Fertilization/ Original Article

Copper use efficiency in soybean cultivars

Abstract – The objective of this work was to evaluate the effect of copper use efficiency in soybean cultivars, grown on a typical Ultisol with a high organic matter content, on soil chemical properties and on plant grain yield, nutritional state, and physiological components. The experiment was carried out in greenhouse conditions, in a 5×4 factorial arrangement, with five soybean cultivars (TMG 1066RR, BRS 360RR, NA 6262RR, BMX Turbo RR, and BRS 359RR) and four Cu rates (0, 2, 4, and 8 mg kg^{-1}). Under the studied soil conditions, the DTPA-TEA and Mehlich-1 extractants were efficient in determining available Cu in the soil. Regardless of the cultivars, Cu application increased grain yield (GY), shoot dry weight yield (SDWY), number of pods per pot, GY/SDWY ratio, photosynthetic rate, stomatal conductance, internal CO_2 concentration, transpiration rate, and chlorophyll content. However, Cu use efficiency varied significantly among the different soybean genotypes. Except for Cu, soil chemical attributes and foliar and grain nutrient contents are not influenced by Cu rates nor by soybean cultivars.

Index terms: *Glycine max*, DTPA-TEA, Mehlich 1, nutritional state, physiological components, yield components.

Eficiência de uso de cobre em cultivares de soja

Resumo – O objetivo deste trabalho foi avaliar o efeito da eficiência do uso de cobre em cultivares de soja, cultivadas em Argissolo típico com alto teor de matéria orgânica, sobre os atributos químicos do solo e a produção de grãos, o estado nutricional e os componentes fisiológicos das plantas. O experimento foi realizado em condições de casa de vegetação, em arranjo fatorial 5×4, com cinco cultivares de soja (TMG 1066RR, BRS 360RR, NA 6262RR, BMX Turbo RR e BRS 359RR) e quatro doses de Cu (0, 2, 4 e 8 mg kg^{-1}). Nas condições de solo estudadas, os extratores DTPA-TEA e Mehlich 1 foram eficientes na determinação do Cu disponível no solo. Independentemente das cultivares, a aplicação de cobre aumentou a produção de grãos (PG), a produção de massa seca da parte aérea (PMSPA), o número de vagens por vaso, a relação PG/PMSPA, a taxa fotossintética, a condutância estomática, a concentração interna de CO_2, a taxa transpiratória e o teor de clorofila. No entanto, a eficiência do uso de Cu variou significativamente entre os diferentes genótipos de soja. Exceto para Cu, os atributos químicos do solo e os teores de nutrientes das folhas e dos grãos não são influenciados pelas doses de Cu nem pelas cultivares de soja.

Termos para indexação: *Glycine max*, DTPA-TEA, Mehlich 1, estado nutricional, componentes fisiológicos, componentes de produção.

Adônis Moreira[(1 ✉)] ,
Larissa Alexandra Cardoso Moraes[(1)] ,
Thiago Assis Rodrigues Nogueira[(2)] and
Bruna Trovo Canizella[(3)]

[(1)] Embrapa Soja, Rodovia Carlos João Strass, s/nº, Acesso Orlando Amaral, Distrito de Warta, Caixa Postal 231, CEP 86001-970 Londrina, PR, Brazil.
E-mail: adonis.moreira@embrapa.br, larissa.moraes@embrapa.br

[(2)] Universidade Estadual Paulista Júlio de Mesquita Filho, Campus de Ilha Solteira, Avenida Brasil, nº 56, Centro, CEP 15385-000 Ilha Solteira, SP, Brazil.
E-mail: tar.nogueira@unesp.br

[(3)] Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, PR 445, Km 380, Campus Universitário, CEP 86057-970 Londrina, PR, Brazil.
E-mail: bruna_canizella@hotmail.com

✉ Corresponding author

Introduction

The introduction of the no-tillage management into crop systems has caused a gradual increase in soil organic matter (SOM), leading to a

higher nutrient availability, water retention, and cation exchange capacity, which allow oxygen diffusion for the development of soil meso- and microfauna and, consequently, a better growth of plant roots (Fageria & Moreira, 2011; Lourente et al., 2011). However, due to its great affinity to SOM, available copper content may decrease (Stevenson & Fitch, 1981; Migocka & Malas, 2018) as a result of the increased formation of complexes and chelate, which can reduce the concentration of the element in the soil solution, mainly when SOM is associated with the clay fraction, since copper retention in the solid phase is largely related to the presence of these two types of materials (Wu et al., 1999).

Copper deficiency has been reported in many agricultural soils in several parts of the world (Fageria, 2009). In South America, for example, it has been observed in cereals and legumes (Fageria et al., 2015), varying according to soil conditions and crop genotypes (Fageria, 2014). In this scenario, knowing the amount of copper required is important, since this micronutrient is essential for the healthy growth of crop plants, being responsible for activating several enzymes, with roles in photosynthesis, respiration, protein and carbohydrate metabolisms, lignification, and pollen formation (Marschner, 2012; Migocka & Malas, 2018).

In the soil, Cu^{2+} ions bind directly to SOM through two or more functional groups, such as the carboxyl, carbonyl, and phenolic ones. For copper, immobilization occurs by the formation of a rigid inner-sphere complex in conditions of low pH value, differently from that observed for other metals, such as manganese, iron, and cobalt, which form an outer-sphere complex with SOM (McBride, 1997). With Cu^{2+}, soil components are absorbed in the following preference order: manganese oxide (MnO), SOM, iron oxide (Fe_2O_3), aluminum ion (Al^{3+}), and clay minerals (Wu et al., 1999). However, soil MnO and SOM are more prone to connect to Cu^{2+} in a non-permutable manner, since, when adsorbed by clay minerals and Fe and Al oxides, copper can be exchanged by other cations and pass to the soluble phase, which does not occur during binding to MnO and SOM (Wu et al., 1999).

