

RESEARCH ARTICLE

Nutritional and morphophysiological responses of soybean to micronutrient fertilization in soil

Rubens Ribeiro da Silva*, Larissa Urzêdo Rodrigues, Rodrigo Ribeiro Fidélis, Álvaro José Gomes de Faria and Vitor L. Nascimento

Federal University of Tocantins, Gurupi, TO, Brazil.
 *Author for correspondence: rrs2002@uft.edu.br

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ABSTRACT

Micronutrient fertilization is essential to meet the nutritional deficiency of plants, providing the full development of crops. The aim of this work was to evaluate the effects of fertilization with micronutrients applied in the soil on the nutrition and morphology of soybean plants. The experimental design was completely randomized, with four replications. The treatments consisted of fertilization with micronutrients applied to soil to reach three levels of availability of B: low ($<0.15 \text{ mg dm}^{-3}$ - control), good (0.61 mg dm^{-3}) and high ($> 0.90 \text{ mg dm}^{-3}$). For this, the following values were applied: 0.00 (control), 56.67 and $211.11 \text{ kg ha}^{-1}$ of the fertilizer containing 1.8% B, 0.85% Cu, 2% Mn and 9% Zn. Fertilization with the good and high treatments did not affect the uptake of B, but increased the Cu and Mn content, and reduced the Zn content in the plants. Higher plants with greater leaf expansion were observed in the high treatment, however, this did not influence the soybean grain yield. In the control, the physiological activity revealed smaller A , whereas g_s and C_i were more effective; reverse effect was observed with high treatment. Fertilization with micronutrients in the soil affects the nutritional status and the photosynthetic activity of the plants due to the imbalance in the concentrations of B, Cu, Mn and Zn supplied with the fertilizer, reflecting in the absence of gains in the production of soybean grains.

Highlighted Conclusions

1. Fertilization with micronutrients in the soil using as criterion the reach of three levels of availability of B does not increase the production of biomass and grains, despite ensuring foliar contents of B ($\bar{Y} = 40.40$) and Cu ($\bar{Y} = 23.06$) suitable for soybean cultivation in the Cerrado.
2. The inequity in the concentrations of B, Cu, Mn and Zn supplied with fertilizer affects the physiological performance of soybean plants.

INTRODUCTION

The success of soybeans production in Brazil depends on a successful fertility program that includes all the essential nutrients for the growth and development of this oilseed (Gaspar et al. 2018). Despite the advances in micronutrient research - driven in large part by the success of fertilization with macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) (Burns 2017); the supply of boron (B), copper (Cu), manganese (Mn) and zinc (Zn) needs to be better understood, given the importance of these nutrients in biochemical and physiological processes vital to plant production (Hänsch and Mendel 2009, Marschner 2012).

Recent studies have shown that soil fertilization with micronutrients increases the levels of these elements in cultivated areas (Barbosa et al. 2016), revealing that the reach of high soybean yields (between 3,328 and 5,583 kg ha^{-1}) is conditioned by the absorption of B, Cu, Mn and Zn in the order of 24.3 to 42.7, 5.3 to 9.4, 57.2 to 113 and 24.5 to 56.8 mg kg^{-1} of biomass (Urano et al. 2007, Enderson et al. 2015). In addition, the effects of micronutrient fertilization on the photosynthetic activity of plants have been widely reported (Wang et al. 2009, Ilyas et al. 2015, Tavallali 2017, Flores et al. 2018a).

Since fertilization costs strongly impact the soybean production system in the Cerrado, representing up to 47.45% of the total invested per hectare (Richetti and Garcia 2017); that the results of the research show a limitation of wide applicability (Gaspar et al. 2018) and that diagnostic tools in assessing the availability of

micronutrients in the plant imply unnecessary fertilization in some regions (Enderson et al. 2015); the knowledge of the effects promoted by soil fertilization with sources of low solubility based on the morphophysiological aspects can help in the nutritional management of the soybean crop, contributing to the improvement of crop growth and yield. The aim of this study was to evaluate the effects of fertilization with micronutrients in the form of oxides applied in the soil on the nutrition and morphophysiology of soybean plants.

