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Side dressing nitrogen fertilization in soybean in association with inoculation

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Abstract

The soybean is a crop that demands high levels of nitrogen (N) to express its productive potential. Supplemental fertilization with N can be a management strategy to increase the yield. This study aims to assess the effect of the N side dressing fertilization at the V4 stage of soybean, with and without inoculation, as well as its effects on the process of biological nitrogen fixation (BNF). The experimental design was randomized complete-block design with four replications in factorial scheme 2×5, where the seeds were either inoculated or not and received 5 doses of N: 0, 10, 20, 30, and 40 kg ha^{-1}. Vegetative growth, nodulation, and yield components were evaluated. The stem diameter, plant height, and grain yield were not affected by inoculation, only by the N doses, with positive linear responses to the N supply. The leaf area responded positively to the seed inoculation and to the N supply. The node mass and viable node mass were not altered by seed inoculation or the supply of mineral N; and, until the dose of 40 kg ha^{-1}, the node formation did not decrease. The number of pods per plant and the thousand-grain weight showed an interaction between inoculation and doses of N. The side dressing N fertilization at the V4 stage, until the dose of 40 kg ha^{-1}, did not affect the vegetative growth and BNF, while it contributed to increase grain yield, even in the presence of inoculation.

Keywords*:* *Glycine max.* Biological nitrogen fixation. Yield components. Morphological traits.

Introduction

Soybean (*Glycine max* [L.] Merrill) is the most produced grain in Brazil. In the 2021/2022 crop, this specific oilseed was cultivated in an extensive area of the country, with 40.98 million ha and a 4.6% variation when compared to the 2020/2021 crop, totaling the production at 124.26 million tons (CONAB, 2021). The grain can be destined for the foreign market or crushed to obtain derived products such as brans, oils, and biofuels (HIRAKURI et al., 2019). To grow this legume, nitrogen (N) is the most required nutrient (MELLO-PRADO, 2021). To produce 1,000 kg of grains, an estimated 80 kg of nitrogen is required (SEDIYAMA et al., 2009).

Nitrogen absorption is the main determinant of soybean grain production (SANTACHIARA et al., 2017). Soybean is a legume that, in association with bacterium, performs biological nitrogen fixation (BNF) (MORETTI et al., 2020). In the BNF process, the gaseous nitrogen present in the atmosphere (N_2) is assimilated and transformed into ammonia (NH_3), which is rapidly incorporated into H+ ions in bacterial cells, transforming into ammonium ions (NH_4^+) by the nitrogenase enzyme (HUNGARY; FIELDS; MENDES, 2001; TAIZ et al., 2017). This process is known for its high capacity in fixing nitrogen and introducing it into agricultural systems. Zuffo et al. (2022) estimated the total accumulation of nitrogen by soybean at more than 220 kg ha^{-1}.

The amount of nitrogen supplied depends on the efficiency of the BNF, i.e., the quality and quantity of the inoculant, as well as some care at inoculation (ZITO et al., 2007). Despite BNF being the main, and often the only, source of nitrogen in soybean production systems in the country, several studies with the use of mineral nitrogen have been developed with the objective of potentiating crop yields. For this, investigations on mineral nitrogen in soybean seek to better understand the dynamics between BNF and additional supplementation with nitrogen. Among the various factors investigated, we found studies that associate nitrogen doses with sowing density (FERREIRA et al., 2016; WERNER et al., 2016); supplementary mineral nitrogen with its effects on oil and/or protein contents in grains (HAMAGUCHI et al., 2020; MOREIRA et al., 2017; PRUSIŃSKI, BATURO-CIEŚNIEWSKA, BOROWSKA, 2020; SZPUNAR-KROK et al., 2021); nitrogen supply with seed quality (ZUFFO et al., 2021); as well as those that investigate the dynamics of nitrogen absorption in the initial stages of soybean, in which the BNF is not yet meeting the requirements of the crop (CONCEIÇÃO et al., 2018); in addition to studies on the effects of fixed nitrogen in the subsequent crop (GOSS et al., 2002; ZUFFO et al., 2022); and studies on foliar nitrogen and their effects on carbon and oxidative metabolism (RODRIGUES et al., 2021).

Bahry et al. (2014) pointed out that, in some edaphoclimatic conditions, nitrogen from BNF is not enough to meet the requirements of the crop and its limitation may compromise the final productivity, evidencing that the application of nitrogen in the reproductive phase of soybean positively influences some yield components. Moreira et al. (2017) emphasized that, under conditions of high productive potential, the BNF may not be sufficient to ensure high nitrogen absorption rates to meet the plant's demand for maximum productivity during seed-filling. The filling of the pods in soybean is the developmental phase of the highest demand for nitrogen (LAMOND; WESLEY, 2001).