In plants, nutrient uptake differs according to species and cultivars, which are classified as efficient and responsive or as inefficient and responsive, for example (Moreira et al., 2015). Moreira et al. (2019) and Moreira & Moraes (2019) found a significant increase in soybean [*Glycine max* (L.). Merr.] and wheat (*Triticum aestivum* L.) yield when copper was applied in a field experiment, in two growing seasons, under subtropical and greenhouse conditions. Moreira et al. (2016) highlighted that nutrient use efficiency in genotypes is a key strategy to improve soybean yield, concluding that the uptake of nutrients, including copper, varies from one soybean genotype to the other.

The objective of this work was to evaluate the effect of copper use efficiency in soybean cultivars, grown on a typical Ultisol with a high organic matter content, on soil chemical properties and on plant grain yield, nutritional state, and physiological components.

Materials and Methods

The experiment was conducted under greenhouse conditions, at an average temperature of 26°C and a day/night photoperiod of 13±0.5/11±0.5 hours, at Embrapa Soja, in the municipality of Londrina, in the state of Paraná, Brazil (23°11'39"S, 51°10'40"W, at 630 m altitude). The soil used was an Argissolo típico (Santos et al., 2013), i.e., a typical Ultisol, collected at 0–20-cm depth, at the municipality of Ponta Grossa, also in the state of Paraná. Before the treatments were applied, soil chemical analysis showed the following attributes (Teixeira et al., 2017): pH ($CaCl_2$) 6.5, 25.1 g kg^{-1} C, 7.5 mg kg^{-1} P (Mehlich-1 extractant), 0.3 $cmol_c$ kg^{-1} K^+, 4.5 $cmol_c$ kg^{-1} Ca^{2+}, 0.5 $cmol_c$ kg^{-1} Mg^{2+}, 0.0 $cmol_c$ kg^{-1} Al^{3+} (KCl 1.0 mol L^{-1}), 4.0 $cmol_c$ kg^{-1} H+Al, 8.0 mg kg^{-1} S-SO_4^-, cation exchange capacity (CEC) ($\sum K^+$, Ca^{2+}, Mg^{2+}, H+Al) of 5.51 $cmol_c$ kg^{-1}, base saturation (V) [($\sum K^+$, Ca^{2+}, Mg^{2+}/CEC)×100] of 56.8%, 0.2 mg kg^{-1} available B (hot water), 1.7 mg kg^{-1} available Cu (Mehlich 1), 3.2 mg kg^{-1} Cu [diethylenetriaminepentaacetic acid-triethanolamine (DTPA-TEA)], 75.5 mg kg^{-1} available Fe (Mehlich 1), 80 mg kg^{-1} available Mn (Mehlich 1), and 21.9 mg kg^{-1} available Zn (Mehlich 1). Soil density was 1.2 g cm^{-3} and clay content was 357 g kg^{-1}.

The experimental design was completely randomized, in a 5×4 factorial arrangement, used to evaluate the response of five soybean transgenic cultivars (TMG 1066RR, BRS 360RR, NA 6262RR, BMX Turbo RR, and BRS 359RR) to four Cu rates (0, 2, 4, and 8 mg kg^{-1}), with four replicates. The choosen cultivars were obtained from different breeders, were representative of the subtropical region, showed an indeterminate cycle, and were classified in the 5.9 to 6.2 relative maturity group.

Thirty days before planting in 3.0-kg soil clay pots, a total of 3.0 Mg ha^{-1} dolomitic lime were applied (13.5% MgO and 95% effective calcium carbonate equivalent). The pots were irrigated daily with deionized water to keep the soil next to 70% of total pore volume. Ten seeds were seeded and, after harvest, two plants were left per pot.

Nitrogen was supplied by the inoculation of seeds with a cocktail of *Bradyrhizobium elkanii* + *Bradyrhizobium japonicum*. The other nutrients – phosphorus, potassium, sulfur, boron, cobalt, iron, manganese, molybdenum, nickel, and zinc – were applied as recommended by Moreira et al. (2011) for experiments conducted in greenhouse conditions: 50 mg kg^{-1} P (monoammonium phosphate), 0.5 mg kg^{-1} B (H_3BO_3), 0.1 mg kg^{-1} Mo ($Na_2Mo_4.2H_2O$), 0.01 mg kg^{-1} Co ($CoCl_2$), 0.01 mg kg^{-1} Ni ($NiSO_4.6H_2O$), 5.0 mg kg^{-1} Mn ($MnSO_4.3H_2O$), and 5.0 mg kg^{-1} Zn ($ZnSO_4.7H_2O$). At the V_2 and V_4 growth stages, 50 mg kg^{-1} K (potassium sulphate) were applied twice as topdressing fertilization, totaling 100 mg kg^{-1} in the cycle. Except for limestone, nutrients were added in solution.

At the R_2 growth stage (Fehr et al., 1971), during the morning period, the third and fourth trefoils were measured, starting at the apex, with the LI-6400XT portable photosynthesis analyzer (LI-COR Biosciences, Lincoln, NE, USA). Photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration rate (Trmmol, mmol H_2O m^{-2} s^{-1}), and intercellular CO_2 concentration (Ci, µmol CO_2 mol^{-1}) were determined, and water use efficiency (µmol CO_2 m^{-2} s^{-1}) was calculated for A/Trmmol. In the same stage and leaves, the soil-plant analysis development (SPAD) index, used to indicate relative chlorophyll content, was also measured with SPAD 502 (Konica Minolta, Inc., Osaka, Japan), being later converted into chlorophyll content (mg cm^{-2}) through the equation $\hat{y} = 16.033 + (7.5774 \times SPAD)$ (Fritschi & Ray, 2007).