MATERIAL AND METHODS

The work was carried out in the experimental area of the Universidade Federal do Tocantins (UFT) campus Gurupi (11°43'45" S and 49°04'07" W, altitude 278 m). The region is classified as Brazilian Cerrado, with annual average temperature of 29.5 ° C and average rainfall of 1,804 mm, being a rainy summer and a dry winter (SEPLAN 2012).

A Red-Yellow Latosol collected from the 20 cm layer was used in the experiment, previously discharged and sieved with a mesh of 2.75 mm and incubated for acidity correction using a 2.5:1 mixture of calcium carbonate (CaCO₃) and magnesium (MgCO₃), whose applied dose was determined in previous assays to reach pH 6.0 (Santos et al. 2013a). The chemical and physical analysis of the soil after the application of the carbonates is in Table 1.

Table 1. Chemical attributes and physical properties of a Red-Yellow Oxisol used in the experiment.

pH (H ₂ O)	Al ³⁺	H+Al	C.E.C. ⁽¹⁾	m ⁽²⁾	V ⁽³⁾	O.M. ⁽⁴⁾
cmol _c dm ⁻³%.....	
6.20	0.00	2.20	5.14	0.00	57.00	1.80
Ca ²⁺	Mg ²⁺	K ⁺	S.B. ⁽⁵⁾	P _{Meh}	S	B
cmol _c dm ⁻³mg dm ⁻³	
2.10	0.70	0.14	2.94	10.50	6.00	0.10
Cu	Fe	Mn	Zn	Sand	Silt	Clay
mg dm ⁻³%.....	
0.80	36.00	1.70	8.70	65.00	5.00	30.00

⁽¹⁾C.E.C.: cation exchangeable capacity in pH 7.0; ⁽²⁾m%: aluminum saturation; ⁽³⁾V%: base saturation; ⁽⁴⁾O.M.: organic matter content; ⁽⁵⁾S.B.: sum of bases.

The experimental units were composed of polyethylene bags (30x45 cm) filled with 7.0 dm³ of soil. Base fertilization (single superphosphate - 18% P₂O₅ and potassium chloride - 60% K₂O) was carried out, with doses of each fertilizer calculated to reach levels classified as good availability for soybean cultivation, according Ribeiro et al. (1999).

The experimental design was completely randomized, with four replications. The treatments consisted of fertilization with micronutrients applied in the soil, adopting as criterion the reach of three levels of B availability: low (<0.15 mg dm⁻³ - control), good (0.61 mg dm⁻³) and high (> 0.90 mg dm⁻³). For this, the following values were applied: 0.00 (control), 56.67 and 211.11 kg ha⁻¹ of the FTE BR12 fertilizer (1.8% B, 0.85% Cu, 2% Mn and 9 % Zn). The concentrations of B, Cu, Mn and Zn in each treatment are shown in Table 2.

Table 2. Concentrations of B, Cu, Mn and Zn for each treatment with the fertilizer applied in the soil.

Treatment	Fertilizer concentration (kg ha ⁻¹)	Micronutrient concentration applied (mg dm ⁻³)			
		B	Cu	Mn	Zn
Low	0.00	-	-	-	-
Good	56.67	0.51	0.24	0.57	2.55
High	211.11	1.90	0.90	2.11	9.50

Six seeds of soybean cv. M8349 IPRO were treated chemically with insecticide Fipronil (i.a.) and fungicide Thiophanate-methyl + Fluazinam (i.a.) and inoculated with *Bradyrhizobium japonicum*. At 15 days after sowing, thinning was done leaving three plants per pot. The plants were drip irrigated three times a day by semi-automated system. When necessary, phytosanitary management was performed with insecticide Acetamipride (i.a.) and fungicides Trifloxystrobin + Prothioconazole (i.a.).