According to Petter et al. (2012), there is an increase in grain yield of the crop with applications of 20 to 40 kg ha^{-1} of nitrogen, applied in the R1 stage of the crop, and a reduction at doses of 80 and 160 kg ha^{-1}. Lamond and Wesley (2001), in their studies conducted in eight cultivars of Kansas, USA, observed that the grain yield of soybean crops increased by about 11% due to nitrogen fertilization (22 and 45 kg ha^{-1}) in the R3 stage of irrigated soybean. On the other hand, Ferreira et al. (2016) did not detect a response in grain yield when investigating side dressing application of 45 kg ha^{-1} of nitrogen in the soybean vegetative stage V2. When studying the high doses of nitrogen in soybean (up to 180 kg ha^{-1}), Goss et al. (2002) verified a reduction in nodulations and nitrogen-derived participation of BNF in the total absorbed, without reflections on grain yield. This suggests that there should be a balance in the supply of nitrogen so that the nutrient does not nullify the effects of BNF or become an uneconomic practice. Thus, there is no uniformity among the responses for better positioning of supplemental nitrogen in soybean crops and its interaction with the various management factors.

Santachiara et al. (2017) investigated the relative importance of BNF and mineral nitrogen from the soil in the performance of 70 soybean cultivars under conditions of high and low availability of mineral nitrogen. The authors concluded that maximum performance in crop productivity and nitrogen absorption of the system is not necessarily associated with the relative maximization of BNF. At the same time, they found a negative correlation between nitrogen from BNF and nitrogen absorbed from the soil. Thus, what should be sought is the maximization of total nitrogen absorption, which is reflected in crop productivity, by better balancing BNF and mineral nitrogen supply via fertilization.

Understanding the physiological processes of BNF and the factors that control it is greatly important, allowing the production chain to adopt the best management system, aiming at increasing the efficiency of nitrogen use and increasing productivity (FAGAN et al., 2007). Thus, this work was developed with the objective of verifying the effect of side dressing soybean crops with nitrogen, with and without inoculation, as well as their effects on the BNF process.

Material and methods

The experiment was carried out between October 2021 and February 2022, in a commercial area of soybean production in the municipality of Ipiranga do Norte, in the state of Mato Grosso (15° 35' 14" South, 56° 5' 51" West, 213 m altitude). The climate of the region is of type Aw according to Köppen classification (1948), characterized by the presence of two well-defined seasons, a rainy season (from October to April) and drought season (from May to September), and by the small annual thermal amplitude, with monthly averages ranging from 24°C to 27°C (PEREIRA et al., 2018). Figure 1 shows the means of the meteorological data of maximum temperature, average temperature, minimum temperatures, and rainfall (mm) accumulated during the period of the experiment, obtained by the meteorological station of the Brazilian Agricultural Research Corporation (Embrapa), the Agrosilvopastoral Unit, located in the municipality of Sinop, in the state of Mato Grosso, 160 km from the experiment.

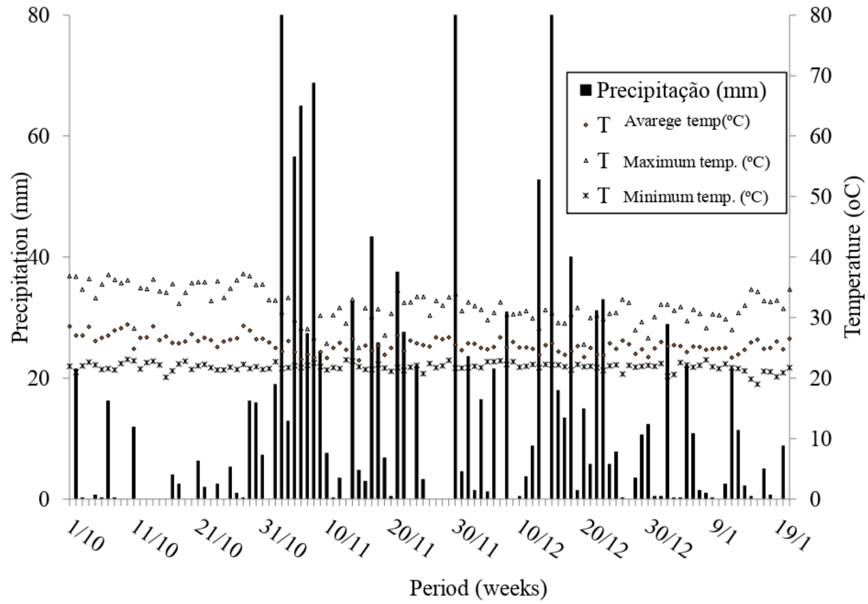
The adopted experimental design was randomized blocks, with four replications, in a factorial scheme 2 × 5, composed of inoculated and non-inoculated treatments with nitrogen-fixing bacteria of the genus *Bradyrhizobium* and five doses of nitrogen: 0 (control), 10, 20, 30, and 40 kg ha^{-1}. The source of nitrogen was urea (45% N), applied at stage V4 of the culture (25 days after sowing), and the inoculant was *Bradyrhizobium* based on the Masterfix® brand, at a dose of 100 mL to 50 kg^{-1} of seeds, applied in the treatment of seeds before sowing.

