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Forms of nitrogen fertilization on soybean in the northern of Mato Grosso State, Brazil

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Abstract. This study aimed to verify the effects of soil and foliar application of N in different growth stages of two soybean cultivars in the Northern of Mato Grosso State, Brazil. The experimental design was randomized complete block design with four replications in factorial scheme 2 x 7. The soybean cultivars were: TMG 132 and TMG 1179. The treatments were: control (without N fertilizer), 20 kg of N applied in the furrow at sowing, 20 kg of N applied on the soil surface at sowing time, 20 kg of side dress N applied in V2 on the soil, 20 kg of side dress N applied in V4 on the soil; 20 kg of N applied via foliar in V2 and lastly 20 kg of N applied via foliar in V4. The N in the soil, even at sowing, reduces nodulation of soybean plants, generating small gains in productivity. The application of 20 kg N ha^{-1} starting in V2 stage increases vegetative growth, nodulation and soybean yields. Foliar application of N at a dose of 20 kg ha^{-1} in V2 or V4 stages is the most efficient way to increase soybean productivity.
Keywords: *Glycine Max*. Inoculation. Nodulation. Leaf spraying.

Introduction

Soybean is one of the most consumed oleaginous grains in the world due to its versatility of use in food for humans, animals and industries (GESTEIRA et al., 2015; PARENTE et al., 2015). Brazil produced 98 million tons of soybean grains in the crop season of 2015/2016, being the world second biggest grain producer; the middle-west region is responsible for 50% of this production (CONAB, 2016).Soybean culture has a great demand on Nitrogen (N), the main element absorbed in the soybean culture (FERREIRA et al., 2016; SILVA et al., 2011). N is involved in vegetal metabolism, photosynthesis, respiration, growth and development, in production of flowers, leaves and grains (DAVENPORT et al., 2015; Taiz & ZIEIGLER, 2013). Soybeans have high protein content, hence very high N requirements (DOZET et al., 2016). This element is found in organic matter, in soil solution and in the air; however its natural quantity is not enough for the producer to obtain an acceptable productivity. 240kg N ha^{-1} is the quantity estimated for soybean to achieve an average productivity of 3000 Kg ha^{-1} (HUNGRIA et al., 2006).

In Brazil, N fertilizers are commonly used in cultures, but it is an uncommon practice in the soybean culture. Due to the improvement in biological N fixation (BNF), through a symbiotic association of plant roots with atmospheric N fixers, bacteria of the genus *Bradyrhizobium* (*Bradyrhizobium japonicum and B. elkanii*), and the soybean varieties in Brazil obtain the N for its development and production from that association. Through the method of inoculation, bacteria of the genus *Bradyrhizobium* are applied in a peat substrate and posteriorly are inoculated in soybean seeds at sowing. After the inoculation in the seeds, these bacteria infect the roots, through root hairs, and create nodules. The nodules are responsible for a mutualistic relation between the bacteria and the plant. This process transforms the atmospheric N_2 in an available form of N during the development of the soybean (BRANDELERO et al., 2009; SILVA et al., 2011).

Addition of N fertilizers in leguminous plants has an adverse effect on biological fixation due to a decrease in oxygen availability in nodule respiration; and on limitation of carbohydrates on the nodule metabolism, once the plant deflects the nodule carbohydrates to absorb the N directly (PARENTE et al., 2015). This process increases the plant productive efficiency; however this process also increases the costs to the producers (MENDES et al., 2008).

Since inoculation is an expensive process to the plant, some studies show an increase in productivity when N is added through fertilization (FAGAN et al., 2007). Moreover, some producers apply certain quantity of N at sowing to prevent the deficiency of this macronutrient, especially in the initial stages; part of the formulated fertilizer containing NPK used in soybean culture is commercialized in a formula containing a small quantity of N (SILVA et al., 2011). However, there are still controversies in respect to this subject, therefore, it is necessary to comprehend better the effect of N fertilization in the physiology of the plant, in order to obtain the balance between the addition of N and the biological fixation (FAGAN et al., 2007).

In this context, this study aims to verify the effect of foliar and soil application of N on the vegetative development, chlorophyll level, nodule formation and productivity of two soybean cultivars (TMG 132 and TMG 1179) from the northern region of Mato Grosso State, at different development stages of the soybean culture.

