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RESEARCH ARTICLE

Vegetative and productive characteristics of soybean under doses of boron and inoculation of *Trichoderma atroviride*

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ABSTRACT

The application of new management techniques with the objective of implementing in agriculture, such as the use of growth-promoting and/or plant-protecting microorganisms and micronutrients, appears as an alternative. Thus, the aim of the study was to evaluate the potential of *Trichoderma atroviride* in association with doses of B, analyzing the effects on nutritional contents, yield components and productivity. The study was carried out in the field, in a randomized block design in a 4 × 5 factorial scheme, whose treatments were combined, consisting of foliar application of *T. atroviride* in three phenological stages (V5, R1 and R5) and no application (SEM), and five doses of B (0, 500, 1000, 1500 and 2000 g ha^{-1}). The data obtained were submitted to ANOVA and, when significant, the study of polynomial regression and Tukey's test was carried out, followed by Pearson's linear correlation analysis ($p < 0.05$). The use of the microorganism and doses of B did not result in a significant increase in the growth and productive yield of soybean (*Glycine max* (L.) Merr.), but the application of the micronutrient significantly increased the number of pods per plant and the foliar content of B. Positive correlations were observed between plant height and pod insertion height (r = 0.51***), pod with branches (r = 0.68***), grains with branches and pod (r = 0.69*** and 0.95*** respectively), as well as a negative correlation between 100-grain mass and plant height (r = -0.24*), and a non-correlation for yield and leaf content of B.

Key words: Endophytic fungi, *Glycine max*, leaf fertilization, mineral elements, production.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) stands out among legumes for its high nutritional value and use in various sectors of the agroindustry, being characterized as an important agricultural commodity worldwide and in Brazil (Xu et al., 2021). In recent years, due to the increase in grain consumption and the difficulty to open new agricultural areas, it has been necessary to implement new agronomic practices in order to provide improvements in soybean growth, development and productivity (Vogel et al., 2021).

In this context, the search for management techniques in agriculture that reduce costs, are efficient and less impacting the environment and human health, has shown the use of microorganisms associated with micronutrients that allow greater absorption and tolerance to nutrients, which can improve activities biochemical properties and resulting in more effective plant development (Landi et al., 2012; Rubio et al., 2017).

From this perspective, fertilization with micronutrients has been one of the ways to meet the plant's needs and mitigate the deficiencies arising from this imbalance (da Silva et al., 2019). The foliar spraying of micronutrients, such as B, has helped in the functions directly linked to improvements in productivity, in which the use of this nutrient allows plants to perform functions related to the formation of pods and increase in grain, root development, cell division, metabolization of carbohydrates and proteins, transport of sugars, as well as carrying out the synthesis of nucleic acids and the formation of cell walls (Cakmak and Römheld, 1997; Reid, 2014).

Thus, several studies have been carried out with microorganisms of the genus *Trichoderma*, characterized as fungi of wide distribution and occurrence in the world, present in different classes of soils and natural habitats, specifically in those containing organic matter (Waghunde et al., 2016). Some strains of *Trichoderma* have caused an increase in the total surface of the root system, allowing access to mineral elements (Hermosa et al., 2013), being agents capable of improving the absorption mechanism and efficiency of the plant to use nutrients, so that, increases resistance to biotic and abiotic stresses (Nieto-Jacobo et al., 2017; Rezende et al., 2021). These microorganisms have also been recognized for being biostimulants in promoting plant growth and increasing productivity (Woo et al., 2014).

Based on the above, the hypothesis in this study is that *Trichoderma atroviride* and B may promote plant growth and protection. Thus, the objective was to evaluate B doses in association with the application of *T. atroviride* in different phenological phases, analyzing the effects of this association on leaf nutritional contents, yield components and productivity.

MATERIALS AND METHODS

The study was conducted in the 2020-2021 harvest in an area located in the municipality of Alta Floresta (10°02'32" S, 56°06'31" W), state of Mato Grosso, Brazil. The climate of the region according to the Köppen classification is tropical rainy, with two distinct seasons, one rainy (September to April) and the other dry (May to August) with an average annual rainfall of 2243 mm (Alvares et al., 2014), with temperatures around 18 to 40 °C, expressing averages around 26 °C.