After measuring these variables, the same trefoils were collected from the apex (diagnostic leaf) for each treatment. They were dried in a hood with forced circulation, at 65°C, to determine total N, P, K, Ca, Mg, B, Cu, Fe, Mn, and Zn contents (Malavolta et al., 1997). During all the vegetative cycle, senescent leaves were collected to obtain shoot dry weight yield (SDWY). After the physiological maturation stage (R8), grain yield (GY), number of pods per pot (NPP), and number of grains per pod (NGP) were quantified. The total contents of N, P, K, Ca, Mg, B, Cu, Fe, Mn, and Zn were also obtained for the grains. After harvest, soil samples from each pot were collected to detemine soil chemical attributes (pH, SOM, P, K$^+$, Ca^{2+}, Mg^{2+}, H+Al, Al^{3+}, S-SO_4^{2-}, B, Cu, Fe, Mn, and Zn), according to the methodologies described by Teixeira et al. (2017). Available Cu contents in the soil were also extracted by DTPA-TEA (Lindsay & Norvell, 1978).

Results from soil chemical attributes, yield components, physiological components (A, C$_i$, g_s, Trmmol, water use efficiency, and chlorophyll content), and plant nutritional status (leaves and grains) were subjected to normality tests, analysis of variance, F-statistics, Pearson's correlation analysis, and polynomial regression, correlation, and regression, at 5% probability.

Results and Discussion

The cultivar × Cu rate interactions were not significant for soil chemical attributes (Table 1). Most of the analyzed attributes (pH, C, P, K$^+$, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Fe, Mn, and Zn) were above or within the range considered adequate for the development of plants in tropical conditions; the exceptions were Al^{3+} and potential acidity that were within the low or very low range, and available B that was below the recommended range (Moreira et al., 2017b).

In the case of available Cu, independently of the extractant used (Mehlich 1 or DTPA-TEA), there was a linear increment in Cu contents with the increase in nutrient rates (Figure 1). Both extractants were affected by these rates and were significantly correlated when used to determine soil nutrient availability (Figure 2). Available Cu contents varied from 5.6 to 7.8 mg kg^{-1}, with an average of 6.5 mg kg^{-1}, with the DTPA-TEA extractant, and from 2.1 to 3.3 mg kg^{-1}, with an average of 2.5 mg kg^{-1}, with Mehlich 1, which means that DTPA-TEA extracted 2.6 times more available Cu than Mehlich 1 ($\hat{y} = 3.972 + 1.034x$, r = 0.59, p≤0.05). Despite these differences, the interpretation ranges for soil Cu availability and the efficiency of both extractants for the interpretation of soil nutrient contents are in alignment with Tecnologias de produção de soja (2011). Even with high soil available Cu (Figure 2), the ranges were within those obtained

by Moreira & Moraes (2019) in soils with different clay and SOM contents.

GY, SDWY, GY/SDWY ratio, and NPP were influenced by the treatments, with a significant cultivar × Cu rate interaction for these variables (Table 2). These results showed that the variation between cultivars was consistent and that the cultivars responded differently to Cu rates. GY varied from 15.0 to 25.2 g per pot; the highest productivity was found for the BRS 360RR cultivar, with an average of 22.4 g per pot, regardless of

the Cu rate, and the lowest for NA 6262RR, with 17.6 g per pot. Moreira et al. (2015, 2017a) also observed that, even when the same amount of a certain nutrient is applied to substrates, soybean cultivars adapted to the same environmental conditions can behave differently regarding GY.

The SDWY of the five evaluated cultivars varied from 29.2 to 53.5 g per pot in response to the Cu rates, with an average of 40.9 g per pot. Among cultivars, in general, NA 6262RR was the least productive and

Table 1. Influence of copper rates and genotypes on the chemical attributes of a typical Ultisol after cropping of soybean (*Glycine max*) cultivars[1].