The experimental units consisted of five rows of spaced cultivation of 0.5 m with 4 m length, totaling 10m^2 of the total plot. The sown soybean was the commercial cultivar 83HO113 TP IPRO, with maturation group 8.3, the habit of indeterminate growth, and lodging resistance. Sowing occurred at the rate of 12 to 12.5 plants per linear meter (population of 240,000 plants ha^{-1}). Sowing was carried out on October 10, 2021, under a no-tillage system.

From the opening of the area, in 2004, to 2012, only soybeans were cultivated; from 2012 to 2019, the first harvest was of soybeans and the second of millet; and from the years 2019 to 2022, the first and second harvests were of soybeans and corn, respectively. The soil was sampled, before the sowing, in the layers of 0-10 and 10-20 cm depth. The soil contained the following chemical and physical parameters in the 0-10 cm layer: pH-$CaCl_2$ 5.6; 465 g kg^{-1} of clay; 2.2, 0.56, and 0 mg dm^{-3} of base cations Ca, Mg, and Al, respectively (extracted by KCl 1 mol L^{-1}); 10.1 and 39.6 mg dm^{-3} of P and K available, respectively (extracted by Mehlich^{-1}); and 3.42 dag kg^{-1} of organic matter. For the 10-20 cm layer, the following physical and chemical parameters were identified: pH-$CaCl_2$ 5.4; 505 g kg^{-1} of clay; 0.94, 0.28, and 0.25 mg dm^{-3} of base cations Ca, Mg, and Al, respectively (extracted by KCl 1 mol L^{-1}); 3.50 and 31.50 mg dm^{-3} of Available P and K, respectively (extracted by Mehlich^{-1}); and 2.21 dag kg^{-1} of organic matter.

The base fertilization for all experimental units consisted of the application of 465 kg ha^{-1} of formulated fertilizer (NPK) 07-24-24, with the application of 32.55 kg ha^{-1} of nitrogen and 111.6 kg ha^{-1} of P_2O_5 and K_2O. The crop was maintained with adequate phytosanitary levels, applying herbicides, insecticides, and fungicides whenever necessary.

Figure 1. Monthly mean maximum temperature (°C), monthly mean average temperature (°C), monthly mean minimum temperatures(°C), and accumulated rainfall (mm) for the 2021/2022 crop.



Source: EMBRAPA Agrosilvopastoral weather station, Sinop, in the state of Mato Grosso, 2022.

The evaluations of the variables regarding vegetative development and soybean nodulation were performed at stage R1, collecting the data of six plants representative of the three central lines (useful area). The following were evaluated: stem diameter (mm), measured in the stem of the plant at the height of 5 cm from the soil with the aid of the digital caliper; plant height (cm), measured with a graduated ruler; leaf area (m^2), measured using leaf area integrator model LICOR (LI-3010); number of viable nodes, by counting viable nodes; mass of nodes (g); shoot dry matter (g); and root dry matter (g). The plants collected from each experimental plot were stored in identified paper bags, taken to the plant nursery laboratory of the Federal University of Mato Grosso (UFMT), Sinop campus, in the state of Mato Grosso, and placed in the greenhouse at 65°C for 48 hours to determine the dry matter.

For the component variables of soybean yield: number of pods, number of seeds per pod, mass of 1,000 grains (g), and grain yield (kg ha^{-1}); the measurements were performed after manual harvesting of plots (80 random plants within the plot's useful area) on February 6, 2022, 119 days after sowing. At harvest, the grains had approximately 180 g kg^{-1} of water. The samples were placed in bags identified with the treatments and taken to Proteplan in Sorriso, in the state of Mato Grosso, where they were mechanically threshed on a motorized stationary thresher. After threshing, the grains were cleaned and sifted manually, then placed in properly identified paper bags. The grain moisture was then corrected to 130 g kg^{-1} water in a forced air circulation oven at 60°C, following official recommendations (BRASIL, 2009).