Climatic data were collected during the conduction of the research (Figure 1), in which, it expressed ideal conditions for soybean (*Glycine max* (L.) Merr.) cultivation during the period, where the ideal temperature range for soybean development is between 20 and 30 °C, whose relative humidity exceeds 60%, and the water requirement is 450 to 800 mm throughout the cycle (Embrapa, 2013).

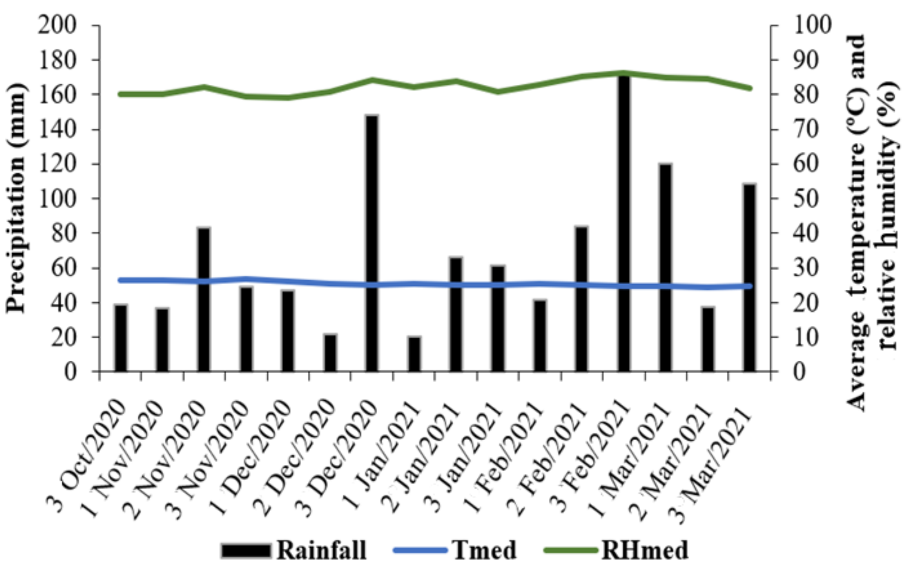

Figure 1. Decennial data of mean temperature (Tmed), mean relative humidity (RHmed) and rainfall recorded in the experimental area during the entire crop cycle.

The soil of the experimental area is characterized as Dystrophic Red-Yellow Latosol, of sandy-clay textual class (Embrapa, 2018), whose taxonomy classification is the Rhodic Hapludox (Soil Survey Staff, 2014). Before the installation of the research, soil samples were collected from the experimental area in the layer from 0 to 20 cm and sent for laboratory analysis. The results of physical and chemical properties in relation to soil fertility are described in Table 1.

It is possible to observe through soil analysis (Table 1), that the content of micronutrient B described by Alvarez et al. (1999), is in low availability (0.31 mg dm^{-3}), where it is close to the lower average limit (0.36 mg dm^{-3}), as well as the organic matter content (18.34 g dm^{-3}), which, according to these authors, is close to the medium availability level, whose minimum limit is 20.1 g dm^{-3}. It is observed (Table 1) that the content of P (3.1 mg dm^{-3}) and K (45.6 mg dm^{-3}) was considered low and medium, respectively, as portrayed by Novais (1999).

In sowing no-tillage, the cv. BMX DESAFIO RR 8473 RSF was used, with a spacing of 0.45 m, aiming to obtain 17 plants m^{-1}. The seeds were treated with bacteria, and the commercial product used as a source of *Bradyrhizobium japonicum* was the inoculant Masterfix, strains SEMIA 5019 and SEMIA 5079 (5×10^9 viable cells g^{-1} or mL^{-1}), and as a source of *Azospirillum brasilense*, Masterfix Gramíneas, Abv5 and Abv6 strains (2×10^8 viable cells mL^{-1}). The fertilization was realized based on the recommendation of Novais (1999), where there was no need to carry out liming in the experimental area due to base saturation being 57.6% (Table 1), whose author recommends that liming be carried out when saturation by bases is less than 50%. Fertilization also consisted of the application of 250 kg ha^{-1} monoammonium phosphate (MAP) (10% to 12% N and 48% to 52% P_2O_5), being 30 kg ha^{-1} N with MAP and 140 kg ha^{-1} potassium chloride (60% K_2O) applied in coverage in the vegetative stage V4, in which, the use of N in sowing occurred due to the area characterizing the no-tillage system containing a lot of corn straw on the soil. Cultural and phytosanitary treatments also consisted of technical recommendations for soybean crop.