To evaluate the effect of treatments on shoot morphophysiology and nutritional status of soybean, leaf samples were collected when 50% of the plants were in the R2 (full flowering) stage and were then sent to the laboratory to determine: plant height (cm); stem diameter (mm); number of branches per plant (un); number of flowers per plants (un); dry weight of shoot, leaves and discs(g): the plants, the leaves, without petiole, and, with the aid of a

perforator, 12 discs were removed from the central part of the leaves, were conditioned in paper bags and dried in a forced circulation oven at 65 °C for 72 h. After this period, they were weighed in a precision scale (0.00 g); leaf area (LA - cm²): the leaf area of each plant was calculated according to Equation 1:

$$LA = \frac{(ND \times DA \times DWL)}{DWD} \quad \text{Equation 1}$$

where in: ND: number of discs; DA: disc's area, in cm²; DWL: dry weight of leaves, in g⁻¹; and DWD: dry weight of the discs, in g⁻¹.

Specific leaf area (SLA - cm² g⁻¹) was determined according to Equation 2:

$$SLA = \frac{TLA}{DWL} \quad \text{Equation 2}$$

Foliar contents of B (mg kg⁻¹ biomass): adapted from Carmo et al. (2000), using 0.1 mol L⁻¹ HCl to extract the extract; foliar contents of Cu, Mn and Zn (mg kg⁻¹ biomass): according Carmo et al. (2000), being the extract obtained by perchloric nitric digestion and the element determined by atomic absorption spectroscopy.

The photosynthetic activity of the soybean plants was analyzed by means of reading fully developed leaves, from each plant ten times, using an Infrared Gas Analyzer - IRGA, LCiSD ADC system®, from 9 to 12 o'clock on days with clear sky, in order to maintain the homogeneous environmental conditions during the evaluations. The photosynthetic pigments were quantified by readings at the center of the leaf using a ClorofiLOG® chlorophyll meter model CFL 1030 Falker, which provides results in dimensionless units, FCI values (Falker Chlorophyll Index). The physiological parameters obtained were: total chlorophyll (FCI); net CO₂ assimilation rate (*A* - μmol CO₂ m⁻² s⁻¹); stomatal conductance (*gs* - mmol H₂O m⁻² s⁻¹); transpiration rate (*E* - mmol H₂O m⁻² s⁻¹); internal CO₂ concentration (*Ci* - μmol CO₂ mol⁻¹ air); water use efficiency (WUE - μmol CO₂ mol⁻¹ H₂O).

At the end of the crop cycle and at the time of harvest, the results from the average of two plants per pot evaluated the yield characteristics of soybean: number of pods per plant (un); number of grains per pod (un); number of grains per plant (un); grain weight per plant (g); hundred grains weight (g).

Data were submitted to analysis of variance with the averages evaluated by the Tukey test at 5% of probability, using the software SISVAR 5.6 (Ferreira 2010).

RESULTS

Table 3 presents the summary of the analysis of variance for the morphophysiological characteristics, nutritional status and yield components of soybean plants submitted to fertilization with micronutrients incorporated in the soil.

Micronutrient fertilization in the treatments of good and high availability of micronutrients promoted significant effects (*p*≤0.05) on the morphological development of soybean plants, increasing up to 19% in plant height, 18% in number of branches per plant, 34% in leaf area and 15% in specific leaf area in relation to control (low availability of micronutrients) (Table 4). On the other hand, stem diameter and dry weight of shoot was not influenced by treatments.

The photosynthetic activity of the plants was influenced (*p*≤0.05) by micronutrient fertilization, with a slight increase in total chlorophyll content (11%) and substantial improvement in WUE (239%) of plants cultivated in the treatment with good availability of micronutrients, whereas *A* was 15% better in the treatment with high levels of micronutrients (Table 5). Furthermore, the increase of micronutrient concentrations in the treatments with good and high levels affected the other physiological parameters evaluated, reducing in three times the value of *gs* and five times the value of *E*, but maintaining almost unchanged the *Ci*, in comparison with the higher values observed in the control.

The nutritional status of soybean plants was significantly influenced (*p*≤0.05) by fertilization with micronutrients applied to the soil, except for the B content, maintained in the average of 40.4 mg kg⁻¹ of biomass (Table 6). Generally, treatments with good and high levels of micronutrients increased about 2.5 times the Cu and Mn content, while reducing the Zn content by 46% compared to the control.