After moisture correction, the mass of 1,000 grains (g) and grain yield (kg ha^{-1}) were determined. For the number of pods, number of grains per pod, mass of nodes, viable nodes, shoot dry matter, and root dry matter, the average of four plants per plot was calculated. For the quantification of the mass of nodes, intact blocks of soil were removed together with the roots of the plants by manually digging with a mattock. After separation and washing of the roots, the total nodes present in the samples were removed and weighed on a high-precision scale. To determine the viable nodes, the total nodes obtained were sectioned in the middle with a stylus, and the viable nodes identified with rosy coloration were weighed on a high-precision scale.

The data obtained were submitted to variance analysis (ANOVA) at the level of 5% probability by the F test, with the help of the statistical program SISVAR (FERREIRA, 2011). The means of treatments, with and without inoculation, were compared by the Tukey test, and the nitrogen doses, when with differences, were submitted to regression analyses.

Results and discussion

We found no significant differences between the inoculated or non-inoculated soybean seeds among the means of the variables stem diameter, plant height, number of viable nodes, mass of nodes, shoot dry matter, root dry matter, and grain yield. We found, however, differences in leaf area, number of pods, number of seeds per pod, and mass of 1,000 grains; for which the treatments with inoculation were statistically superior to the non-inoculated treatments (Table 1).

After studying the supply of nitrogen in soybean according to cultivars and years, Prusiński, Baturo-Cieśniewska, and Borowska (2020) reported responses in plant height and leaf area to doses of 30 and 60 kg ha^{-1} of N applied in pre-planting, regardless of seed inoculation. Using a similar dose of nitrogen (45 kg ha^{-1}) and also applying it via the soil during the vegetative stage of soybean, Werner et al. (2016) reported responses in plant height for only one of the years of evaluation. The authors described that this variable is very dependent on climatic conditions and therefore has inconsistent responses.

The response to inoculation is also highly dependent on the number of rhizobium already established in the soil. Thus, the area of the experiment has been cultivated in the summer crop with soybean seeds inoculated with *Bradyrhizobium* since 2004, which explains the presence of nodes in the root of the non-inoculated and the low response to the inoculation treatment, which presented values slightly higher than the non-inoculated for most variables, such as stem diameter, plant height, number of viable

Table 1. Stem diameter (mm), plant height (cm), leaf area (cm^2), mass of nodes (g plant^{-1}), mass of viable nodes (g plant^{-1}), shoot dry matter (g plant^{-1}), root dry matter (g plant^{-1}), number of pods, number of seeds per pod, weight of 1.000 seeds (g), and grain yield (kg ha^{-1}) in relation to inoculated or non-inoculated seeds. Ipiranga do Norte, in the state of Mato Grosso, harvest 2021/2022.

Treatments	Diameter (mm)	Height of plants (cm)	Leaf area (m^2)	Mass of nodes (g plant^{-1})	Mass of viable nodes (g plant^{-1})	Shoot dry matter (g plant^{-1})
Not inoculated	6.60 a	76.85 a	1.46 b	10.16 a	1.34 a	19.10 a
Inoculated	6.60 a	77.07 a	1.71 a	10.37 a	1.45 a	29.20 a
Mean	6.60	77.27	1.59	10.27	1.39	24.15
C. V.	3,19	1,76	19,40	20,74	26,47	12,50

Treatments	Root dry matter (g plant^{-1})	Number of pods	Number of seeds per pod	Mass of 1.000 grains (g)	Grain yield (kg ha^{-1})
Not inoculated	3.72 a	40.30 b	2.55 b	167.50 b	3113.85 a
Inoculated	5.80 a	46.75 a	2.61 a	172.50 a	3168.33 a
Mean	4.76	43.52	2.58	170.00	74.41
C. V.	13.89	8.82	1.64	3.20	13.65

Source: Elaborated by the authors

*The means followed by the same letters in the columns do not differ from each other to the 5% probability level according to the Tukey test.