Table 1. Physical analysis and chemical properties of soil in the 0 to 20 cm depth layer of the experimental area. MO: Organic matter; CEC: cation exchange capacity; BS: base saturation.

pH ($CaCl_2$)	5.30
P, mg dm^{-3}	3.10
K, mg dm^{-3}	45.60
Ca, $cmol_c$ dm^{-3}	2.49
Mg, $cmol_c$ dm^{-3}	0.75
Al, $cmol_c$ dm^{-3}	0.00
H+Al, $cmol_c$ dm^{-3}	2.50
Zn, mg dm^{-3}	1.50
Cu, mg dm^{-3}	1.60
Fe, mg dm^{-3}	191.80
Mn, mg dm^{-3}	50.80
B, mg dm^{-3}	0.31
S, mg dm^{-3}	15.80
MO, g dm^{-3}	18.34
CEC, $cmol_c$ dm^{-3}	5.80
BS, %	57.30
Sand, %	47.60
Silt, %	8.50
Clay, %	43.70

The experiment was carried out in a randomized block design with four replicates, where the treatments were arranged in a 4 × 5 factorial scheme with a combination of factors, which were constituted by the association of *Trichoderma atroviride* at three times of the phenological stage of the culture, being the vegetative stage with five nodes (V5), early flowering stage (R1) and in the reproductive stage of grain formation and filling (R5) and non-application (SEM), associated with five doses of B (0, 500, 1000, 1500 and 2000 g ha^{-1}) applied at the beginning of flowering stage (R1).

The used source of B was boric acid (17% B), where the preparation of the solution was characterized by the dilution of the product in water. For treatments with *T. atroviride*, suspensions were prepared following the standard amount of 2×10^7 conidia mL^{-1}. The treatments were applied via foliar using a manual sprayer, in which constant pressure was calibrated, and 300 mL spray solution was applied per plot, equivalent to 222 L ha^{-1}.

For the analysis of leaf B content, the collection was carried out at the reproductive stage of full flowering (R2), and the fully developed trifoliates were sampled at the apex of the main branch, according to the protocol described by Malavolta et al. (1997). The determinations followed the methodology proposed by da Silva et al. (2009) through dry digestion, and later through the inductively coupled plasma method used by Hamurcu et al. (2019).

Soybean was harvested at the complete maturity stage (R9), when morphological components, yield and productivity of the crop were determined. Ten plants were collected from the useful area of each plot, being evaluated plant height (PH) and the height of insertion of the first pod (FPIH) with the aid of a tape measure; number of branches per plant (BP), number of pods per plant (PP) and number of grains per plant (GP) by counting in each plant. To obtain the mass of 100 grains (M100) and productivity (PROD), all plants were harvested in 3 m of the useful area of the plot, which were mechanically threshed and manually cleaned to separate the grains. From this, weighing was carried out on a precision scale (Marte UX6200H, Shimadzu, Kyoto, Japan) and the determination of grain moisture by means of a moisture meter (Gehaka 600, São Paulo, Brazil), in which the production was calculated in kg ha^{-1} with the humidity corrected to 13%.

The data obtained were subjected to ANOVA and when significant, a polynomial regression study was carried out for the quantitative factor and the Tukey average comparison test for the qualitative factor, with the help of the statistical software R Core Team (R Foundation for Statistical Computing, Vienna, Austria). Therefore, the Pearson correlation test ($p < 0.05$) was performed using the SigmaPlot software (Systat Software, San Jose, California, USA).