Cu rates (mg kg^{-1})	pH CaCl$_2$	C (g kg^{-1})	P (mg kg^{-1})	K$^+$	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	CEC	V (%)	S-SO$_4^{2-}$	B	Fe	Mn	Zn
											--------------------(mg kg^{-1})----------------------				
				---------------------(cmol$_c$ kg^{-1})-----------------------											
						TMG 1066RR									
0	7.0	27.3	31.1	0.3	10.1	1.5	0.0	2.0	13.9	85.4	93.0	0.2	58.8	219.8	23.1
2	7.1	24.8	31.5	0.3	9.7	1.6	0.0	2.1	13.7	85.3	56.6	0.2	76.7	224.2	19. 6
4	7.0	25.4	30.6	0.4	9.7	1.6	0.0	2.0	13.6	85.3	66.2	0.2	78.9	228.8	20.3
8	7.1	25.8	32.5	0.3	10.3	1.5	0.0	2.2	14.3	86.1	52.8	0.2	66.4	218.4	18.8
Average	7.1	25.8	31.4	0.3	9.9	1.5	0.0	2.1	13.9	85.5	67.2	0.2	70.2	222.8	20.4
						BRS 360RR									
0	7.1	25.3	30.0	0.4	9.8	1.5	0.0	2.1	13.6	85.6	52.8	0.2	69.5	206.0	19.6
2	7.0	25.7	30.3	0.2	10.6	1.3	0.0	2.0	14.1	85.9	70.6	0.2	62.9	214.2	20.7
4	7.1	27.1	31.1	0.2	10.5	1.4	0.0	2.1	14.2	86.0	43.8	0.2	62.0	208.6	20.3
8	7.0	24.6	34.0	0.2	9.7	1.2	0.0	2.0	13.1	84.4	43.4	0.2	88.4	223.1	19.1
Average	7.1	25.7	31.4	0.3	10.1	1.4	0.0	2.0	13.77	85.5	52.7	0.2	70.7	213.0	19.9
						NA 6262RR									
0	7.1	26.2	33.6	0.6	9.5	1.6	0.0	2.2	13.8	85.6	59.5	0.3	69.8	208.7	19.9
2	7.1	24.2	32.0	0.5	9.7	1.7	0.0	2.0	13.9	85.7	50.0	0.3	70.9	216.9	19.2
4	7.1	24.8	31.2	0.5	9.9	1.7	0.0	2.1	14.2	85.9	69.0	0.3	67.8	206.6	19.0
8	7.0	26.5	29.1	0.5	9.9	1.5	0.0	2.0	13.9	85.5	59.5	0.3	69.6	216.8	19.9
Average	7.1	25.4	31.5	0.5	9.7	1.6	0.0	2.1	14.0	85.7	59.5	0.3	69.5	212.3	19.5
						BMX Turbo RR									
0	7.0	25.8	27.5	0.4	9.4	1.6	0.0	2.0	13.4	85.1	47.6	0.3	77.7	215.1	18.4
2	7.0	27.4	32.9	0.6	9.1	1.6	0.0	2.1	13.3	85.2	61.3	0.3	82.5	219.8	19.1
4	7.0	26.4	30.9	0.3	9.6	1.5	0.0	2.0	13.3	85.2	63.0	0.3	69.7	207.4	19.3
8	7.1	27.3	28.7	0.3	10.2	1.6	0.0	2.1	14.1	86.0	55.6	0.2	63.5	205.6	18.8
Average	7.0	26.7	30.0	0.4	9.6	1.6	0.0	2.0	13.5	85.4	56.9	0.3	73.3	212.0	18.9
						BRS 359RR									
0	7.0	26.2	32.2	0.3	10.1	1.6	0.0	2.0	13.9	85.7	62.2	0.3	79.8	217.0	19.1
2	7.0	29.8	31.0	0.2	10.0	1.4	0.0	2.1	13.7	85.1	63.7	0.3	57.0	192.1	18.3
4	6.8	25.2	30.0	0.3	9.1	1.5	0.0	2.0	12.8	84.7	58.4	0.4	66.0	212.3	21.6
8	7.0	26.7	33.5	0.3	8.4	1.4	0.0	2.1	12.2	83.2	64.2	0.3	82.9	223.8	21.1
Average	6.9	27.0	31.7	0.3	9.4	1.5	0.0	2.0	13.1	84.7	62.1	0.3	71.4	211.3	20.0
						F-test									
Cultivar (a)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rates (b)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
a × b	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	8.12	14.71	9.15	9.47	14.19	12	47	11.63	15.44	12.66	9.78	11.46	16.84	15.88	13.62

[1]CEC, cation exchange capacity: ∑K$^+$, Ca^{2+}, Mg^{2+}, H+Al; V, base saturation: (∑K$^+$, Ca^{2+}, Mg^{2+})/CEC; and CV, coefficient of variation. [ns]Nonsignificant by the F-test, at 5% probability.
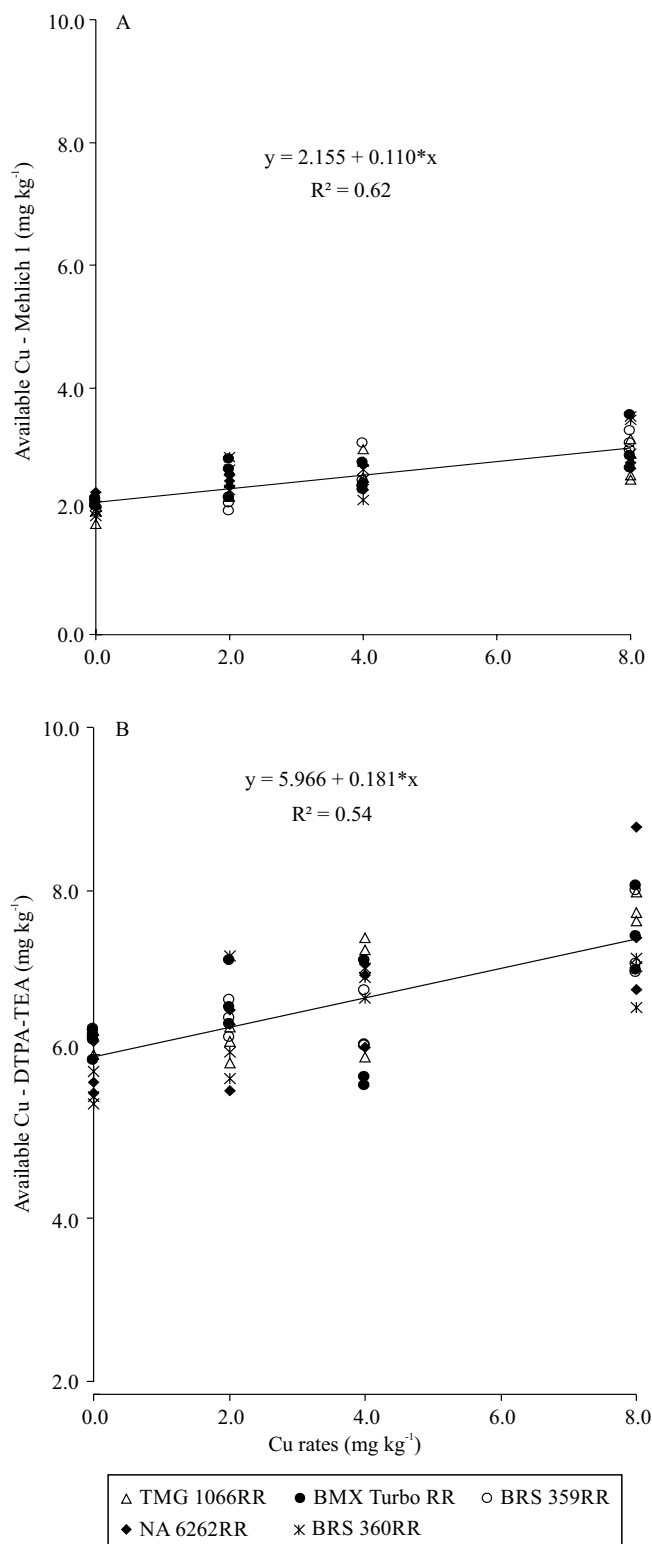
Figure 1. Available copper in the soil obtained with the Mehlich-1 (A) and diethylenetriaminepentaacetic acid-triethanolamine (DTPA-TEA) (B) extractors in response to copper rates after cropping of soybean (*Glycine max*) cultivars. *Significant by the F-test, at 5% probability.