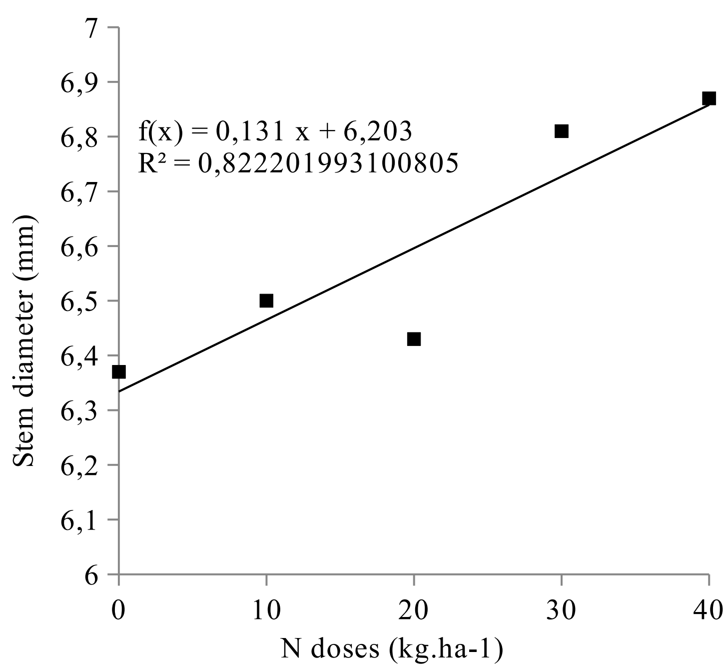
nodes, mass of nodes, shoot dry matter, root dry matter, and grain yield, in which there were no significant differences between treatments. The mean for shoot dry matter, root dry matter, number of pods, number of seeds per pod, and mass of 1,000 grains – for which the inoculation treatments were statistically superior to the non-inoculation treatments – were similar to the values found by Pereira et al. (2018). The authors found that the supplemental fertilization with foliar application of N (10 kg ha^{-1}), in the stages V2 and R1 of soybean, wsd more efficient in increasing grain yield when compared with the control treatment with inoculation.

For the variable stem diameter, there was a linear response with the increase of the applied doses (Figure 2). Thus, within the range of the studied nitrogen variation, the stem diameter increases linearly at the rate of 0.0131 mm for each kg of N, until the dose of 40 kg ha^{-1}. Pereira et al. (2018), in turn, observed a significant difference in stem diameter with soil and foliar application in the R1 stage with doses of 10 kg ha^{-1}, but not with the application at the V4 stage. Notably, the larger stem diameter increases the plant's tolerance to tipping due to mechanical or environmental damage (RAIMUNDI, MOREIRA, TURRI; 2013).

Plant height also had a linear response with the increase of nitrogen dosage. For each 1 kg increase in nutrient, we registered an increase of 0,2375 cm in the stature of the crop (Figure 3) Silva et al. (2011) also observed an increase in plant height with the application of up to 40 kg ha^{-1} of N in the V2 stage. Franchini et al. (2015), however, reported an increase in plant height with the application of 30 kg ha^{-1} of N during the sowing.

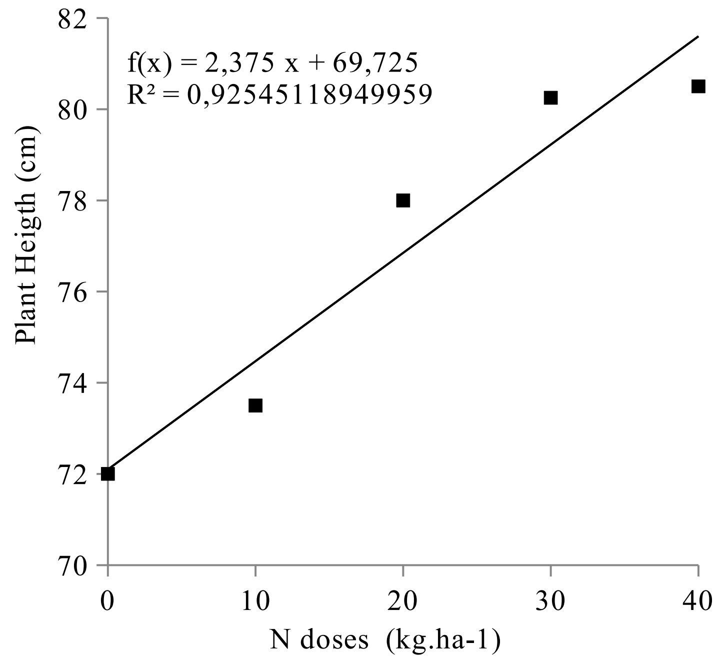
For the variable leaf area, the treatments with increasing doses of nitrogen with inoculation were also different; there was no interaction of nitrogen dose levels with inoculation (Figure 4). The results found in this study corroborate those obtained by Yokoyama et al. (2018), who reported an increase in leaf area with fertilization of 80 kg ha^{-1} of N in soybean sowing.

Figure 2. Stem diameter (mm) according to nitrogen doses. Ipiranga do Norte, in the state of Mato Grosso, harvest 2021/2022.