Methods

This study was conducted between October 2014 and February 2015, in the experimental area of the Federal University of Mato Grosso – *Campus* Sinop, localized in the state of Mato Grosso (MT); at 11º85'35'' south latitude, 55º 51'15'' west longitude and 380m altitude. In this region, the climate is classified by the Koppen-Geiger system as Aw type, which consists in two very defined seasons. The wet season occurs between October and April, while the dry season occurs between May and September (ROLIM et al., 2007). The annual average temperature varies between 24º and 27ºC.

The experimental design was in randomized complete blocks design (RBD) with four replicates in factorial scheme 2x7. The first factor was composed by two soybean varieties, TMG 132 and TMG 1179; the second factor was composed by different forms and times of N application; the control group did not receive the N application (only an inoculation of *Bradhirrhizobium japonicum*). The treatments were: 20 kg of N applied in the furrow at sowing, 20 kg of N applied on the soil surface at sowing time, 20 kg of side dress N applied in V2 on the soil, 20 kg of side dress N applied in V4 on the soil; 20 kg of N applied via foliar in V2 and lastly 20 kg of N applied via foliar in V4. For the treatments, urea was used as a source of N. The plots were composed by four lines of 5m of length and 0.45m of row spacing. Two central lines, excluding the 0.5m of the borders, composed the useful plot. Before sowing, the seeds were inoculated with *Bradyrhizobium japonicum*, using a peat inoculant and were treated with the micronutrients Co and Mo, at a dose of 3 g ha^{-1} and 30 g ha^{-1}, respectively.

The soil of the experimental area is known as Dystrophic Yellow Red Latosol, which has a clayey texture (Santos et al., 2013). The results of soil analysis (0-0.2 m depth) were: pH in H_2O (5.7); P (9.6 mg dm^{-3}); K (42.0 mg dm^{-3}); Ca (24.0 mmol$_c$ dm^{-3}); Mg (8.0 mmol$_c$ dm^{-3}); V (42.5 %); organic matter (20.0 g kg^{-1}); S-SO^{-4} (10.0 mg dm^{-3}); Zn^{2+} (16.7 mg dm^{-3}); Mn^{2+} (7.1 mg dm^{-3}); Cu^{2+} (3.7 mg dm^{-3}) and B (0.3 mg dm^{-3}). The culture treatments (control of weeds, blight and diseases), previous and posterior to the sowing, were conducted following the recommendations for soybean culture. The control of weeds occurred within the recommended period, that is, up to 28-34 days after emergence, without affect the yield potential (SILVA et al., 2015). The areas were initially dissected with an application of 960g ha^{-1} of glyphosate. After emergency, an application of 960g ha^{-1} of glyphosate, 80 g ha^{-1} of Haloxyfop–p–methyl and 600ml ha^{-1} of mineral oil was performed using a manual costal spray (100 L ha^{-1}) at 7 and 27 days after emergence.

The chlorophyll index was evaluated in the stage of entire inflorescence using a chlorophylometer of the brand clorofiLOG (Model CFL-1030). For this variable five random plants were evaluated in each plot, from three intact leaves in the median region of the plants. This stage of entire inflorescence was also used to obtain plant height, number of leaves, foliar area, stem diameter and aerial part dry matter of the plant. Plant height was obtained with a measuring tape, through the average height of five plants; the measurement started from the ground and ended at the apical meristem. Stem diameter was obtained from five plants, at 5 cm of height from the ground level, with the use of a digital caliper rule. The number of nodules and leaves were obtained through the counting of the present structures. After the measurements in the field, the plants were cut at ground level, stored in paper bags and dried in a greenhouse forced air circulation (at 60ºC until constant weight) to obtain the aerial part dry matter of the plants (PEREIRA et al., 2014).

The evaluations of nodules formation (number of nodules and dry matter) were performed in the entire inflorescence stage, R1. To this, five plants localized in the first line, at the right side of the useful plot, were selected. Through a small hole of approximately 0.20 x 0.20 x 0.20m, without damaging the plant radicular system, these five plants were entirely removed from the ground with its complete radicular system and the aerial part. After harvesting, the roots were separated from the plant aerial part with scissors. After washing, the soil adhered to the roots were removed and the roots with the nodules and the plant aerial part were separated in paper bags. Subsequently, the samples were dried in a greenhouse forced air circulation (at 60º C, until constant weight) to obtain the dry matter aerial part of the plant, dry matter of the roots and posteriorly, after a new manual separation, to obtain the number and the dry matter of the nodules.

