aos 111DAS (04/04/17) (A111), número de perfilhos vegetativos aos 111 DAS (NPV111) e clorofila (unidade SPAD) aos 111 DAS em BRS Ipyporã no primeiro ensaio, sob diferentes doses de boro e épocas de aplicação (Base-B, Cobertura 41 DAS-C1) e no segundo ensaio, sob diferentes doses de boro e épocas de aplicação (Base+Cobertura aos 125 DAS-B+C2, Cobertura aos 41 DAS+Cobertura aos 125 DAS-C1+C2). Campo Grande-MS, 2017.

	First test					
	A111 (cm)		**VTN111**		SPAD	
D/E	B	TD1	**B**	**TD1**	B	TD1
0.0	40aA[1]	40aA	**26aA**	**26aA**	28aA	28aA
0.5	39aA	45aA	**25aA**	**23aA**	29aA	31aA
1.0	50aA	45aA	**20aA**	**26aA**	27aB	33aA
2.0	44aA	41aA	**24aB**	**31aA**	31aA	30aA
4.0	35aA	42aA	**21aB**	**27aA**	32aA	31aA
Means	43		**25**		30	
Control treatment	40		**26**		28	
CV	13.76		**13.95**		8.38	
	Second test					
	A111 (cm)		VTN111		**SPAD**	
D/E	**B+TD2**	**TD1+TD2**	B+TD2	TD1+TD2	**B+TD2**	**TD1+TD2**
0.0	**40aA[1]**	**40aA**	26aA	26aA	**28aA**	**28aA**
1.0	**38aA**	**42aA**	22aB	29aA	**27aB**	**34aA**
2.0	**41aA**	**36aA**	17aB	30aA	**27aB**	**32aA**
4.0	**38aA**	**41aA**	25aA	27aA	**26aB**	**31aA**
8.0	**42aA**	**36aA**	18aB	27aA	**29aA**	**28aA**
Means	**39**		24		**29**	
Control treatment	**40**		26		**28**	
CV	**11.01**		16.22		**9.61**	

[1] Means followed by the same uppercase letter in the row and lowercase letters in the column do not differ by the test of Tukey at 5% probability.

[1] Médias seguidas pela mesma letra maiúsculas nas linhas e minúscula nas colunas não diferem entre si pelo teste de Tukey, a 5% de probabilidade.

Table 5. Inflorescence number (IN), reproductive tiller number (RTN), and inflorescence number per reproductive tiller (IN/RTN) in BRS Ipyporã in the first test under different boron doses and time of application (Base-B, Topdressing 41 DAS-TD1) and in the second test using different doses of boron and time of application (Base+Topdressing at 125 DAS-B+TD2, Topdressing at 41 DAS+Topdressing at 125 DAS-TD1+TD2). Campo Grande-MS, 2017.

Tabela 5. Número de inflorescência (NI), número de perfilho reprodutivo (NPR) e número de inflorescência por perfilho reprodutivo (NI/NPR) em BRS Ipyporã no primeiro ensaio, sob diferentes doses de boro e épocas de aplicação (Base-B, Cobertura 41 DAS-C1) e no segundo ensaio, sob diferentes doses de boro e épocas de aplicação (Base+Cobertura aos 125 DAS-B+C2, Cobertura aos 41 DAS+Cobertura aos 125 DAS-C1+C2). Campo Grande-MS, 2017.

	First test					
	IN		**RTN**		IN/RTN	
D/E	B	TD1	**B**	**TD1**	B	TD1
0.0	21aA[1]	21aA	**11aA**	**11aA**	1.9aA	1.9aA
0.5	26aA	20aA	**12aA**	**10aA**	2.3aA	2.2aA
1.0	23aA	19aA	**11aA**	**10aA**	2.1aA	1.9aA
2.0	23aA	22aA	**12aA**	**10aA**	1.9aA	2.1aA
4.0	45aA	21aA	**13aA**	**11aA**	1.8aA	2.0aA
Means	23		**11**		2.0	
Control treatment	21		**11**		1.9	
CV	35.45		**36.63**		13.99	
	Second test					
	IN		RTN		**IN/RTN**	
D/E	**B+TD2**	**TD1+TD2**	B+TD2	TD1+TD2	**B+TD2**	**TD1+TD2**
0.0	**21aA[1]**	**21aA**	11aA	11aA	**1.9aA**	**1.9aA**
1.0	**16aA**	**21aA**	8aA	13aA	**2.0aA**	**1.6aA**
2.0	**29aA**	**37aA**	11aA	15aA	**2.5aA**	**2.4aA**
4.0	**20aA**	**37aA**	11aA	15aA	**1.8aA**	**2.2aA**
8.0	**17aA**	**20aA**	8aA	10aA	**2.1aA**	**2.1aA**
Means	**25**		11		**2.1**	
Control treatment	**21**		11		**1.9**	
CV	**46,63**		28,63		**16.55**	

[1] Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ by the test of Tukey, at 5% probability.