Source: Elaborated by the authors

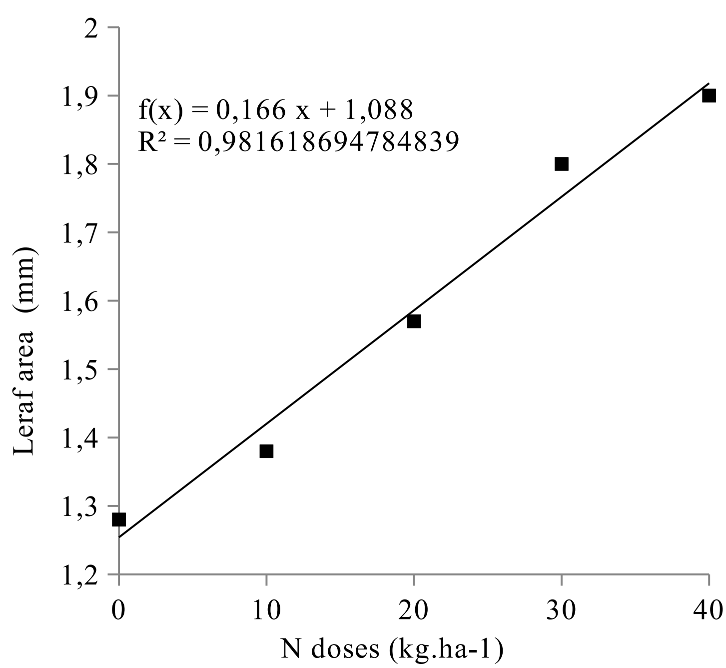
Figure 3. Plant height (cm) according to nitrogen doses. Ipiranga do Norte, in the state of Mato Grosso, crop 2021/2022.



Source: Elaborated by the authors

We found no significant differences for the variables mass of nodes, mass of viable nodes, shoot dry matter, and root dry matter – with means of 10.26, 1.39, 24.15, and 4.76 g, respectively – regarding the increase of nitrogen dosage. Parente et al. (2015) observed that the

Figure 4. Leaf area of soybean plants (cm^{-2}) as a function of nitrogen doses. Ipiranga do Norte, state of Mato Grosso, crop 2021/2022.



Source: Elaborated by the authors

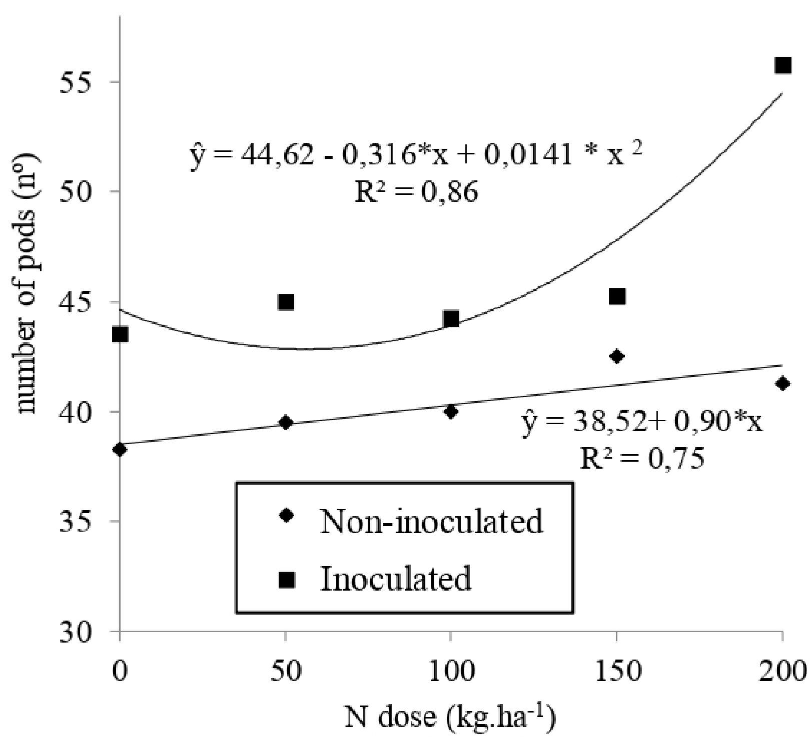
BRS Valiosa RR cultivars had a higher mass of nodes when fertilized in R1 if compared with fertilization at sowing. The authors also concluded that nitrogen fertilization in R1 allowed the development of a greater number of nodes and higher volume compared to fertilization performed at sowing. A similar result was reported by Goss et al. (2002), who did not find a reduction in the dry mass of nodes with doses up to 60 kg ha^{-1} of N via soil. The mean values for shoot and root dry matter are similar to those found by Pereira et al. (2018), in which the supplementary fertilization with 10 kg ha^{-1} of N via foliar application during the V2 and R1 stages of soybean was of higher efficiency in increasing the productivity of soybean grains when compared with the control treatment with inoculation.