The soybean grain harvesting, separation and threshing were performed manually, when the grains were approximately with 18% of humidity. In order to perform the production evaluations, after this stage, the soybean had its humidity adjusted to 13%. The initial grain water amount was determined by a direct method, in a greenhouse forced air circulation, at 105°C, for 24 hours (BRASIL, 2009).

The mass of 1000 grains, number of pods and productivity were obtained after harvesting. The mass of 1000 grains was obtained through a summation of the average weight of ten samples of 100 grains of each plot. The number of pods was obtained summing the number of pods of all plants in the useful plot. The productivity was quantified through the grain production in the plot useful area and transformed to kg ha^{-1}.

The data collected was submitted to an analysis of variance at a level of 5% in probability using the software SISVAR (FERREIRA, 2011). If there was any significance through the F-test from the analyses of variance the averages were compared using Turkey test at 5%.

Results and discussions

The variables chlorophyll index and stem diameter were significantly altered with the N treatments, however no difference was observed between the soybean cultivars (Table 1). The chlorophylls levels were higher with the foliar applications of N in V2 and V4 stages, differing from the chlorophylls levels from the control group. The results of all other treatments did not differ from the results of the control group. In V4, plant leaves chlorophyll levels under foliar applications of N were 12.34 units superior to the level found in plants under soil application of N at sowing. This result compared to the control group showed a difference of 23.84 units. For the variable stem diameter, all treatments (with addition of N) showed higher values in comparison with the control group (without N), except the application of N at sowing.

Table 1. Vegetative characteristics: Chlorophyll index (CLO) and stem diameter SD (mm) of the soybean cultivars TMG 132 and TMG 1179 under different forms and times of N application. Crop of 2014/2015, Sinop – MT.

Treatments:	Variables	
	CLO	SD (mm)
Control	64.50 a	5.01 a
N in the furrow at sowing	67.12 a	5.23 a
N on the soil at sowing	67.31 a	6.25 b
Side dress N in V2	72.92 a	6.54 b
Side dress N in V4	76.00 a	6.65 b
N in V2 via foliar	86.06 b	6.68 b
N in V4 via foliar	88.34 b	6.91 b
CV (%)	7.07	13.59

Averages followed by equal letters in the columns do not differ from each other by the Tukey test at 5% of probability.

The relation between N fertilization and chlorophyll content are demonstrated in the results. Davenport et al. (2015) highlights the relation between N metabolism and the photosynthetic enzymes and metabolites. This association is important to optimize the culture photosynthetic activity and consequently the production of photo assimilates, the compounds that will increase productivity.

Results of the treatments that received soil fertilization did not differ statistically from the results of the control group, in the variable chlorophyll index. This can be directly correlated to the fact that great part of the N absorbed in this phase is used for the production of other structures in the plant, and not only for the synthesis of chlorophyll. However, Franchini et al. (2015) stressed that N applied at sowing can reduce the levels of chlorophyll in the leaves due to a decrease in nodulation, and hence, in N fixation.

In a study conducted by Salgado et al. (2012), who evaluated the N in beans under stress, the chlorophyll index (a, b, and total) did not show interaction with the factors genotype and environment. However, this study showed a different result, in which the forms of N application showed direct interference in the chlorophyll index.

Soybean is a leguminous species with the capacity of biological N fixation (BNF), and so, inoculation is important and adopted in soybean production (DOZET et al., 2015, PARENTE et al., 2015). When inoculated that bacteria will present in the soil and will recognize the roots of the host plant. This event results in formation of nodules and fixation of atmospheric N_2 (HUNGRIA et al., 2006; ZILLI et al., 2010).

For the variables plant height, number of leaves, foliar area, number of nodules and dry matter of nodules, a significant interaction among the treatments and the cultivars TMG 132 and TMG 1179 was observed (Tables 2 and 3). The height of the cultivar TMG 132 was higher in the plants under application of N, independently of the stage they were found. A similar result was reported by Franchini et al. (2015) concerning plant height in soybean integrated with livestock production systems. In relation to the number of leaves, all plants that received N differed from the control group. The application on the V2 stage showed a significant difference presenting approximately the double value, when compared to the value found in the control group. With regard to the foliar area, the plants under foliar application in V4 showed the best developments.