TMG 1066RR, the most (Table 2). According to the regression equation ($\hat{y} = 38.260 + 2.090x - 0.225x^2$, $R^2 = 0.86$, p≤0.05), the highest SDWY was obtained with the Cu rate estimated at 4.6 mg kg^{-1}, which is three times above the value recommended by Allen et al. (1976) and Moreira et al. (2011) as the proper amount for plant cultivation in experiments conducted in greenhouse conditions.

The GY/SDWY ratio and NPP were also influenced by Cu rates and soybean cultivars (Table 2), presenting a quadratic effect for the highest cultivar averages obtained with 4.0 and 3.3 mg kg^{-1} Cu, respectively. Among the cultivars, the highest and lowest GY/SDWY ratios of 0.56 and 0.45 were found for NA 6262RR and TMG 1066RR, respectively, with an average value of 0.50. For NPP, the inverse was observed, with values ranging from 41 for NA 6262RR to 64 for TMG 1066RR, with an average of 53 among cultivars; BRS 360RR, BMX Turbo RR, and BRS 359RR presented intermediate values. Moreira et al. (2017a) and Moreira
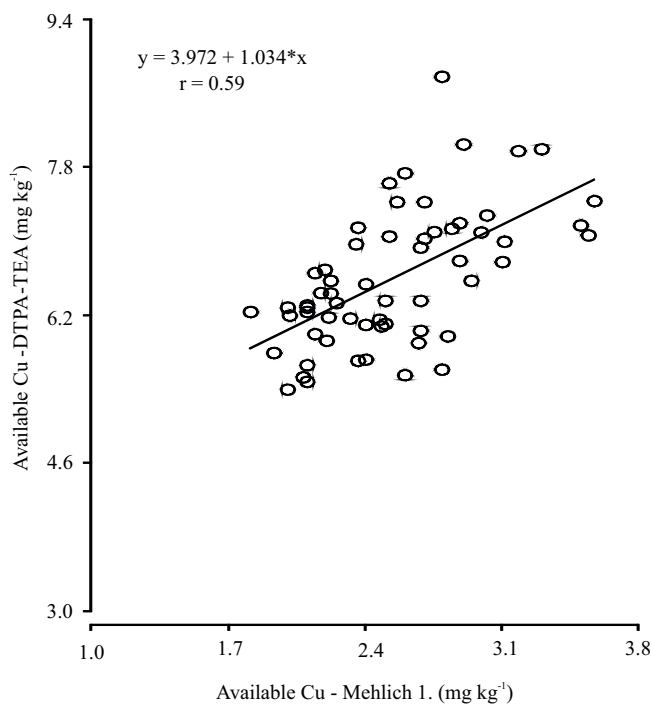

Figure 2. Relationship between the Mehlich-1 and diethylenetriaminepentaacetic acid-triethanolamine (DTPA-TEA) extractors, at pH 7.3, in determining available copper in the soil after cropping of soybean (*Glycine max*) cultivars. *Significant at 5% probability by the F-test.

& Moraes (2016) also found that NPP varied among soybean cultivars, regardless of the used fertilization. The NGP varied from 2 to 3, a number similar to

that obtained by Moreira et al. (2015) and Moreira & Moraes (2016) for different cultivars. Fageria et al. (2010) attributed differences in NGP in leguminous plants to genetic factors, which could explain the differences between genotypes of varying origins and characteristics.