[1] Médias seguidas pela mesma letra maiúsculas nas linhas e minúscula nas colunas não diferem entre si pelo teste de Tukey, a 5% de probabilidade.

There was no difference between treatments for pollen grain germination in both tests (Table 6) and the values did not exceed 20%. Such values are relatively low concerning those found when quantifying and regarding viability. This fact may indicate that the micronutrient did not contribute to the increase of this variable or, even, that the methodology adopted was not efficient for the evaluation, suggesting the need for further studies, with different medium and incubation environments.

According to Franzon & Raseira (2006), *in vitro* germination is influenced by different factors, such as differences between species regarding the required conditions (constituents of the culture medium, temperature, and incubation time), flower development stage upon collection, and storage conditions.

Another important topic observed when evaluating pollen germination was a large amount of pollen leaked into the culture medium. Also, according to Franzon & Raseira (2006), the basic medium for these tests consists of sugar and boric acid, in which sugar is responsible for promoting the osmotic balance between the pollen and the germination medium and for providing energy for the development of the pollen tube. On the other hand, boron stimulates the growth of the pollen tube and reduces the probability of pollen ruptures, indicating the need for further studies to define the number of components used in the culture medium.

Regarding pollen grain viability in the first test, no difference was observed between treatments and viability was, on average, greater than 50%. In the second trial, the best time of application was topdressing at 41 DAS + topdressing at 125 DAS (TD1+TD2), with 82.0% viability, and the lowest value was 62.5%, occurring with the application at the base + topdressing at 125 DAS (B+TD2). Regarding pollen quantification (PQ), no difference was observed among treatments for the two tests.

It is suggested that the high viability, combined with the low germination of the pollen grain, may indicate problems with pollen tube formation in the genotype, which was not influenced by the presence of the micronutrient, even at high doses.

Table 6. Pollen grain germination (%PG), pollen grain viability (PV), and pollen grain quantification (PQ) in BRS Ipyporã in the first test using different doses of boron and time of application (Base-B, Topdressing 41 DAS-TD1) and in the second test using different doses of boron and time of application (Base+Topdresssing at 125 DAS-B+TD2, Topdressing at 41 DAS+Topdressing at 125 DAS-TD1+TD2). Campo Grande-MS, 2017.

Tabela 6. Germinação do grão de pólen (%GP), viabilidade do grão de pólen (VP) e quantificação do grão de pólen (QP) em BRS Ipyporã no primeiro ensaio, sob diferentes doses de boro e épocas de aplicação (Base-B, Cobertura 41 DAS-C1) e no segundo ensaio, sob diferentes doses de boro e épocas de aplicação (Base+Cobertura aos 125 DAS-B+C2, Cobertura aos 41 DAS+Cobertura aos 125 DAS-C1+C2). Campo Grande-MS, 2017.

First test						
	%PG[1]		**PV**		PQ	
D/E	B	TD1	**B**	**TD1**	B	TD1
0.0	11.7aA	11.7aA	**74.7aA**	**74.7aA**	2417aA	2417aA
0.5	13.0aA	4.6aA	**57.9aA**	**44.2aA**	2000aA	1875aA
1.0	11.2aA	3.6aA	**75.4aA**	**75.6aA**	3333aA	2500aA
2.0	2.5aA	7.8aA	**72.7aA**	**71.6aA**	3917aA	3416aA
4.0	5.9aA	5.7aA	**74.9aA**	**68.2aA**	3000aA	3583aA
Means	6.8		**67.6**		2953	
Control treatment	11.7		**74.7**		2417	
CV	37.22		**22.78**		43.45	
Second test						
	%PG[1]		**PV**		PQ	
D/E	B+TD2	TD1+TD2	**B+TD2**	**TD1+TD2**	B+TD2	TD1+TD2
0.0	11.7aA	11.7aA	**74.7aA**	**74.7aA**	2417aA	2417aA
1.0	5.2aB	19.7aA	**45.4aB**	**85.8aA**	2250aB	4167aA
2.0	4.8aA	6.3aA	**56.5aB**	**82.8aA**	2916aA	2916aA
4.0	5.1aA	8.1aA	**73.3aA**	**79.1aA**	3000aA	3000aA
8.0	12.1aA	7.3aA	**74.9aA**	**80.3aA**	3250aA	3417aA
Means	8.6		**72.3**		3115	
Control treatment	11.7		**74.7**		2417	
CV	31.05		**19.38**		36.67	