The application of N did not interfered in the number of radicular nodules in the cultivar TMG 132. The dry mass of the nodules was significant in plants in V2, as much under foliar application as under soil application. The treatment that resulted in the greatest mass of nodules was the treatment under foliar application in V4. This treatment achieved a mass of 3.52g plant^{-1}, 414% superior to the mass of 0.85g plant^{-1} of the control group (Table 2).

The application of N altered the number of radicular nodules in the cultivar TMG 1179. The most efficient time of N application was in V4 via foliar, which resulted in 97.25 nodules. The dry mass of the nodules was consequently more efficient in the treatment under foliar application of N in V4, achieving a dry mass of 8.0g plant^{-1} (Table 3). Corroborating the present results with Parente et al. (2015) also found a relation between time of N application on soybean and number of nodules and dry mass of nodules. These authors stated that N applications in R1 stage were more efficient in that variables than at sowing due to a beneficial effect in nodulation and biological fixation process.

Table 2. Vegetative characteristics: plant height (PH); number of leaves (NL); foliar area (FA); number of nodules (No) and dry mass of nodules (MSNo) of the cultivar TMG 132 under different forms and times of N application. Crop of 2014/2015, Sinop – MT.

Treatments:	Variety TMG 132				
	PH (m)	NL	FA (m²)	No	MSNo (g plant^{-1})
Control	0.36 a	13.5 a	0.24 a	7.50 a	0.85 a
N in the furrow at sowing	0.40 a	18.00 b	0.38 b	5.75 a	0.98 a
N on the soil at sowing	0.50 b	19.75 c	0.38 b	7.50 a	1.35 a
Side dress N in V2	0.54 b	26.00 d	0.43 b	9.50 a	3.52 b
Side dress N in V4	0.57 b	22.25 c	0.51 c	8.25 a	2.27 b
N in V2 via foliar	0.57 b	20.25 c	0.53 c	9.25 a	2.43 b
N in V4 via foliar	0.59 b	22.25 c	0.57 c	10.25 a	3.52 b
CV (%)	10.28	12.50	10.14	35.49	32.41

Averages followed by equal letters in the columns do not differ from each other by the Tukey test at 5% of probability.

Table 3. Vegetative characteristics: plant height (PH); number of leaves (NL); foliar area (FA); number of nodules (No) and dry mass of nodules (DMNo) of the cultivar TMG 1179 under different forms and time of N application. Crop of 2014/2015, Sinop – MT.

Treatments:	Variety TMG 1179				
	PH (m)	NL	FA (m²)	No	DMNo (g plant^{-1})
Control	0.38 a	16.75 a	0.25 a	29.25 a	1.71a
N in the furrow at sowing	0.50 b	14.50 a	0.31 b	26.25 a	2.40 a
N on the soil at sowing	0.49 b	21.5 b	0.33 b	40.00 a	3.51 b
Side dress N in V2	0.51 b	21.5 b	0.37 c	29.75 b	3.42 b
Side dress N in V4	0.44 a	15.75 a	0.42 c	32.00 a	4.00 b
N in V2 via foliar	0.50 b	18.75 a	0.44 c	44.75 a	4.22 b
N in V4 via foliar	0.50 b	25.50 c	0.43 c	97.25 c	8.00 c
CV (%)	10.28	12.50	10.14	35.49	32.41

Averages followed by equal letters in the columns do not differ from each other by the Tukey test at 5% of probability.

TMG 1179 presented a quite higher quantity of nodules than TMG 132. This difference shows how the varieties behave in a dissonant form in that parameter. The formation of smaller nodules in N fertilized plants occurs due to a reduction in the efficiency of the bacteria *Bradyrhizobium* in the presence of the mineral N (VIEIRA NETO et al., 2008; NOGUEIRA et al., 2010).

N fertilization was positive in all parameters studied in the soybean culture. The cultivar 1179 responded more positively in the nodulation and the TMG 132 presented better results in vegetative terms. Brito et al., (2015) performed a similar study of N fertilization in common bean culture and they found different results concerning different cultivars. Dozet et al. (2016) still reported results indicating an effect of N fertilization on the quality properties, as oil and protein content, of soybean.

