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STARTER NITROGEN FERTILIZATION IN DIFFERENT SOYBEAN CULTIVARS

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Abstract

Although several studies have demonstrated that biological nitrogen fixation can provide the nitrogen required by soybean, doubts regarding the need for nutrient application at sowing, specifically "starter N," in early soybean cultivars persist, particularly in high-yielding crop fields. Thus, this study aimed to evaluate the effect of starter nitrogen fertilization on soybean cultivars with different maturity cycles. The experiment was conducted at Fazenda Recanto, in Carmo do Rio Claro, Minas Gerais state, during two crop seasons (2016/2017 and 2017/2018), using a randomized complete block design with split-plot arrangement and four replications. In the 2016/17 cropping season, the plots were comprised of three soybean cultivars (M6410 IPRO, NS7300 IPRO, and TEC7849 IPRO). In the 2017/18 cropping season, the plots consisted of four cultivars (M5917 IPRO, M6410 IPRO, AS3680 IPRO, and NS7670 RR). The subplots were assigned four nitrogen doses at sowing (0, 10, 20, 40 kg ha^{-1}). Grain yield, number of nodules per plant, mass of dry nodules per plant, number of grains and pods per plant, plant height and insertion of the first legume were evaluated. Experimental data were subjected to analysis of variance, and means were compared using the Scott-Knott test at 5% probability. The nitrogen rates at sowing did not influence soybean yields and agronomic characters of cultivars evaluated in the cropping 2016/17 and 2017/18. Increasing doses of nitrogen inhibited soybean nodulation.

Keywords: biological fixation; *Glycine max*; nodulation; grain yield.

ADUBAÇÃO NITROGENADA DE ARRANQUE EM DIFERENTES CULTIVARES DE SOJA

Resumo

Apesar de vários estudos já terem demostrado que a fixação biológica de nitrogênio é capaz de fornecer o nitrogênio (N) requerido pela soja, dúvidas sobre a necessidade de aplicação no nutriente na semeadura “N de arranque”, em cultivares precoces de soja ainda são recorrentes, principalmente em lavouras com alto potencial de produtividade. Dessa forma, objetivou-se com esse estudo avaliar o efeito da adubação nitrogenada de arranque em cultivares de soja com diferentes ciclos. O experimento foi realizado na Fazenda Recanto, em Carmo do Rio Claro, Estado de Minas Gerais, em duas safras (2016/2017 e 2017/2018), em delineamento em blocos casualizados, em esquema de parcelas subdivididas, com quatro repetições. As parcelas foram compostas na safra 2016/17 por três cultivares de soja (M6410 IPRO, NS7300 IPRO e TEC7849 IPRO), e na safra 2017/18 por quatro cultivares (M5917IPRO, M6410IPRO, AS3680IPRO e NS7670RR). A subparcelas foram constituídas por quatro doses de N na semeadura (0, 10, 20, 40 kg ha^{-1}). Foi avaliada a produtividade de grãos, número de nódulos por planta, massa de nódulos secos por planta, número de grãos e vagens por planta, altura de plantas e de inserção do primeiro legume. Os dados experimentais foram submetidos à análise de variância, e as médias comparadas através do teste de Scott-Knott a 5% de probabilidade. As doses de N em semeadura não influenciaram a produtividade e os caracteres agronômicos das cultivares avaliadas nas safras 2016/17 e 2017/18. Doses crescentes de nitrogênio inibiram a nodulação da soja.

Palavras-chave: fixação biológica; *Glycine max*; nodulação; produtividade.

Introduction

Since the beginning of soybean cultivation expansion in the 1970s, particularly in Brazilian Cerrado region, farmers have raised concerns regarding the sufficiency of solely relying on biological nitrogen fixation (BNF) to meet the soybean's nitrogen requirements and achieve high yields (FAGAN *et al.*, 2007). The application of mineral N in soybean crops increases the production cost and is not supported by research, although several producers use it because ammonium monophosphate (MAP) is the main fertilizer used in the crop sowing.