In leaves, differences were observed among cultivars for N, P, K, Ca, Fe, Mn, and Zn contents and for the cultivar × Cu rate interaction for Cu content; in grains, differences were verified among cultivars for Ca and Fe contents and also for the interaction between treatments for Cu content (Tables 3 and 4). In leaves, nutrient contents varied from 22.2 to 35.5 g kg^{-1} N, 2.8 to 9.9 g kg^{-1} P, 13.3 to 23.0 g kg^{-1} K, 1.7 to 3.4 g kg^{-1} Ca, 3.0 to 4.8 g kg^{-1} Mg, 3.2 to 11.7 mg kg^{-1} Cu, 29.2 to 122.5 mg kg^{-1} Fe, 40.1 to 115.5 mg kg^{-1} Mn, and 12.5 to 23.0 mg kg^{-1} Zn; in grains, from 26.2 to 40.8 g kg^{-1} N, 4.0 to 8.0 g kg^{-1} P, 15.2 to 27.4 g kg^{-1} K, 1.6 to 2.9 g kg^{-1} Ca, 1.1 to 1.9 g kg^{-1} Mg, 3.6 to 15.7 mg kg^{-1} Cu, 78.1 to 185.7 mg kg^{-1} Fe, 45.2 to 83.4 mg kg^{-1} Mn, and 12.1 to 37.1 mg kg^{-1} Zn. Except for the Cu contents that varied with the Cu rates and for the N well below the contents indicated as adequate by Malavolta et al. (1997), the values obtained for P, K, Ca, Mg, Fe, Mn, and Zn, in the present study, were similar to those reported by Moreira et al. (2015, 2016) for different soybean cultivars in the same environmental conditions.

Considering the average of soybean cultivars and Cu rates, the order of the macronutrient contents in the leaves was N > K > P > Mg > Ca, while that of micronutrients was Fe > Mn > Zn > Cu (Table 3). In the grains, the order of the macronutrient contents was N > K > P > Ca > Mg, and that of micronutrients was similar to the one observed for leaves (Table 4). Likewise, Moreira et al. (2015, 2017a) also reported higher N, K, and P contents in leaves and grains. In relation to micronutrients in grains, Moreira et al. (2015) obtained a similar order, considering the average of different soybean cultivars. Among the evaluated cultivars, BRS 360RR presented the highest N, P, Ca, Fe, Mn, and Zn contents in the grains (Table 4), indicating a greater uptake efficiency, in agreement with Fageria et al. (2016), who observed that the uptake capacity of each cultivar and/or species affected the internal nutrient content of the plant. Probable divergences between the results of the present study and of other researches, such as that of Moreira & Moraes (2016), can be explained

Table 2. Grain yield (GY), shoot dry weight yield (SDWY), GY/SDWY ratio, number of pods per pot, and grains per pod of five soybean (*Glycine max*) cultivars in response to copper fertilization rates.

Cu rates	GY	SDWY	GY/ SDWY	Pods per pot	Grains per pod
(mg kg^{-1})	-------(g per pot)-------			---------(No.)----------	
TMG 1066RR					
0	19.4	46.1	0.42	64	3
2	24.0	53.5	0.45	70	3
4	24.1	49.9	0.48	61	3
8	21.2	48.3	0.44	61	2
Average	22.2	49.4	0.45	64	3
BRS 360RR					
0	20.6	43.4	0.47	49	3
2	21.8	51.5	0.42	65	3
4	23.7	48.1	0.49	61	2
8	23.3	47.9	0.49	62	3
Average	22.4	47.7	0.47	59	3
NA 6262RR					
0	16.0	29.2	0.55	37	2
2	17.2	30.6	0.56	41	3
4	18.3	34.9	0.52	48	2
8	18.7	30.1	0.62	37	2
Average	17.6	31.2	0.56	41	2
BMX Turbo RR					
0	15.0	33.5	0.45	40	2
2	20.7	30.8	0.67	42	2
4	21.0	40.0	0.52	50	2
8	16.7	35.7	0.47	37	3
Average	18.4	35.0	0.53	42	2
BRS 359RR					
0	21.2	38.9	0.54	53	3
2	21.6	42.0	0.51	56	3
4	25.2	41.5	0.61	59	2
8	18.7	36.6	0.51	51	3
Average	21.7	39.7	0.54	55	3
Average					
0	18.4	38.2	0.48	52	3
2	21.1	41.7	0.51	54	3
4	22.5	42.9	0.52	54	2
8	19.7	40.6	0.48	50	3
Average	20.4	40.9	0.50	53	3
F-test					
Cultivar (a)	*	*	*	*	ns
Rate (b)	*	*	*	*	ns
a × b	*	*	*	*	ns
CV (%)[1]	12.52	13.84	13.51	16.43	8.17

[1]CV, coefficient of variation. *Significant by the F-test, at 5% probability. [ns]Nonsignificant.

by the amount of nutrients applied to the soil and by the differences among the assessed cultivars.

The analysis of variance also showed a positive effect of Cu rates on plant physiological attributes (Table 5). The highest average values for A, g_s, Trmmol, and chlorophyll content were found for cultivar BRS 359RR, while those for C_i and water use efficiency were obtained for TMG 100RR. In the average

Table 3. Nitrogen, phosphous, potassium, calcium, magnesium, copper, iron, manganese, and zinc contents in the leaves of five soybean (*Glycine max*) cultivars in response to copper fertilization rates.

