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Agronomic characteristics of soybean cultivars with late-season nitrogen application in supplementation to the inoculation of *Bradyrhizobium* spp.

Características agronômicas de cultivares de soja com aplicação tardia de nitrogênio em suplementação à inoculação de *Bradyrhizobium* spp.

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ABSTRACT

Late season nitrogen (N) applications may serve as cultural practices to increase soybean yield and grain protein concentration in modern high-yielding cultivars. A study to evaluate the response of soybean cultivars to late N application in supplementation to *Bradyrhizobium* spp. inoculation was conducted in two Brazilian Cerrado agricultural soils with medium and high fertility during the 2019/2020 growing season. Treatments were arranged in a randomized block design in a 2×2×5 factorial scheme: two agricultural production environments [UFMS 1 (medium fertility) and UFMS 2 (high fertility)], two soybean cultivars (TMG 7067 IPRO and BMX Bônus IPRO), and five late N application rates (0, 50, 100, 150 and 200 kg N ha–1), with four replicates. Canonical correlation analysis (CCA) was used to investigate the interrelationships between the groups of independent (agricultural production environments, soybean cultivars, and N application rates) and dependent (soybean agronomic traits) variables. Nitrogen rates were applied in topdressing at the R5.3 soybean growth stage. Late N applications did not affect soybean agronomic traits (plant height, first pod height, pod number per plant, grain number per pod, and 1,000-grain mass) and did not increase the grain protein concentration or yield of the soybean cultivars, regardless of the fertility level of the agricultural area. We conclude that when efficient strains of *Bradyrhizobium* spp. are used in soybean cropping in medium- and high-fertility Cerrado soils, there is no need to apply late rates of N fertilizer.

Index terms: Biological N fixation; *Glycine max* (L.) Merrill.; protein; urea.

RESUMO

A aplicação tardia de nitrogênio (N) pode ser uma prática agronômica para aumentar o rendimento da soja e o teor de proteína dos grãos em cultivares modernos de alto potencial produtivo. Um estudo para avaliar a resposta de cultivares de soja em relação à aplicação tardia de N em suplementação à inoculação de *Bradyrhizobium* spp. foi conduzido em dois solos do Cerrado de média e alta fertilidade durante a safra 2019/2020. Os tratamentos foram dispostos no delineamento de blocos casualizados em esquema fatorial 2 × 2 × 5: dois ambientes de produção agrícola [UFMS 1 (média fertilidade) e UFMS 2 (alta fertilidade)], duas cultivares de soja (TMG 7067 IPRO e BMX Bônus IPRO) e cinco doses de aplicação tardia de N (0, 50, 100, 150 e 200 kg N ha^{-1}), com quatro repetições. As doses de N foram aplicadas em cobertura no estádio fenológico $R_{5.3.}$ Análise de correlação canônica foi utilizada para avaliar a relação entre o grupo de variáveis independentes (ambientes de produção agrícola, cultivar de soja e doses de aplicação de N) e dependentes (características agronômicas da soja). As aplicações tardias de N não afetaram as características agronômicas (altura de planta, altura da primeira vagem, número de vagens por planta, número de grãos por vagem e massa de 1.000 grãos) e não aumentaram a produtividade e o teor de proteína dos grãos das duas cultivares de soja, independentemente do nível de fertilidade (médio ou alto) da área produção agrícola. Conclui-se que quando estirpes eficientes de *Bradyrhizobium* spp. são utilizadas no cultivo da soja em solos do Cerrado de média e alta fertilidade, não há necessidade de aplicar doses tardias de fertilizante nitrogenado.

Termos para indexação: Fixação biológica de N; *Glycine max* (L.) Merrill.; proteína; ureia.

INTRODUCTION

Soybean [*Glycine max* (L). Merrill] is an oilseed crop with substantial amounts of protein (36-40%) and oil (18-20%) in its grains (Lima et al., 2015). The crop has a large nitrogen (N) requirement throughout the growing season due to the high grain protein content. Approximately 80 kg N is required for each ton of soybean grain produced (Kaschuk et al., 2016). Soybean N requirements have higher demand rates during pod development and grain filling (Hungria; Mendes, 2015), which are generally fulfilled by biological N fixation (BNF) through symbiosis with strains of *Bradyrhizobium* spp. (Zilli et al., 2021), in addition to N uptake from the soil (Zuffo et al., 2018).

Technological advances combined with greater investment in soybean crop management have led to significant increases in crop productivity in recent decades in Brazil (Korber et al., 2017). However, this increase in soybean grain yield (above 3,000 kg ha^{-1}) has not resulted in grain and meal protein concentrations at levels compatible with the standard required by the international market, which is approximately 47% protein (Assefa et al., 2019). According to Silva et al. (2015), the soybean protein concentration may change due to edaphoclimatic conditions, crop management practices, cultivar genetic characteristics, and the occurrence of drought stress. Therefore, N supplementation can be an alternative to increase the soybean protein concentration. This is because N is responsible for several metabolic processes and reactions in plants and is a structural component of chlorophyll molecules, enzymes, and proteins (Bang et al., 2021).