Phosphate fertilization with MAP at sowing is used due to the lower cost per kilogram of phosphorus (P) and its greater availability (MOREIRA *et al.*, 2023), compared to other commercial formulas of phosphate fertilizers (RODRIGUES *et al.*, 2016). When MAP is used in areas with adequate or high P contents, the fertilizer rate is reduced (MACEDO *et al.*, 2021; MOREIRA *et al.*, 2023) and, consequently, the amount of N applied per hectare is low. However, MAP is also used in opening areas, where the P content is low, consequently, high doses of fertilizer are used. Hence, it becomes essential to understand the impact N on the BNF process (CARDOSO *et al.*, 2018).

Although research has shown that the application of mineral N in the seed furrow can reduce BNF nodulation and efficiency when it exceeds 20 kg ha^{-1} (HUNGRIA *et al.*, 2006; ARATANI *et al.*, 2008; KASCHUK *et al.*, 2016; HUNGRIA *et al.*, 2017; CORDEIRO; ECHER, 2019), there are recommendations in the literature for the use of small rates of N (20 to 30 kg of N ha^{-1}), applied at sowing (ARAÚJO; CARAVALHO, 2015). These are called starting rates, whose purpose is to make N available to plants until the beginning of nodulation process.

Data regarding BNF rates in soybeans exhibit significant variability attributed to diverse research environments, methodologies, and strains employed. With continuous advancements in plant and microorganism genetic enhancements, coupled with the adoption of production-boosting technologies, BNF has demonstrated values ranging from 72% to 94% of the crop's total nitrogen requirements. This process has the capacity to fix up to 300 kg ha^{-1} of N, representing 70% to 85% of the overall N accumulated by the plants (HUNGRIA, 2006; CORDEIRO; ECHER, 2019).

Currently, there are concerns regarding the ability of BNF to meet the N requirements for achieving high yields, especially with crop yields reaching around 6000 kg ha^{-1}. Considering the extraction of 84 kg of N per 1000 kg of grain to achieve these high yields, approximately 500 kg ha^{-1} of N would be necessary needed (CARDOSO *et al.*; 2018; EMBRAPA, 2020).

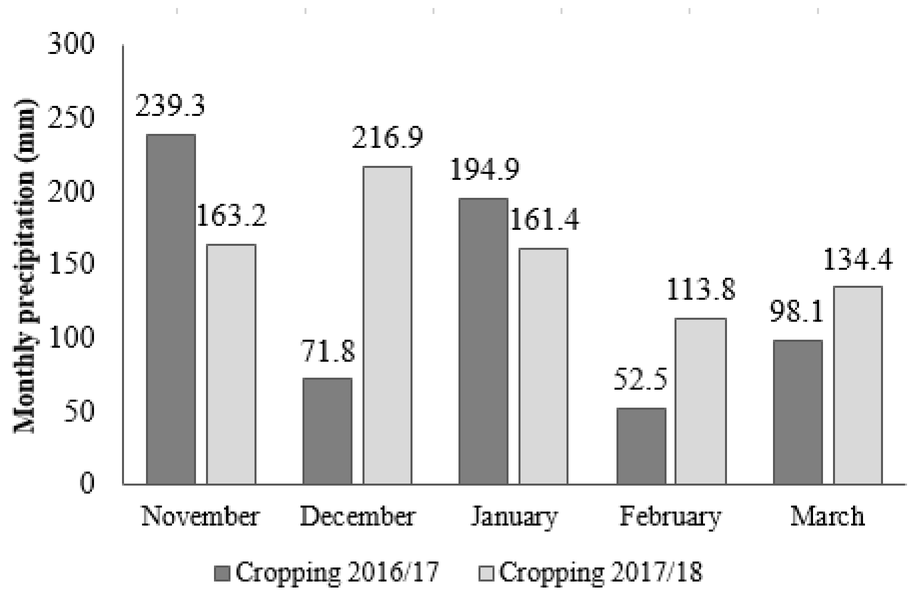
Due to the high demand of N for high yields, some researchers have been evaluating the need for application of supplemental N in soybean crops. Moreno *et al.* (2018), for example, applied 60 kg ha^{-1} of N (1/3 at sowing and 2/3 at reproductive stage R4) and there was a 47% increase in soybean yield. Similar responses were observed in other studies (ARATANI *et al.*, 2008; BARRANQUEIRO; DALCHIAVON, 2017). These facts have questioned the real need to make additional N fertilization in soybean.