Means followed by the same uppercase letter in the row and lowercase letter in the columns do not differ from each other by the test of Tukey at 5% probability. [1] Arcsine transformed data $((X+0.5)/100)^{1/2}$, original means shown.

Médias seguidas pela mesma letra maiúsculas nas linhas e minúscula nas colunas não diferem entre si pelo teste de Tukey, a 5% de probabilidade.

[1] Dados transformados arcsen$((X+0,5)/100)^{1/2}$, médias originais apresentadas.

Table 7. Number of full seeds (FSN) and percentage of number of full seeds (%FS) in BRS Ipyporã in the first test using different doses of boron and time of application (Base-B, Topdressing 41 DAS-TD1) and in the second test using different doses of boron and time of application (Base+Topdressing at 125 DAS-B+TD2, Topdressing at 41 DAS+Topdressing at 125 DAS-TD1+TD2). Campo Grande-MS, 2017.

Tabela 7. Número sementes cheias (NSC) e porcentagem do número de sementes cheias (%SC) em BRS Ipyporã no primeiro ensaio, sob diferentes doses de boro e épocas de aplicação (Base-B, Cobertura 41 DAS-C1) e no segundo ensaio, sob diferentes doses de boro e épocas de aplicação (Base+Cobertura aos 125 DAS-B+C2, Cobertura aos 41 DAS+Cobertura aos 125 DAS-C1+C2). Campo Grande-MS, 2017.

First test					Second test				
	FSN		**%FS**			**FSN**		%FS	
D/E	B	TD1	**B**	**TD1**	D/E	**B+TD2**	**TD1+TD2**	B+TD2	TD1+TD2
0.0	71aA[1]	71aA	**17.0aA**	**17bA**	0.0	**71aA**	**71bA**	17abA	17aA
0.5	112aA	71aA	**19.3aA**	**14.7bA**	1.0	**66aA**	**68bA**	22.0abA	14.0aB
1.0	98aA	85aA	**20.4aA**	**17.6bA**	2.0	**66aB**	**125aA**	15.7bA	22.9aA
2.0	84aA	69aA	**18.5aA**	**17.8bA**	4.0	**94aA**	**110abA**	25.9aA	22.3aA
4.0	52aB	147aA	**15.5aB**	**58.6aA**	8.0	**54aA**	**65bA**	14.8bA	18.6aA
Means	90		**22,8**		Means	**81**		19.5	
Control treatment	71		**17**		Control	**71**		17	
CV	49.97		**53.76**		CV	**24.07**		21.83	

[1] Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other by the test of Tukey, at 5% probability.

[1] Médias seguidas pela mesma letra maiúsculas nas linhas e minúscula nas colunas não diferem entre si pelo teste de Tukey, a 5% de probabilidade.

The number of full seeds per pot (FSN) for the first trial was not influenced by any treatment, unlike in the second (Table 7) test, in which the best treatment was for a dose of 2.0 kg.ha^{-1} of boron applied in topdressing at 41 DAS + 125 DAS (TD1+TD2). Brunes *et al.* (2015), in a similar study of boric fertilization for the production of wheat seeds, observed similarities between the fertilization dates (sowing and tillering) regarding the number of seeds per plant and the boron dose that presented the highest efficiency for these parameters was of 2.49 kg B ha^{-1}.

For the percentage of the number of full seeds (%FS), both in the first and second tests, there was an interaction between doses and times. In the first, the dose of 4.0 kg.ha^{-1} of boron provided the best result when applied in topdressing at 41 DAS (58.6%) and, in the second test, the best result was with the dose of 2.0 kg .ha^{-1} in B+TD2, 25.9%; however, with a lower result than the first test.

Such results show a positive response to boric fertilization with the highest dose evaluated when it is applied in topdressing at 41 DAS, before the pre-flowering of BRS Ipyporã plant.