Micronutrients applied in soil significantly affected (*p*≤0.05) soybean yield components, where the increase of concentrations in the treatments with good and high availability increased, on average, 23% number of flowers per plants (Table 7). For number of pods per plant and number of grains per plant the best results did not differ between the control and the treatment with high levels of micronutrients, presenting average gains in the order of 20% and 9% in relation to the treatment with good levels of micronutrient.

Table 3. Analysis of variance of the traits plant height (PH), stem diameter (SD), number of branches per plant (BP), dry weight of shoot (DWS), leaf area (LA), specific leaf area (SLA), total chlorophyll (Chl), net CO₂ assimilation rate (A), stomatal conductance (gs), transpiration rate (E), internal CO₂ concentration (Ci), water use efficiency (WUE), foliar contents of B, Cu, Mn and Zn, number of flowers per plants (FP), number of pods per plant (PP), number of grains per pod (GPo), number of grains per plant (GPI), grain weight per plant (GW), hundred grains weight (HG) of soybean plants submitted to soil fertilization with micronutrients.

Traits	Degrees of freedom	Residual	Average	C.V. (%)
	2	9		
PH	47.63 [*]	3.84	38.69	5.06
SD	0.01 ^{ns}	0.08	3.80	7.57
BP	3.24 [*]	0.59	10.37	7.40
DWS	0.38 ^{ns}	0.13	4.38	8.21
LA	43,129.00 ^{**}	4,130.00	717.04	8.96
SLA	1,221.11 ^{**}	3.59	233.85	0.81
Chl	13.28 ^{**}	1.16	34.45	3.13
A	15.69 ^{**}	0.22	9.62	4.84
gs	0.133 ^{**}	0.00	0.38	13.49
E	45.70 ^{**}	0.21	5.48	8.37
Ci	432.81 ^{**}	17.26	321.42	1.29
WUE	9.17 ^{**}	0.04	2.41	7.76
B	11.32 ^{ns}	6.88	40.40	6.49
Cu	387.58 ^{**}	0.86	23.08	4.02
Mn	27.15 ^{**}	0.27	6.10	8.59
Zn	117.30 ^{**}	0.36	15.88	3.77
FP	114.78 ^{**}	12.2	47.67	7.33
PP	5.99 ^{**}	0.52	11.49	6.31
GPo	0.07 ^{ns}	0.02	2.24	6.73
GPI	6.79 ^{**}	0.54	25.55	2.88
GW	0.06 ^{ns}	0.04	4.61	4.50
HG	5.49 ^{ns}	3.17	17.98	9.91

Table 4. Plant height, stem diameter, number of branches per plant, dry weight of shoot, leaf area and specific leaf area of soybean plants submitted to soil fertilization with micronutrients.

Morphological traits	Treatments		
	Low	Good	High
Plant height (cm)	35.38 b	38.44 ab	42.26 a
Stem diameter (mm)	3.83	3.74	3.82
Number of branches per plant (un)	9.38 b	11.13 a	10.61 ab
Dry weight of shoot (g)	4.04	4.44	4.65
Leaf area (cm ²)	607.93 b	728.54 ab	814.64 a
Specific leaf area (cm ² g ⁻¹)	219.56 c	228.66 b	253.33 a

Means with the same lowercase letters in the row do not differ by the Tukey test at 5%.

Table 5. Total chlorophyll, net CO₂ assimilation rate (A), stomatal conductance (gs), transpiration rate (E), internal CO₂ concentration (Ci), and water use efficiency of soybean plants submitted to soil fertilization with micronutrients.

Physiological traits	Treatments		
	Low	Good	High
Total chlorophyll (ICF)	32.56 b	36.19 a	34.59 ab
A (μmol CO ₂ m ⁻² s ⁻¹)	9.91 b	7.51 c	11.44 a
gs (mmol H ₂ O m ⁻² s ⁻¹)	0.54 a	0.18 c	0.42 b
E (mmol H ₂ O m ⁻² s ⁻¹)	8.54 a	1.85 c	6.04 a
Ci (μmol CO ₂ mol ⁻¹ ar)	331.17 a	310.48 c	322.61 b
WUE (μmol CO ₂ mol ⁻¹ H ₂ O)	1.21 c	4.11 a	1.90 b

Means with the same lowercase letters in the row do not differ by the Tukey test at 5%.