RESULTS AND DISCUSSION

In the present study, the responses of the morphological and yield variables in the soybean crop, depending on the time of application of *Trichoderma atroviride* in association with B doses, expressed a significant difference only for pods per plant in relation to B doses (Table 2).

Table 2. Mean values for plant height (PH), first pod insertion height (FPHI), branches per plant (BP), pods per plant (PP), grains per plant (GP), mass of 100 grains (M100) and soybean yield (PROD) as a function of *Trichoderma atroviride* application time and B doses. *Significant at 5% by the F test; ns: nonsignificant; SEM: non-application; V5: vegetative stage with 5 nodes; R1: early flowering stage; R5: reproductive stage of grain formation and filling.

	Agronomic characteristics						
	PH	FPIH	BP	PP	GP	M100	PROD
	cm		nr			g	kg ha^{-1}
Seasons (S)							
SEM	76.5	15.6	1.6	40.3	104.8	20.3	4966.2
V5	78.8	15.7	1.6	39.6	102.1	20.4	5330.8
R1	80.0	17.0	1.7	40.9	106.2	20.4	5224.3
R5	78.2	15.9	1.6	38.9	100.9	20.4	4953.8
F value	1.71^{ns}	0.70^{ns}	0.23^{ns}	0.38^{ns}	0.44^{ns}	0.12^{ns}	1.65^{ns}
Doses of B (D)							
0	81.1	15.9	1.6	39.3	102.9	20.5	5067.5
500	77.1	16.0	1.6	38.6	99.3	20.4	5123.2
1000	78.9	16.5	1.5	40.3	103.5	20.1	5246.1
1500	77.5	15.3	1.7	44.1	112.8	20.3	5273.2
2000	77.4	16.5	1.7	37.8	98.9	20.5	4884.0
F value	1.77^{ns}	0.36^{ns}	0.42^{ns}	2.60*	1.86^{ns}	1.46^{ns}	0.91^{ns}
S × D							
F value	1.05^{ns}	1.20^{ns}	0.89^{ns}	1.05^{ns}	1.17^{ns}	1.06^{ns}	0.86^{ns}
CV, %	6.41	21.57	30.59	15.44	15.85	2.48	12.79

The absence of significant results in relation to the application of *T. atroviride*, for all variables (Table 2) may be due to the fact that fungi of the genus *Trichoderma* exert beneficial effects in several crops in terms of productivity improvement, aiding in the absorption of nutrients and stimulating the plant's defense, especially against abiotic stress, such as drought (Mendoza-Mendoza et al., 2018), and in the present research, it is noted by observing the data (Figure 1), that the culture did not undergo abiotic stress throughout the development period, which would justify the non-observance of significant effects under the inoculation of the microorganism.

The nonsignificant results for the application of B doses may be due to the fact that, despite the B content in the soil (Table 1) is in low availability (0.31 mg dm^{-3}) and close to the lower mean limit (0.36 mg dm^{-3}) (Alvarez et al., 1999), as well as the organic matter content (18.34 g dm^{-3}), which, according to these authors, is close to the level of medium availability, whose minimum limit is 20.1 g dm^{-3}, may have contributed to increase the availability of B in the soil and thus adequately supply the plants. In this perspective, several studies have pointed to a correlation between the constituents of soil organic matter (SOM) in the dynamics of B absorption, in which the SOM decomposition promotes an increase in the organic C content, which allows for greater B chelation due to the formation of complexes B-diol, and there may also be the production of organic acids that solubilize B, thus improving its availability in the soil solution when removed by absorption or leaching (Dey et al., 2017).

In this sense, some studies also showed that the foliar supply of B did not have a significant effect on plant height (Rodrigues et al., 2019), branches per plant (Souza et al., 2016), pod insertion height, mass of 100 grains, grains per plant and productivity (Nakao et al., 2018), thus resembling the results found in this research.