The introduction of new cultivars with super-early and precocious cycles has prompted technicians and farmers to question the response of this new cultivars to N fertilization at sowing, and if their behavior is similar to those where N fertilization happens at later maturity cycle. Adopting the hypothesis that new cultivars do not respond to N fertilization at sowing, the goal of this study was to evaluate the effect of N fertilization in soybean cultivars with different cycles.

Material and Methods

Two experiments were carried out at Fazenda Recanto, in the municipality of Carmo do Rio Claro, Minas Gerais State, in two consecutive cropping years (2016/2017 and 2017/2018). The farm is located at latitude 21º 02' 44" S and longitude 45º 59' 53" W with 780 m of altitude. The soil of this farms is classified as Latossolo Vermelho-Amarelo in the Brazilian Soil Classification System (SANTOS *et al.*, 2018), and as Typic Hapludox in Soil Taxonomy System (Soil Survey Staff, 2014). The mesoregion climate is Cwa (dry-winter humid subtropical climate according with Köppen Climatic Classification), with a cold and dry winter and a hot and humid summer. The average annual temperature is around 20.4 °C, with dry and mild winters and rainy summers with high temperatures. The average annual precipitation is 1,487 mm. In Figure 1, the monthly precipitation during the period of the experiment is presented.

Figure 1. Monthly precipitation in the period in which the experiment was carried out. INMET (2018).



Both areas of the experiments had a history of soybean cultivation during five cropping seasons, three years under no-till system, without mechanical intervention by harrowing or subsoiling, but soil corrections were carried out annually. In both cropping seasons, the liquid inoculant was applied through the furrow, using the commercial product Masterfix L® from Stoller company, which has a concentration of 5 x 109 Bradyrhizobium japonicum bacteria per mL of the commercial product, with strains SEMIA 5019 and SEMIA 5079. In the experiment, a rate of 400 mL per hectare of the inoculant Masterfix L® was used. Before cultivation, sampling and analysis of chemical attributes were performed, as shown in Table 1.

Table 1. Results of soil analysis in the areas where the experiment was carried out.

pH	P	K	Ca	Mg	Al	H+Al	CEC	m	BS	MO	B	$S\text{-}SO_4$
H_20	----mg dm^{-3}--		---------------$cmol_c$ dm^{-3}-------					---------%-------			--mg dm^{-3}--	
Cropping season 2016/2017												
5.9	31	168	4.9	1.2	0	3.6	10.1	0.0	65	3.9	1.1	20
Cropping season 2017/2018												
6.3	121	144	5.2	1.5	0	2.7	9.8	0.0	72	3.6	0.9	7.0

pH - pH in water (1:2.5 soil/solution); OM - soil organic matter ($Na_2Cr_2O_7$ 4 mol L^{-1} + H_2SO_4 5 mol L^{-1}) (SILVA, 2009); P and K mixed resin (van RAIJ *et al.*, 1986); Fe, Zn, Mn and Cu (SILVA, 2009); Ca, Mg e Al (KCl 1 mol L^{-1}); S - Sulfur extracted as sulfate and the result was converted to S, (H+Al) - potential acidity (SMP). CEC - cation exchange capacity at pH 7.0 obtained by adding Ca, Mg, K and H+Al; m= Al saturation [m = ((Al)/CECefetive)) × 100] and base saturation [BS = ((Ca+Mg+K+H+Al)/CEC)) × 100].