The result for the mass of viable nodes corroborates those observed by Parente et al. (2015), in which they did not verify a difference in this variable with the application of nitrogen. As for the root dry mass, the results differ from that found by Zuffo et al. (2019), who found lower values of root dry mass with 40 kg ha^{-1} of nitrogen.

The number of pods was statistically significant with the interaction of inoculation with nitrogen doses, there was an increase in pods with linear behavior for non-inoculation treatments and a quadratic behavior for inoculation treatments, with the response variable increasing with the use of doses in all analyses. For the non-inoculated treatments, the response in the number of pods was linear, with an increase of 0.9 pods for each kg of nitrogen applied. For the inoculated treatments, there was a quadratic response, with an increase in the number of pods from the dose of 11 kg ha^{-1} of nitrogen (Figure 5).

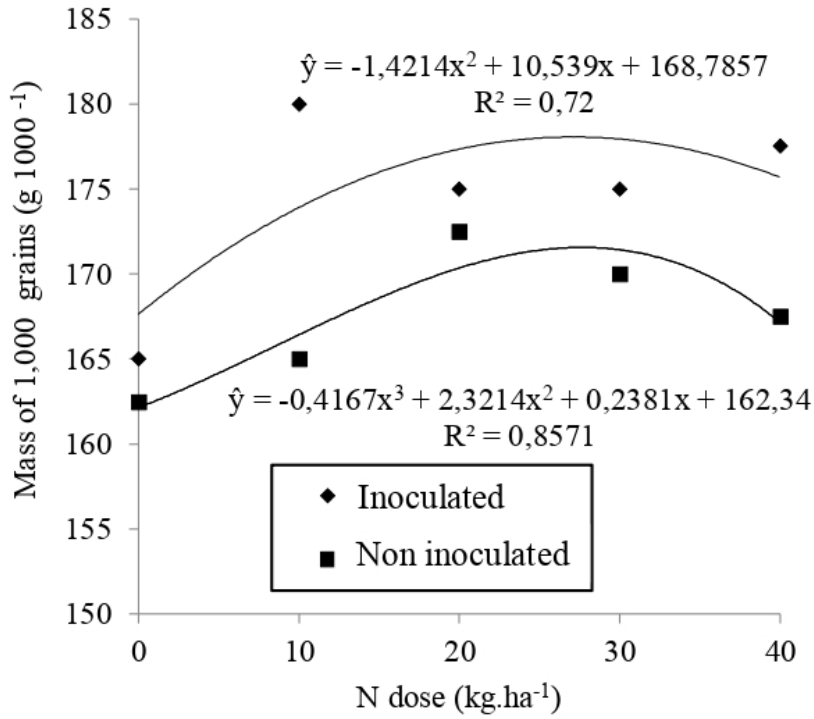
The results corroborate those found by Petter et al. (2012), in which they found that the number of pods per plant was influenced

Figure 5. Number of pods according to nitrogen doses. Ipiranga do Norte, in the state of Mato Grosso, harvest 2021/2022.



Source: Elaborated by the authors

Figure 6. Mass of 1,000 grains (g) as a function of nitrogen doses. Ipiranga do Norte, in the state of Mato Grosso, harvest 2021/2022.



Source: Elaborated by the authors

by nitrogen fertilization at doses of 20 and 40 kg ha^{-1}, but was reduced with applications of 80 and 160 kg ha^{-1} in, for three soybean cultivars with different cycles. In common bean plants, Carvalho et al. (2001) reported a significant increase in the number of pods with the application of nitrogen.

We also found an interaction for the variable mass of 1,000 grains. Inoculated treatments have always presented a higher mass than non-inoculated treatments (Figure 6). The results obtained were similar to that observed by Petter et al. (2012), in which they verified an increase in the mass of 1,000 grains with applications of 20 and 40 kg ha^{-1} of N. With the foliar application of nitrogen in soybeans, Rodrigues et al. (2021) also found an increase in the mass of 1,000 grains in two consecutive years of evaluation. Zuffo et al. (2021) reported that the best nitrogen dose for a higher mass of 1,000 grains, with inoculation, was that of 50 kg ha^{-1} applied via soil at the R2 stage.