Table 6. Foliar contents of B, Cu, Mn and Zn of soybean plants submitted to soil fertilization with micronutrients.

Nutritional traits	Treatments		
	Low	Good	High
B (mg kg ⁻¹ biomass)	39.10	39.80	42.30
Cu (mg kg ⁻¹ biomass)	11.75 b	28.00 a	29.50 a
Mn (mg kg ⁻¹ biomass)	4.23 b	5.00 b	9.08 a
Zn (mg kg ⁻¹ biomass)	22.06 a	13.56 b	12.00 c

Means with the same lowercase letters in the row do not differ by the Tukey test at 5%.

Table 7. Number of flowers per plants, number of pods per plant, number of grains per pod, number of grains per plant, grain weight per plant, and hundred grains weight of soybean plants submitted to soil fertilization with micronutrients.

Yield components	Treatments		
	Low	Good	High
FP (un)	41.50 b	50.33 a	51.17 a
VP (un)	12.05 a	10.09 b	12.33 a
GV (un)	2.19	2.39	2.14
GP (un)	26.27 a	24.04 b	26.33 a
MGP (g)	4.71	4.66	4.47
M100 (g)	18.80	18.49	16.64

Means with the same lowercase letters in the row do not differ by the Tukey test at 5%.

DISCUSSION

In this study using oxides as a source of micronutrients, the best observed results for the developmental characteristics of the culture may be related to the accumulation of B (\bar{Y} = 40.40 mg kg⁻¹) and Cu (\bar{Y} = 23.08 mg kg⁻¹) foliar, whose average contents are considered enough for the full development of the culture in the Cerrado (Galvão 2004). In this way, the reflexes of adequate nutrition of B manifested in the vegetative and reproductive growth of the plants, as observed for plant height, leaf area, number of flowers per plants, number of pods per plant and number of grains per plant (Tables 4 and 7), with highlight to the role of this micronutrient in the synthesis and elongation of cell wall and pollen tube growth (Dechen and Nachtigall 2006, Hänsch and Mendel 2009).

The acceptable accumulation of Cu in soybean leaves reveals the presence of a sophisticated mechanism that maintains its capture, mobilization and storage (Clemens 2001), even in the control (low soil availability - Table 1). According Ando et al. (2013), the absorption and transport of Cu is done through the roots to the xylem, from where the Cu is translocated to the shoot through different transporters in the plasma membrane, suggesting the dynamic regulation of the intracellular concentrations in response to the physiological demand of plants (Adress et al. 2015, Vatansever et al. 2016a). However, similarly to results demonstrated in this work, Barbosa et al. (2016) observed that the use of 132.32 kg ha⁻¹ of a source of micronutrients (mixture of salts, sulphates and oxides - sulphate) did not promote any effect on the leaf content of Cu of soybean, although increasing the availability of the element in the soil. For some authors, this reinforces the different levels of Cu requirement between cultivars for the normal development of plants (Mantovi et al. 2003).

Regarding the physiological traits, this has been widely reported photosynthetic responses to nutrient supply (Warren 2004, Wang et al. 2009, Tavallali 2017). It is known that micronutrients act in the process of photosynthesis in different ways, in synthesis: participating in the metabolism of carbohydrates (B), fixing carbon and forming enzymes (Fe and Mn), integrating the electron transport chain (Cu) and acting as an activator of photosynthetic enzymes of the oxidase class (Zn) (Cakmak and Römheld 1997, Santos et al. 2013b). In this sense, the photosynthetic activity of the plants evaluated in R2 presented the best total chlorophyll index and WUE, especially when the soil was fertilized with good availability of micronutrients (Table 5). Considering that both B deficiency and excess affect root growth (Chen et al. 2012, Silva et al. 2015), the results of total chlorophyll by the supply of B in the control and high availability of micronutrients can be attributed to an eventual low nodulation and absorption of water and nutrients by the roots, which affects the assimilation of N - important component of the chlorophyll molecules (Prado 2008, Silva et al. 2015).