The experiments were carried out in a randomized block design, in a split-plot scheme, with four replications. The main plots being constituted by the different soybean cultivars and the subplots constituted by the 4 rates of N at sowing (0, 10, 20, 40 kg ha^{-1}). In the first cropping season, cultivars M6410 IPRO, with relative maturation degree (MD) 6.4; NS7300 IPRO (MD 7.3), and TEC7849IPRO (MD 7.8), and in the second cropping season, cultivars M5917IPRO (MD 5.9); M6410IPRO (MD 6.4); AS3680IPRO (MD 6.8), and NS7670 RR (MD 7.6), totaling 48 plots in the 2016/17 cropping year and 64 plots in the 2017/18 cropping year. Each plot had 35 m^2, consisting of 7 lines spaced 0.5 m by 10 m in length.

Fertilization at sowing was the same during the two cropping season, using 200 kg ha^{-1} of P_2O_5, source NPK 03-17-00. The fertilizer was applied in the furrows at a depth of 10 cm, being seven centimeters beside and below the seeds. Regarding K, the nutrient was applied at a variable rate, before broadcast sowing, aiming to raise the percentage of K in the CEC (cation exchange capacity at pH 7.0) to 5%.

The sowing of the 2016/2017 cropping year was carried out in the first half of December, with cultivars M6410 IPRO, NS7300 IPRO, and TEC7849 IPRO. The sowing of the 2017/2018 cropping year was carried out in mid-November with cultivars M5917IPRO, M6410IPRO, AS3680IPRO, and NS7670RR. All cultivars were sown with the seed population recommended by the companies that own the cultivars.

The treatments corresponding to N fertilization at sowing (0, 10, 20, 40 kg ha^{-1} of N) were applied under the sowing furrow. The application was carried out soon after sowing, manually, using urea as a source of N.

The phytosanitary management of the crops was carried out with previous monitoring and the products used followed the standard treatment of the farm. In the evaluations, the following parameters were considered: plant height, insertion of the first pod, number of nodules per plant, weight of nodules, number of pods per plant and number of grains per plant. The average height of five plants per plot, randomly chosen in the area, was given by measuring the distance between the plant's collar and the end of the main stem. The height of insertion of the first pod was obtained in the same way, measuring the height of insertion of the first pod from the ground surface.

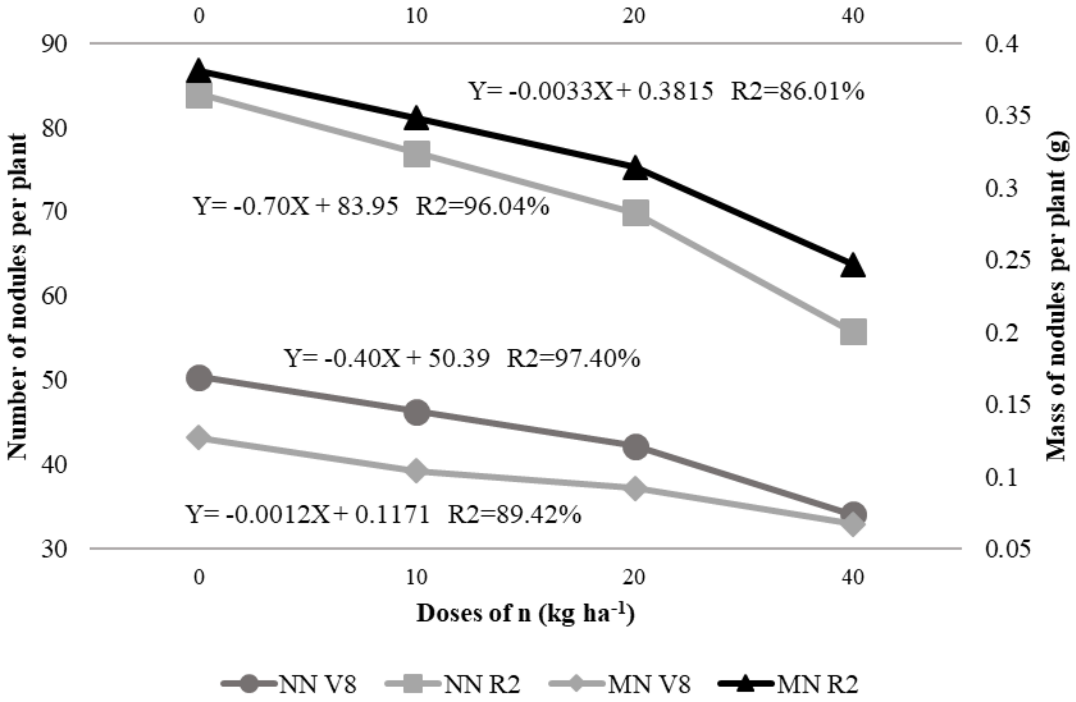
The harvest of both cropping years was carried out in the second half of March, the characters plot weight, plot moisture, number of pods and grains were evaluated to obtain yield. To estimate yield, a harvest of $5m^2$ was carried out for each plot. Grain moisture was standardized to 13%.