Plant productivity was altered by the application of N, independently of the phonological moment or stage. The application of N increased soybean productivity; however this increase did not differ between the cultivars TMG 132 and TMG 1179, although Parente et al. (2015) have already found interaction for that variable between soybean cultivars and time of N application. For the variable number of pods, the foliar application of N in V2 and in V4 was the most efficient, resulting 66 and 66.62 pods, respectively (Table 4). The other treatments were distributed in two distinct groups, being the worst result obtained in the treatments with N at sowing. These results did not differ statistically from the results of the control group. Corroborating with that, Zilli et al. (2011) did not find significance difference between mineral N fertilization at soil and only the inoculation on soybean yield.

Table 4. Productive characteristics: number of pods (Nº of pods), grains per pod (Grain pod^{-1}), Mass of 1000 grains (Mass 1000) and productivity (Prod.) of the cultivars TMG 132 and TMG 1179, under different forms and time of N application. Crop of 2014/2015. Sinop – MT.

Treatments	Variables			
	Nº of pods	Grain pod^{-1}	Mass 1000	Prod. (kg.ha^{-1})
Control	45.00 a	3.25 a	117.87 a	1723.90 a
N in the furrow at sowing	49.12 ab	3.25 a	119.37 a	1898.31 ab
N on the soil at sowing	50.00 abc	3.50 ab	125.25 ab	2190.64 abc
Side dress N in V2	52.50 bc	3.75 ab	131.62 b	2586.59 bc
Side dress N in V4	56.12 c	3.87 ab	143.25 c	3120.90 cd
N V2 via foliar	66.00 d	4.12 ab	146.00 c	4019.90 de
N V4 via foliar	66.62 d	4.37b	148.87 c	4337.07 e
CV (%)	8.17	15.67	4.68	11.72

Averages followed by equal letters in the columns do not differ from each other by the Tukey test at 5% of probability.

In a study conducted by Petter et al. (2012), 20 and 40 kg N ha^{-1} applied in late planted soybean increased the number of pods per plant, while 80kg and 160kg N ha^{-1} decreased the number of pods. N has potentialities to increase the capacity of the plant in producing reproductive buds. However, these results will be obtained in function of the characteristics of the production compounds of each soybean cultivar.

For the variable grains per pod, the results were obtained from two separated groups. The group with the highest value was obtained from the treatment that received foliar N application in V4. The only that differ statistically from the control treatment.

The variable mass of 1000 grains presented a significant difference, assorting the averages in three distinct groups. One of the groups did not differ statistically from the control group; this group includes the treatments that received the N at the sowing time. Differently of the results presented here, Ferreira et al. (2016) and Franchini et al. (2015) did not observe any effect of N fertilization on soybean in mass of 1000 grains, yield and number of pods.

For the variable productivity, an increase in productivity yield was achieved in the treatment under foliar fertilization in V4, achieving 4337.07 kg ha^{-1}. The treatment under foliar fertilization in V2 also resulted in a high yield of 4019.90 kg ha^{-1}. All other treatments were efficient, differing from the control group; only the treatments with N at sowing time did not differ from the control group. Brito et al. (2015) also confirmed a superiority of treatments associating inoculation and mineral N fertilization in common bean in different Brazilian biomes. These author still verified that the results of inoculation is high variable depending of the historic of the area, because the native soil bacteria can compete with the inoculated ones.

Mendes et al (2008) also verified an increase in soybean productivity with N fertilization, but this study points a decrease in soybean productivity under high levels of N application , as in the study performed by Hungria et al. (2006), who verified a reduction after the application of 50 kg N ha^{-1}. On the other hand, Werner et al. (2016) did not find response in soybean yield with doses of 45 kg N ha^{-1} via soil in V2 stage, considering two seasons of growing, as well as Silva et al. (2011) with other N doses.

In a study conducted by Petter et al. (2012), soil application of N at a dose of 80 kg ha^{-1} and 160 kg ha^{-1} reduced significantly the yield of the culture. This fact can be directly correlated to a possible reduction in the formation of nodules and consequently in the efficiency of BNF. However, in this study the N applications were efficient in gain of productivity, besides that, this study was performed in new areas of growing.

Conclusions

Application of N in the furrow at sowing reduces soybean plant nodulation, resulting in little increase in productivity.

Application of N at a dose of 20kg ha^{-1}, starting in V2, increases vegetative growth, nodule formation and soybean productivity.

Foliar applications of N at a dose of 20kg ha^{-1} in V2 or in V4 are the most efficient ways to increase soybean productivity.

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