Cu rates (mg kg⁻¹)	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
	------------(g kg⁻¹)--------------					--------(mg kg⁻¹)-----------			
TMG 1066RR									
0	35.6	3.1	19.9	2.6	3.7	3.2	112.8	66.8	13.5
2	34.2	3.0	18.4	2.2	4.0	3.9	97.9	57.1	15.8
4	31.9	2.9	17.0	1.8	3.0	6.8	81.1	40.1	17.0
8	30.3	2.8	18.4	2.5	3.4	11.7	94.2	59.1	15.2
Average	33.0	2.9	18.4	2.3	3.5	6.4	96.5	55.8	15.4
BRS 360RR									
0	32.1	9.0	13.8	2.7	3.2	3.3	119.8	92.5	23.0
2	42.5	9.9	15.8	3.4	2.8	4.0	110.5	106.9	22.2
4	31.3	9.2	13.7	2.7	3.6	4.6	88.2	66.7	19.9
8	32.8	8.6	13.3	2.5	3.7	10.6	92.0	61.5	13.3
Average	34.7	9.2	14.0	2.8	3.3	5.6	102.6	81.9	19.6
NA 6262RR									
0	34.4	4.7	19.7	3.1	3.1	5.9	74.1	115.5	20.5
2	27.3	3.1	16.9	2.4	3.1	7.3	29.2	45.6	13.5
4	22.2	3.4	17.9	2.7	3.8	7.6	39.3	64.7	16.5
8	27.4	3.8	19.2	2.3	3.4	9.5	116.8	81.3	15.4
Average	27.8	3.7	18.4	2.4	3.3	7.6	64.9	76.8	16.5
BMX Turbo RR									
0	30.5	4.2	18.6	2.2	4.1	4.0	59.7	68.3	12.3
2	29.1	3.9	17.4	2.1	3.6	4.2	102.2	101.4	22.2
4	29.6	5.0	21.4	2.0	3.7	6.8	98.8	80.7	19.8
8	31.2	4.1	21.7	2.0	3.7	10.3	122.5	77.8	16.0
Average	30.1	4.3	19.8	2.0	3.8	6.3	95.8	82.1	17.6
BRS 359RR									
0	28.1	4.8	21.0	2.0	3.7	3.6	104.4	90.7	16.3
2	31.9	5.1	21.8	1.7	3.6	5.6	95.7	63.3	17.4
4	31.0	5.6	21.5	1.8	4.8	6.7	89.8	82.6	17.0
8	30.6	5.5	23.0	1.9	3.9	10.8	83.9	78.6	15.6
Average	30.4	5.2	21.8	1.9	3.8	6.7	93.4	78.8	16.6
Average									
0	32.1	5.1	18.6	2.5	3.6	4.0	94.2	86.8	17.1
2	33.0	5.0	17.9	2.3	3.4	5.0	87.1	74.9	18.2
4	29.2	5.2	18.3	2.2	3.6	6.5	79.4	67.0	18.1
8	30.4	4.9	19.2	2.3	3.6	10.6	101.9	71.6	15.1
Average	31.2	5.1	18.5	2.3	3.6	6.5	90.7	75.1	17.1
F-test									
Cultivar (a)	*	*	*	*	ns	*	*	*	*
Rate (b)	ns	ns	ns	ns	ns	*	ns	ns	ns
a × b	ns	ns	ns	ns	ns	*	ns	ns	ns
CV (%)[1]	12.27	15.41	13.91	9.92	11.63	9.78	18.97	14.96	16.58

[1]CV, coefficient of variation.*Significant by the F-test, at 5% probability. [ns]Nonsignificant.

Table 4. Nitrogen, phosphorus, potassium, calcium, magnesium, copper, iron, manganese, and zinc contents in the grains of five soybean (*Glycine max*) cultivars in response to copper fertilization rates.

Cu rates (mg kg⁻¹)	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
	--------------(g kg⁻¹)--------------					---------(mg kg⁻¹)-----------			
TMG 1066RR									
0	35.6	5.3	15.5	2.0	14.2	6.7	99.9	67.7	23.0
2	40.8	5.4	15.2	2.3	19.1	7.3	91.7	77.4	22.2
4	23.5	7.0	17.1	2.5	17.3	9.1	94.8	80.1	29.6
8	30.3	7.2	16.7	2.5	17.7	8.9	105.0	64.4	23.7
Average	32.5	6.2	16.1	2.3	17.1	8.0	97.8	72.4	24.6
BRS 360RR									
0	41.2	6.8	17.0	2.3	17.2	8.3	172.6	66.3	27.0
2	36.0	9.7	16.8	2.7	14.0	12.2	185.7	83.4	37.1
4	31.3	5.5	18.1	2.3	15.3	13.7	111.9	49.6	26.6
8	36.5	8.1	16.8	2.9	13.8	10.6	167.2	55.7	19.8
Average	36.3	7.5	17.2	2.5	15.1	11.2	159.4	63.7	27.6
NA 6262RR									
0	34.4	5.8	17.1	2.3	12.0	8.1	87.1	45.2	20.5
2	27.3	5.2	16.6	2.7	16.0	11.2	93.6	62.3	20.5
4	25.5	6.2	17.0	2.4	13.0	13.1	87.3	60.0	16.9
8	27.6	8.0	17.7	2.3	15.1	15.7	96.9	69.3	26.5
Average	28.7	6.3	17.1	2.4	14.0	12.0	91.2	59.2	21.1
BMX Turbo RR									
0	29.6	6.4	16.9	2.0	15.9	9.6	87.7	45.3	25.6
2	29.1	7.4	17.8	1.6	12.4	10.1	173.1	61.6	12.1
4	28.4	6.5	22.6	1.7	12.2	10.3	92.9	57.0	19.8
8	31.2	6.4	18.6	1.8	12.7	14.6	98.3	46.2	17.8
Average	29.6	6.7	19.0	1.8	13.3	11.2	113.0	52.5	18.8
BRS 359RR									
0	27.9	5.3	20.5	1.7	12.2	3.6	97.3	50.7	21.1
2	26.2	3.9	25.6	1.6	11.4	10.5	78.5	52.6	21.0
4	26.7	4.5	26.3	1.8	11.7	10.9	78.1	71.4	20.7
8	26.7	4.0	27.4	1.9	12.8	13.6	92.0	71.2	19.9
Average	26.9	4.4	25.0	1.8	12.0	9.7	86.5	61.5	20.7
Average									
0	33.7	5.9	17.4	2.1	14.3	7.3	108.9	55.0	23.4
2	31.9	6.3	18.4	2.2	14.6	10.3	124.5	67.5	22.6
4	27.1	5.9	20.2	2.2	13.9	11.4	93.0	63.6	22.7
8	30.4	6.7	19.5	2.3	14.5	12.7	111.9	61.3	21.6
Average	30.8	6.2	18.9	2.2	14.3	10.4	109.6	61.9	22.6
F-test									
Cultivar (a)	ns	ns	ns	*	ns	*	*	ns	ns
Rate (b)	ns	ns	ns	ns	ns	*	ns	ns	ns
a × b	ns	ns	ns	ns	ns	*	ns	ns	ns
CV (%)[1]	7.99	9.65	8.87	9.45	8.23	7.84	11.42	10.63	12.57