Data were statistically analyzed by applying the F test on the analysis of variance, followed by the application of the Scott-Knott test of means (SCOTT; KNOTT, 1974), $p > 0.05$, or the regression analysis using the statistical program SISVAR (FERREIRA, 2019).

Results and Discussion

The results will be presented by cropping season, since the same cultivars were not used in each cropping year. Initially, the information obtained in the 2016/17 cropping year will be discussed. In this first season, there was no significant interaction between cultivars of different degrees of relative maturity and N rates at sowing. During V8 and R2 stages there was a reduction in the number and mass of nodules as a function of N rates at sowing (Figure 2). The same behavior was observed in other studies (NOVO *et al.*, 1999; CARDOSO *et al.* 2018; ZUFFO *et al.*, 2019), in which different rates and times of nitrogen fertilizer negatively influenced nodulation and nodule mass in different soybean cultivars.

Figure 2. Number of nodules (NN) and mass of nodules (MN) per plant, at stages V8 (plants with eight nodes) and R2 (full flowering), as a function of N doses (kg ha^{-1}) at sowing soybean.



Redutions in the number of nodules in soybean roots because of N fertilizer application may be attributed to alterations in photoassimilate translocation within the plant. In the absence of N, a majority of the produced photoassimilates are translocated to the nodules, followed by the primary roots, and a smaller proportion to the secondary roots. However, in the presence of N, there is a shift in the translocation pattern of photoassimilates, with the secondary roots becoming the primary sink, and the nodules receiving a lower amount of photoassimilates (SAITO *et al.*, 2014). This shift results in a reduced number and mass of nodules, as observed in the present study.

Furthermore, Hungria *et al.* (2007) also concluded that N fertilization at sowing leads to a reduction in BNF in soybean. In the studies conducted by Mendes *et al.* (2000), the addition of N rates during soybean sowing resulted in significant reductions of up to 50% in initial nodulation, particularly when 30 and 40 kg ha^{-1} of N were applied. In addition, high levels of N available in the soil tend to reduce BNF, due to the presence of the nutrient in sufficient quantities for the nutrition of the plant (BRADY; WEIL, 2013). Thus, even with the inoculation using bacteria of the genus *Bradyrhizobium* sp. (ALMEIDA, 2015), there is no signaling for bacteria to initiate nodule formation, consequently reducing BNF (SAITO *et al.*, 2014).

In conditions of low N availability in the soil, the stimulus for root infection by the bacteria from *Bradyrhizobium* sp genus is higher, once in this condition, roots excrete flavonoids, secondary metabolites of the polyphenol class, which induce the transcription of nodulation genes (nod, nol, noe) in rhizobia, establishing the symbiotic relationship between plant and bacteria, thus initiating the formation of nodules (ALMEIDA, 2015).