The average grain yield in the experiment was 3141 kg ha^{-1}, with a significant and linear increase with the applied nitrogen doses (Figure 7). We noticed that each kg of nitrogen side dressing promoted an increase of 7.98 kg of grains. Messa et al. (2022) also found no effect on the interaction of the inoculation treatments with nitrogen fertilization in soybean. The authors, however, reported no effect of the application of 60 kg ha^{-1} of nitrogen on yield and mass of 1,000 grains and a superiority of inoculation treatments only for the variable grain yield. In turn, Prusiński, Baturo-Cieśniewska, and Borowska (2020) found no effect on grain yield for the application of 30 or 60 kg ha^{-1} of N in non-inoculated soybean, which indicates the interaction of factors in the study developed by them.

Similar data were observed by Parente et al. (2015) with the cultivar BRS Valiosa RR at a dose of 20 kg ha^{-1} of N in R1. Bahry et al. (2013) found a higher yield of soybean grains with a dose of 30 kg ha^{-1} in the R5.2 stage, thus recommending its use. Also investigating nitrogen supplementation in the reproductive stage of soybean, Moreira et al. (2017) found a positive response in grain yield with the supply of only 10 kg ha^{-1} with foliar application. The authors, however, did not find an effect of this additional supply of nitrogen in the number of pods per plant and mass of 1,000 grains, differing from the results found here with the supply of higher doses, with soil application. On the other hand, Hamaguchi et al. (2020) did not find the effect of higher availability of mineral nitrogen, via urea fertilization, regarding soybean grain yield and grain mass, attributing this to the ability of the BNF of compensating low supply of nutrients in the medium.

Ferreira et al. (2016), upon evaluating mineral nitrogen applied via soil for soybeans at vegetative stage V2, found no response for the application of 45 kg ha^{-1} of nitrogen in grain yield, number of seeds per pod, mass of 1,000 grains, and oil and protein contents in the grains in two years of evaluation for a cultivar of indeterminate growth habit in four different sowing densities.

Conclusions

Stem diameter, plant height, and grain yield were not affected by inoculation, only by nitrogen doses with positive linear responses to the side dressing application of nitrogen at stage V4.

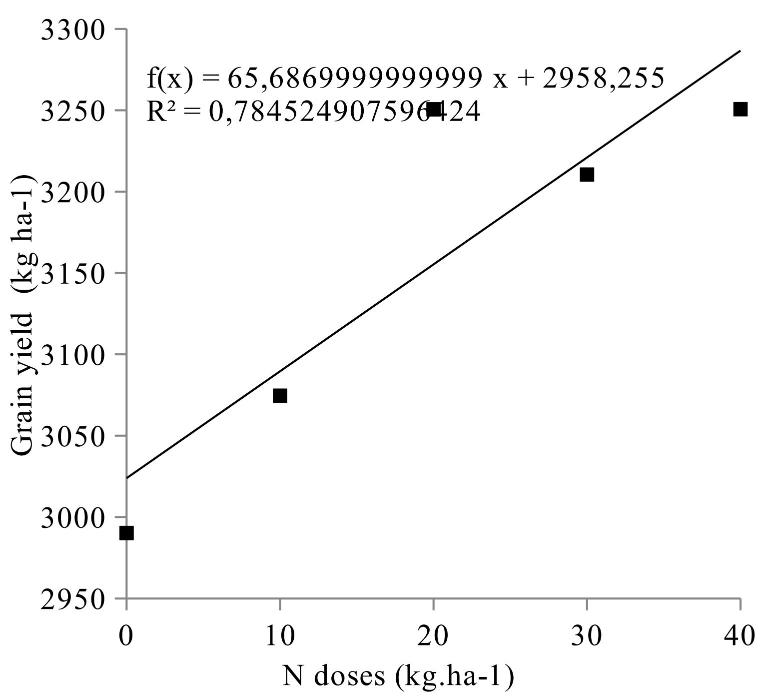
The leaf area had a positive response to seed inoculation and nitrogen supply in v4 stage side dressing.

The mass of nodes and mass of viable nodes were not affected by seed inoculation, and the side dressing application of mineral nitrogen at stage V4 up to the dose of 40 kg ha^{-1} did not reduce node formation.

The number of pods per plant and the mass of 1,000 grains had interaction with both inoculation and with doses of nitrogen side dressing at stage V4.

The nitrogen side dressing fertilization in V4, up to the dose of 40 kg ha^{-1}, did not affect the plant's development or BNF and also contributed to the increase of productivity, even in the presence of inoculation.

Figure 7. Grain yield (kg ha^{-1}) according to nitrogen doses. Ipiranga do Norte, in the state of Mato Grosso, harvest 2021/2022.



Source: Prepared by the authors (2022).

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