Regarding the number of pods per plant, there was a significant difference between the doses applied, corroborating the results obtained by Gowthami et al. (2018), a fact that is explained due to the influence and role of the micronutrient in yields in relation to cell differentiation, development, efficiency in the translocation of photosynthates and growth regulators, leading to an increase and higher seed yield.

It can be seen in Pearson's linear correlation analysis (Figure 2), whose magnitudes were classified according to Carvalho et al. (2004), following the intensities: > 0.00 to ≤ 0.30 (weak), > 0.30 to ≤ 0.60 (moderate) and > 0.60 to ≤ 1.00 (strong). The results of this research showed a significant positive moderate correlation between plant height and height of insertion of the 1st pod (r = 0.51). This result agrees with some studies, where the authors reported that the increase in the population density of plants per hectare had an influence by providing an increase in soybean height for light interception (Bomtempo et al., 2021).

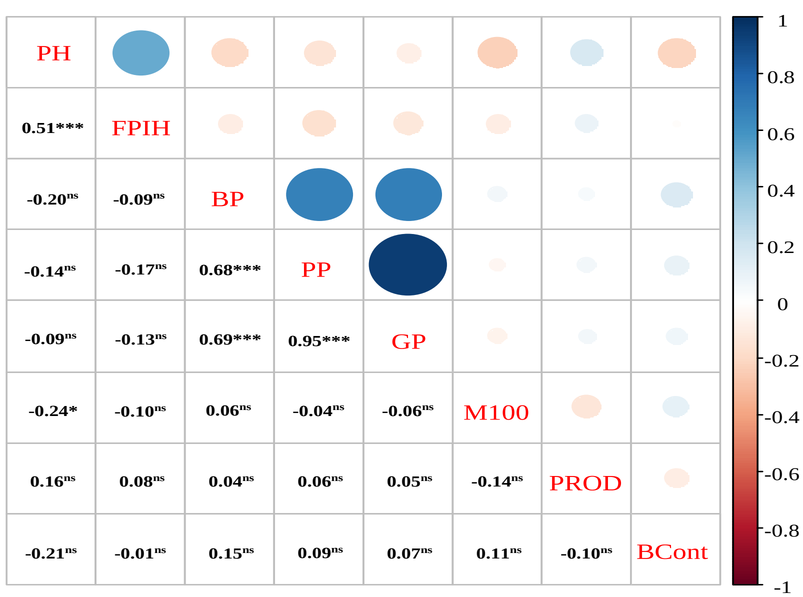

Figure 2. Pearson's linear correlation coefficients between nutritional content, yield components and productivity. *, ***Significant at 5% and 1%, respectively; ns: nonsignificant; PH: plant height; FPIH: first pod insertion height; BP: branches per plant; PP: pods per plant; GP: grains per plant; M100: mass of 100 grains; PROD: productivity; BCont: B content.

However, there was a strong positive correlation between pod and branches per plant (r = 0.68), as well as, grains per plant with branches and pod per plant (r = 0.69; r = 0.95 respectively), result also evidenced by Mahbub et al. (2015), whose pod per plant was the component that most contributed to productivity. Therefore, this is due to the greater efficiency in the capture and distribution of solar energy, where it is converted into photoassimilates, providing stem growth, increase in the formation of branches, pods and grains in the plant (Keerthana et al., 2021).

In contrast, the mass of 100 grains was the only variable that showed a significant negative correlation with plant height (r = -0.24). On the other hand, Kujane et al. (2021) found a significant and positive relationship between 100-grain mass and plant height in soybean.

Soybean yield and B content did not present significant correlations in the present study, where Rosolem et al. (2008) described that the lack of correlation between leaf B levels and productivity can be explained by the difficulty of removing the micronutrient retained in the leaf cuticle or that bound in the pectic layer of the cell wall, in which the metabolic function is not exercised by B in the plant.

The result of leaf B content (Table 3) showed that treatments involving borate fertilization provided significant effects of the micronutrient on leaves.

Table 3. Mean values of leaf B content in soybean plants as a function of *Trichoderma atroviride* application time and B doses. *Significant at 5% by the F test; ns: nonsignificant; SEM: non-application; V5: vegetative with 5 nodes; R1: early flowering stage; R5: reproductive stage of grain formation and filling.