Cultivar M6410 had a higher number of nodules per plant compared to the others in the vegetative stage V8 (Table 2). In turn, in the flowering phase (R2), the three cultivars showed similar number of nodules. For mass of nodules per plant there was no variation in both stages (Table 2). One of the main factors that can account for differences in the number of nodules among cultivars at the V8 stage is the specificity of infection between *Bradyrhizobium* bacteria and different soybean cultivars. Commercially available strains have a recognition mechanism in the association between *Bradyrhizobium* and the legume, which involves a complex array of genetic information between the plant and the bacteria (PERRET *et al.*, 2000). In soybean, several genes are involved in ensuring a beneficial association between the bacteria and the host plant (CARDOSO; FREITAS, 1992; YUSUF *et al.*, 1999). Thus, during soybean development, the first nodules formed by *Bradyrhizobium* infection occur in the main root, and from there, the nodular growth in terms of mass and number increases, reaching its peak at the R2 stage, as observed in the present study.

Throughout the entire crop cycle, there is continuous formation and renewal of nodules in the root system, which can vary in number and mass depending on the specificity between the bacteria and the cultivar, according to the needs of each plant. However, there is a decrease in the formation of new nodules after the R5 stage, which is the period when nitrogen (N) begins to be mobilized and accumulated in the reproductive organs such as pods and grains (CASSINI; FRANCO, 2006). This may explain the similar number of nodules observed among the cultivars in this study during the reproductive period since the formation of new nodules is halted, and the specificity between bacteria and the plant no longer influences the number of nodules (Table 2).

Table 2. Means number of nodules per plant (NNP) and mass of nodules per plant (MNP) (g) at stages V8 and R2, number of pods per plant (NPP), number of grains per plant (NGP), plant height (PH), height of insertion of the first legume (HIFL) and grain yield (bags ha^{-1}) in the 2016/17 cropping season.

Evaluated characters	Cultivars		
	M6410IPRO	**NS7300IPRO**	**TEC7849IPRO**
NNP V8	54.59 a[1]	31.21c	43.66 b
NNP R2	83.61a	59.77a	71.52 a
MNP V8 (mg)	116.00 a	93.00 a	80.00 a
MNP R2 (mg)	330.00 a	310.00 a	330.00 a
NVP	50.80 b	47.30 b	67.00 a
NGP	124.70 b	105.00 c	182.40 a
AP (cm)	93.50 b	92.90 b	109.0 a
AIPL (cm)	19.10 a	19.40 a	17.10 b
Productivity	76.90 a	67.60 a	72.30 a

[1] Means followed by the same letter on the line do not differ by the Skott-Knott test at 5% significance. Each bag has 60 kilograms of grain.

As for the agronomic characters, the N rates applied at sowing, did not modify the number of pods and grains per plant, as well as the height of the plants and the height of insertion of the first legume. Similarly, Silva *et al.* (2011) also did not observe the influence of N rates at sowing on the agronomic characters of soybean, except for plant height, a fact that was attributed by them, to differences in the environmental conditions under which the experiments were conducted.

The agronomic characters are strongly influenced by the genetic characteristics of each cultivar, as observed by Gesteira *et al.* (2015) in cultivars evaluated in southern Minas Gerais. In this present study, the same was observed for some agronomic characters among the studied cultivars (Table 2). For plant height and height of insertion of the first legume, was observed for each cultivar studied, a standard behavior (Table 2).

Cultivar TEC7849 showed a higher plant size, number of pods per plant and number of grains per plant. This is due to the genetic characteristic of this material, as well as its higher degree of relative maturity compared to the others. The average height of the plants, the height of insertion of the first pod and the architecture of the soybean plants are characteristics, genetically defined, which can be influenced by several factors, such as sowing time, spacing, population density, supply of water, air temperature and soil fertility (VAZQUEZ *et al.*, 2008; ROCHA *et al.*, 2012; MATSUO *et al.*, 2015).

Although the number and mass of nodules per plant were reduced by N doses (Figure 2), the cultivar productivity were not modified by N doses applied at sowing moment (Table 2), according also observed by other authors (HUNGRIA; VARGAS, 2000; MENDES *et al.*, 2000; CRISPINO *et al.*, 2001; HUNGRIA *et al.*, 2007). The amounts of nodules and dry nodule mass per plant were always above 15 to 30 and 100 to 200 mg, respectively, described by Hungria *et al.* (2007) as being sufficient to obtain high grain productivity.