The weight of full seeds per pot (FSW) in the first test did not show differences among treatments, in contrast to the percentage of full seed weight (%FSW) (Table 8), where the result was achieved with the dose of 4.0 kg.ha^{-1}, in topdressing, at 41 DAS. This result is similar to that found in the evaluation of the percentage of the number of full seeds.

For the second trial, the FSW differed for doses, reaching the highest values at 2.0 and 4.0 kg.ha^{-1} (0.88 and 0.91, respectively) and, in this case, the %FSW did not differ. However, the means of the treatments did not differ from the mean of the control treatment, which demonstrates that the boron in the substrate was sufficient for the maximum expression of the characteristics of the BRS Ipyporã plant. For both the first and the second tests, it was not possible to perform statistical analysis on the data obtained by the standard germination test, germination speed index (GSI), and first germination count (FGC) because of the insufficient amount of seeds, which prevent achieving the minimum number of repetitions. Even so, it was possible to observe that the germination was approximately 15%, even with average viability results of 70%, which indicates the presence of partial dormancy in the seeds. The FGC was equal to zero for all treatments and GSI achieved a mean equal to 0.9. Also, the seed viability was not negatively affected by treatments. Brunes *et al.* (2016) similarly concluded that boron fertilization in wheat at different times of application did not impair the yield, germination, and vigor of the produced seeds.

Table 8. Weight of full seeds (FSW) and percentage of weight of full seeds (% FSW) in BRS Ipyporã in the first test using different doses of boron and time of application (Base-B, Coverage 41 DAS-TD1) and in the second trial using different boron doses and time of application (Base + Coverage at 41 DAS-B + TD1; Coverage at 41 DAS + Coverage at 125 DAS-TD1 + TD2). Campo Grande-MS, 2017.

Tabela 8. Peso de sementes cheias (PSC) e porcentagem do peso de sementes cheias (%PSC) em BRS Ipyporã no primeiro ensaio, sob diferentes doses de boro e épocas de aplicação (Base-B, Cobertura 41 DAS-C1) e no segundo ensaio, sob diferentes doses de boro e épocas de aplicação (Base+Cobertura aos 41 DAS-B+C1, Cobertura aos 41 DAS+Cobertura aos 125 DAS-C1+C2). Campo Grande-MS, 2017.

	First test					Second test			
	FSW		%FSW			FSW		%FSW	
D/E	B	TD1	B	TD1	D/E	B+TD2	TD1+TD2	B+TD2	TD1+TD2
0.0	0.7aA[1]	0.7aA	46.6aA	46.6abA	0.0	0.7abA	0.7aA	46.6abA	46.6aA
0.5	1.0aA	0.5aB	47.2aA	30.7bA	1.0	0.6abA	0.6aA	51.7abA	36.3aB
1.0	0.9aA	0.7aA	50.2aA	41.4abA	2.0	0.8abA	0.9aA	47.4abA	48.9aA
2.0	0.7aA	0.6aA	45.3aA	43.4abA	4.0	0.9aA	1.0aA	58.1aA	51.1aA
4.0	0.4aA	0.8aA	41.6aA	58.5aA	8.0	0.4bA	0.6aA	36.7bA	45.8aA
Means	0.7		44.8		Means	0.7		47.0	
Control treatment	0.7		46.6		Control	0.7		46.6	
CV	31.47		21.90		CV	23.01		17.95	

[1] Means followed by the same uppercase letter in the row and lowercase letter in the column do not differ from each other by the test of Tukey, at 5% probability.

[1] Médias seguidas pela mesma letra maiúsculas nas linhas e minúscula nas

In summary, these data obtained in the two tests allow the raising of some hypotheses, since it was observed that the doses of the micronutrient boron had no influence on the production of the aerial part neither on the reproductive characteristics of the BRS Ipyporã plant, showing the influence of the time of application only and positive response in root production. The first hypothesis is that the assessed doses were very low in relation to the plant's needs; another theory would be that the boron in the soil is enough for the maximum expression of the plant's characteristics, also considering that there is another factor influencing the low production of full seeds of BRS Ipyporã rather than only the lack of boron, so further studies to identify them are necessary.

4 Conclusion

Boron, applied at different doses and dates, did not affect the production (productivity and quality) of BRS RB331 Ipyporã seeds; the phytotechnical components of the hybrid BRS RB331 Ipyporã were not negatively influenced by the addition of boron and; Boron was beneficial for root production of the hybrid BRS RB331 Ipyporã.

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