	B content (mg kg^{-1})
Seasons (S)	
SEM	81.44
V5	80.70
R1	81.31
R5	79.34
F value	0.30^{ns}
Doses of B (D)	
0	64.67
500	71.04
1000	79.11
1500	92.46
2000	96.20
F value	48.05*
S × D	
F value	1.22^{ns}
CV, %	9.67

In response to fertilization with B, the concentration of this micronutrient in the leaf varied between 64.67 and 96.20 mg kg^{-1}. Therefore, increasing the applied dose, there was a linear increase in B levels in soybean leaves, increasing 0.0169 mg in leaf content per gram of B applied (Table 3, Figure 3). In the literature, there are studies describing that the foliar application of B on soybeans also increased the concentration of the nutrient in the leaves, with levels between 68.15 and 143.33 mg kg^{-1}, where they remained above the recommended level of 21 to 55 mg kg^{-1}, considering the control (Trautmann et al., 2014). This fact is due to the content of organic matter in the soil, which is the main source of B, which, despite its low content in the soil, may have provided adequate amounts of the nutrient for soybean plants (Jacoby et al., 2017).

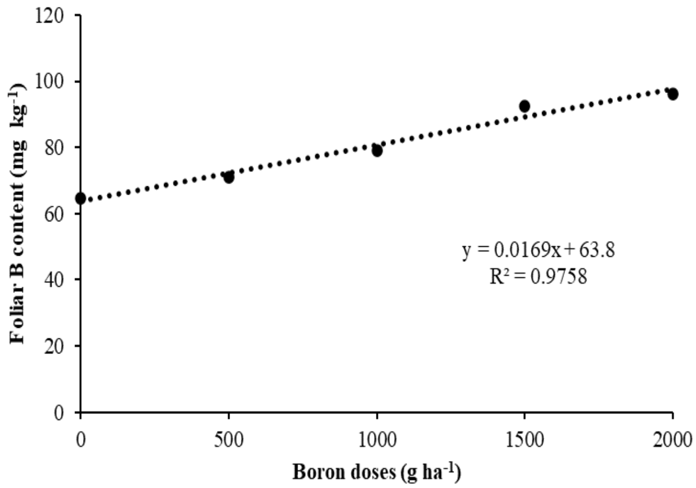

Figure 3. Boron content in soybean leaves as a function of the application of B doses.

CONCLUSIONS

The B supply, regardless of the time of application of *Trichoderma atroviride*, did not demonstrate a growth-promoting action on the components of soybean yield and productivity, but fertilization with the micronutrient increased the number of pods per plant and the foliar content of B.

Yield components are positively correlated, highlighting the relationship between plant height and pod insertion height, pod per plant and branches per plant, between grains per plant with branches and pod per plant. However, there is a negative correlation between the weight of 100 grains and plant height, since yield and B content have no correlation in soybean.

Author contributions

Conceptualization: G.C.G., M.A.C.dC., G.F.F. Methodology: G.C.G., M.A.C.dC., G.F.F., C.H. Software: M.A.C.dC., G.F.F. Validation: G.C.G., M.A.C.dC., G.F.F., C.H., S.A.R.L. Formal analysis: G.C.G., M.A.C.dC., G.F.F. Investigation: G.C.G., M.A.C.dC., G.F.F., C.H., S.A.R.L. Resources: G.C.G., M.A.C.dC., G.F.F., G.C., O.M.Y., C.H., S.A.R.L. Data curation: G.C.G., C.H., S.A.R.L. Writing-original draft: G.C.G., M.A.C.dC., G.F.F. Writing-review & editing: G.C.G., M.A.C.dC., G.F.F., O.M.Y., G.C. Visualization: G.C.G., M.A.C.dC., G.F.F. Supervision: G.C.G., M.A.C.dC., G.F.F. Project administration: G.C.G., M.A.C.dC., G.F.F., C.H. Funding acquisition: M.A.C.dC., G.F.F. All co-authors reviewed the final version and approved the manuscript before submission.

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