The high nodule numbers and high nodule mass observed, probably occurred because this was a five-year-old soybean growing area and possibly, there is a good population of *Bradyrhizobium* already established in the place. It is possible that the results were different in an area of first soybean cultivation, as occurred in the study developed by Zilli *et al.* (2006).

In 2017/18 cropping season, differently from the first year, there was no effect of N rates on plant nodulation (number of nodules and nodule mass) for the periods evaluated, but there were significant differences between cultivars (Table 3).

Table 3. Means of number of nodules per plant (NNP) and mass of nodules per plant (MNP) (g), at flowering stage R2, number of pods per plant (NPP), number of grains per plant (NGP), plant height (PH), height of insertion of the first legume (HIFL) and yield (bags/ha) in the 2016/17 cropping season.

Evaluated characters	**Cultivars**			
	M5917IPRO	**M6410IPRO**	**AS3680IPRO**	**NS7670RR**
NNP	79.50 b[1]	102.50 a	84.60 b	76.50 b
MNP (mg)	480.00 a	360.00 b	490.00 a	510.00 a
NVP	36.10 c	45.50 b	52.10 b	74.90 a
NGP	83.70 c	112.20 b	122.0 b	146.90 a
AP (cm)	81.50 b	87.40 a	95.30 a	102.70 a
AIPL (cm)	15.80 a	14.40 a	14.00 a	14.60 a
Productivity	54.40 b	63.40 a	63.70 a	66.20 a

[1] Means followed by the same letter on the line do not differ by the Skott-Knott test at 5% significance. Each bag has 60 kilograms of grain.

Following the previous season (2016/17), the cultivar M6410 showed a higher number of nodules per plant, but a lower nodule mass compared to the other cultivars (Table 3). These results were probably due to the genetic characteristics of cultivar M6410, as the establishment of bacteria and the beginning of nodulation occur due to the genes existing in each soybean cultivar and their compatibility with the bacteria (PERRET *et al.*, 2000). However, in all cultivars, the quantity of nodules, as well as the dry nodule mass, were much higher than those values described by Hungria *et al.* (2007), to obtain high yields, as also observed in the first crop (Table 2).

During the month of December 2017/18 season crop, when soybean was in the vegetative period, were observed high rainfall (Figure 1), which may have favored the leaching of most part of the N applied in sowing. Thus, there would not be a high amount of N available in the soil to negatively affect the beginning of biological nitrogen fixation (BNF) (ALMEIDA, 2015; SANTOS, 2018).

It is important to also mention that regarding to the agronomic traits, these were not affected by N doses (Table 3), once it was more directly linked to the genetics of each material, as well as to the favorable environment of production (GESTEIRA *et al.*, 2015). The experiment was carried out in an area with a history of no-till, built-up fertility, and good rainfall (Figure 1), and probably with a population of nitrogen fixing bacteria already established.

The soybean cultivars of late and medium cycle for the region showed higher productivity, NS7670 (66.20 bags ha^{-1}), AS3680 (63.7 bags ha^{-1}) and M6410 (63.4 bags ha^{-1}), when compared to the cultivar with earliest cycle M5917 (54.4 bags ha^{-1}) (Table 3). In addition to these factors, Gesteira *et al.* (2015), report that differences in productivity between different soybean materials occur due to the genotype x environment interaction, and these authors observed a variation for productivity of 25 cultivars, evaluated in southern Minas Gerais, affirming the interaction between such genotypes and the crop/growing environment as the mainly factor for the variation in productivity that occurred.

Worth to emphasize that in both evaluated crops, the average of productivity was superior than the national average of 58,9 bags/hectare (3537 kg/ha) (CONAB, 2023). With this, it is possible to understand that even in the case of high productivity for the soybean crop, BNF can contribute with enough N, not being necessary to complement with this nutrient in the sowing moment.

Conclusions

Nitrogen fertilizer in sowing is not necessary, once it can reduce the number of nodules and the mass of dry nodules per plant and does not increase the crop yield, regardless of the relative maturity degree of the cultivars used.

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