[1]CV, coefficient of variation.*Significant by the F-test, at 5% probability. [ns]Nonsignificant.

of cultivars, A presented a significant correlation with GY ($\hat{y} = 6027.30 + 692.17x - 17.93x^2$, r = 0.85, p≤0.05). Among other functions, Cu participates in the electron transportation chain of photosystem I and in cytochrome oxidase in respiration (Malavolta et al., 1997; Fageria, 2009; Moreira et al., 2019). In the plant, approximately 50% of all Cu present is found in the chloroplast connected to that protein, whose proportion in relation to the number of chlorophyll molecules is 3 to 4 for 1,000 of the pigment; with

Table 5. Photosynthetic rate (A), stomatal conductance (g_s), intercellular CO_2 (C_i) concentration, transpiration rate (Trmmol), water use efficiency (WUE), and chlorophyll content of five soybean (*Glycine max*) cultivars in response to copper fertilization rates.

Cu rates (mg kg^{-1})	A (µmol CO_2 m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (µmol mol^{-1})	Trmmol (mmol m^{-2} s^{-1})	WUE (µmol m^{-2} s^{-1})	Chlorophyll (mg m^{-2})
			TMG 1066RR			
0	15.16	0.28	260.58	3.37	4.52	201.55
2	16.53	0.32	261.74	3.30	5.06	219.93
4	18.01	0.41	276.01	3.31	5.46	237.61
8	18.62	0.39	258.81	4.32	4.37	206.48
Average	17.08	0.35	264.28	3.58	4.85	216.39
			BRS 360RR			
0	16.89	0.31	251.92	4.02	4.22	180.08
2	20.71	0.47	262.52	5.46	3.81	210.84
4	17.25	0.38	269.09	5.12	3.35	247.96
8	15.90	0.30	254.87	4.56	3.48	233.13
Average	17.69	0.36	259.60	4.79	3.72	218.00
			NA 6262RR			
0	17.46	0.66	293.09	6.17	2.83	195.43
2	18.41	0.38	252.84	5.30	3.51	198.46
4	16.63	0.30	249.73	4.89	3.39	233.82
8	14.51	0.29	254.55	4.74	3.11	221.13
Average	16.75	0.41	262.55	5.28	3.21	212.21
			BMX Turbo RR			
0	12.69	0.16	197.90	3.22	4.14	218.93
2	15.19	0.19	207.16	3.99	3.90	258.71
4	19.20	0.39	259.82	6.36	3.03	235.27
8	19.24	0.40	255.69	6.13	3.17	222.84
Average	16.58	0.28	230.14	4.92	3.56	233.94
			BRS 359RR			
0	18.23	0.38	262.09	6.09	2.99	205.27
2	18.68	0.39	268.56	6.24	2.99	262.73
4	19.90	0.41	251.16	6.59	3.02	237.19
8	17.17	0.33	250.27	5.64	3.05	237.36
Average	18.50	0.38	258.02	6.14	3.01	235.64
			Average			
0	16.99	0.39	254.01	4.66	3.93	225.46
2	17.90	0.35	250.56	4.86	3.86	218.50
4	17.29	0.35	260.26	5.17	3.46	224.79
8	17.09	0.34	254.84	5.08	3.44	224.19
Average	17.32	0.36	254.92	4.94	3.67	223.24
			F-test			
Cultivar (a)	*	*	*	*	*	*
Rate (b)	*	*	*	*	*	*
a × b	*	*	*	*	*	*
CV (%)[1]	11.61	12.98	10.44	9.58	12.93	14.22

[1]CV, coefficient of variation. *Significant by the F-test, at 5% probability.

this, during Cu deficiency, there is a reduction in the concentration of plastocyanin, as well as in the activity of photosystem I (Fageria, 2009; Hänsch & Mendel, 2009; Marschner, 2012). The effects of Cu on plant physiological components were also described by Migocka & Malas (2018), evidencing the importance of Cu in the plant's nutritional state.

Conclusions

1. In a typical Ultisol with a high organic matter content, the grain yield, shoot dry weight yield, grain yield/shoot dry weight yield ratio, and number of pods per pot of soybean (*Glycine max*) cultivars are positively affected by copper fertilization, but Cu use efficiency varies significantly among the different soybean genotypes evaluated (TMG 1066RR, BRS 360RR, NA 6262RR, BMX Turbo RR, and BRS 359RR).

2. Photosynthetic rate, internal CO_2 concentration, stomatal conductance, transpiration rate, and chlorophyll content present a positive correlation with the available Cu content in the soil.

3. The cultivar × Cu rate interactions are not significant for the studied soil chemical attributes.

4. The Mehlich-1 and diethylenetriaminepentaacetic acid-triethanolamine (DTPA-TEA) extractants are efficient in determining available Cu in the soil; however, DTPA-TEA extracts 2.6 times more available Cu than Mehlich 1.

Acknowledgments

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