The variations in the effects for the chlorophyll content in the plants of soil fertilization treatments with varied doses of micronutrient (adopting B as reference) revealed that the supply of up to 4.0 kg ha⁻¹ of the micronutrient did not affect the chlorophyll index at 28 and 46 days after emergence (Flores et al. 2018a), the fertilization with

4.39 kg ha⁻¹ promoted a higher chlorophyll index at 40 days after germination (Flores et al. 2017), while doses up to 6.0 kg ha⁻¹ reduced pigment rates in common bean leaves (Flores et al. 2018b).

Atmospheric CO₂ must diffuse through the stomatal opening to the carboxylation sites inside the plant cells for photosynthesis to occur, where *C_i* is lower as a function of *A* and *g_s* rates (Aalto and Juurola 2002). In this aspect, the evaluation of the physiological performance of the soybean showed that the control treatment produced a lower *A*, whereas *g_s* and *C_i* were more effective, revealing an inverse effect to that observed in the treatment with high availability of micronutrients (Table 5). Investigating such physiological parameters, Warren (2004) observed that the positive response of photosynthesis to nutrient supply was not accompanied by changes in *g_s* and *C_i*. In the work of Flores et al. (2018a), increasing doses of micronutrients (sources with wide-ranging solubilities) applied to the soil also did not affect *C_i* and *g_s*; however, in different soils, the application of Zn, Cu and B at different levels improved *A*, *g_s*, total chlorophyll and fruit production (Ilyas et al. 2015). It should be noted that in the present study grain yield per plant (MGP) was not affected by treatments (Table 7), a fact similar to that observed by Mallarino et al. (2013).

The soil used in this study presents the initial contents of Mn classified as low and high Zn as high, by Ribeiro et al. (1999) interpretation (Table 1), the concentrations of these micronutrients supplied in the treatments maintained a disparity in the levels of availability recommended by these authors as an ideal. For Barbosa et al. (2016), this reinforces the low efficiency of Mn fertilization in the soil compared to other micronutrients, requiring high doses to increase the absorption by plants. Our results demonstrated that not even the largest accumulation of leaf Mn (9.08 mg kg⁻¹ biomass) supply this nutrient to the soybean crop, which is in the range between 20 and 200 mg kg⁻¹ (Galvão 2004), being detected symptoms typical of Mn deficiency, such as necrosis and chlorosis internerval (Dechen and Nachtigall 2006). In this scenario, the Mn deficiency causes damages in the chloroplast structure due to a reduction in the levels of the enzyme MnSOD (Allen et al. 2007) and an increase in oxygen free radicals (Marschner 2012, Salvador et al. 2013), affecting photosynthesis rate and reducing the amount of chlorophyll, which decreases the level of soluble carbohydrates in plants (Ndakidemi et al. 2011), as observed for total chlorophyll, *A* and dry weight of shoot (Tables 4 and 5).

The evaluation of the nutritional state of soybean plants also showed that fertilization with increasing doses of fertilizer applied to the soil reduced the absorption of Zn by the plants (Table 6), where only the control guaranteed enough leaf content, between 20 and 50 mg kg⁻¹ (Galvão 2004). This behavior resembles that obtained by Barbosa et al. (2016), although they found an increase in the level of Zn in the soil and a positive effect on soybean yield. For some authors, Zn homeostasis in plants is maintained mainly by the coordinated regulation of a wide network of homologous transporters in different plant species (Moreau et al. 2002, Ramesh et al. 2003, Vatansever et al. 2016b). In this sense, physiological and biochemical studies have demonstrated that the responsiveness of Zn is linked to the establishment of defense mechanisms, such as chelation (Bertrand and Poirier 2005, Solymosi and Bertrand 2010), presuming the effects observed by the excessive doses applied to the soil.

Allied to a series of genetic and environmental factors influencing the response of plants submitted to adverse soil fertility conditions (Gaspar et al. 2018), the nutritional demand for soybean is still hampered by the difference in micronutrient concentration with the doses supplied through a single source of slow release in the soil, such as oxides. This suggests that the benefits of these fertilizers in providing B, Cu, Mn and Zn need longer times than this research, or even requires foliar supplementation with sources of high solubility, especially for the care of Mn by the crop.

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