Some recent research has reported that the application of N fertilizer has no beneficial effect on enhancing soybean grain yield (Fipke et al., 2016; Korber et al., 2017; Zuffo et al., 2018). According to Kaschuk et al. (2016), the positive soybean response to N fertilization depends on the N application management practices and the conditions of the plant growth environment. In this sense, there is no way to generate a definitive and comprehensive conclusion on this agricultural practice. On the other hand, other research has shown that N fertilization can improve plant growth and soybean grain yield (Barranqueiro; Dalchiavon, 2017; Moreno et al., 2018; Chiluwal et al., 2021). Therefore, this agricultural practice can be a viable alternative in some soybean production systems, especially with late N application, to optimize grain filling and protein concentrations.

These contradictory results of the soybean response to N topdressing are generally attributed to the symbiotic process efficiency, cultivars, sowing time, N source, soil textural class, soil organic matter content, and climatic factors (Aratani et al., 2008). In Brazil, early soybean cultivars with high grain yield potential and indeterminate growth have increased in recent years. Using these early cultivars allows farmers to grow two crops per agricultural year (i.e., soybean/maize or soybean/cotton). However, these early high-yielding cultivars have a higher requirement for N from the soil–plant system (Moreno et al., 2018). Therefore, special attention needs to be given to the proper management of plant N nutrition. However, the response of high-yielding cultivars to late N application is still incipient and inconclusive.

Late N application in supplementation to *Bradyrhizobium* spp. inoculation has been used by some Brazilian soybean farmers to increase soybean yield and grain protein concentration, especially in modern high-yielding cultivars, which have low protein concentrations in their grains (Chiluwal et al., 2021). The importance of N availability to soybean during reproductive growth is indicated by its high rates of N accumulation in the seed during the grain filling phase (Hungria; Mendes, 2015; Moreno et al., 2018). This greater N requirement during the reproductive phase of soybean may not be entirely met by biological N_2 fixation alone, requiring N fertilizer supplementation. Indeed, Moreno et al. (2018) showed that the application of 40 kg N ha^{-1} during pod development (R_4 stage) resulted in greater grain yield when compared to plants unfertilized with N. In a study conducted in Minnesota and Arkansas in the United States of America, Chiluwal et al. (2021) reported that late N application after the R5 stage increased grain protein concentration and soybean yield in comparison with the unfertilized control. Therefore, planning N fertilization during periods of higher N demand is essential for optimizing crop yield and decreasing the cost of N fertilizer application (Petter et al., 2012). However, the effects of late N application on soybean cultivation under Brazilian tropical conditions are still incipient and inconclusive. In addition, no study has analyzed the interrelationship of this agronomic practice with the agronomic traits of soybean using multivariate analysis methods. Canonical correlation analysis measures the existence and intensity of the association between two groups of primary and secondary agronomic characteristics (Saed-Moucheshi et al., 2013). This multivariate technique has been widely used due to the possibility of simultaneously integrating multiple information or dependent variables. In addition, this approach has helped many researchers choose the most appropriate agronomic practices to be recommended to farmers, especially in studies involving the quantification of several dependent variables.

This study evaluated the response of two soybean cultivars to late N application in supplementation to the inoculation of efficient *Bradyrhizobium* spp. strains in two Brazilian Cerrado agricultural soils under no-till conditions during the 2019/2020 growing season.

MATERIAL AND METHODS

Study site description

The field experiments were conducted in two areas of the agricultural experiment station of the Federal University of Mato Grosso do Sul, called UFMS 1 and UFMS 2, in Chapadão do Sul, MS, Brazil (18°46′18″S, 52°37′25″W with an altitude of 810 m), during the 2019/2020 growing season. The regional climate, according to the Köppen classification, is tropical with a winter dry season (Aw). The mean annual temperature is 24.0 °C, with a minimum of 18.8 °C (July) and a maximum of 29.0 °C (January). The mean annual rainfall is 1,850 mm. The climatic conditions during the soybean growing season included a mean air temperature of 27.1 °C (± 2.1 °C), mean relative humidity of 76% (± 8%), and total rainfall of 1,260 mm.

The soils of the agricultural experimental stations were classified as Latossolo Vermelho – LV (Santos et al., 2018) or Rhodic Hapludox (Soil Survey, 2014). The occurrence of Rhodic Hapludox in the northeast region of Mato Grosso do Sul state is common, and this class of soil has no restrictions with respect to agricultural use and management (Santos et al., 2018). The two agricultural experimental areas were cultivated under no-till conditions for two years with soybean/maize rotations in the 2017/2018 and 2018/2019 growing seasons. Before starting the experiments, soil samples were collected from the 0–0.20 m layer using a hole auger at five points per plot. The results of the soil chemical analysis did not show significant variability between the plots with respect to the soil chemical properties of the experimental area. The chemical analysis showed that the soils have medium and high fertility levels and medium and low acidity levels with pH values of 5.3 and 5.7, which allowed adequate availability of nutrients to plants and had no negative impact on the biological N_2 fixation process. According to Bakari et al. (2020), a soil pH lower than 5.0 limits soybean nodulation due to the toxic effects of Al and Fe ions, causing poor nodule formation and functioning. The average values of the soil chemical analysis are shown in Table 1.

Experimental design and treatments

The experiments were arranged in a completely randomized block design in a 2×2×5 factorial scheme, with four replicates. Treatments consisted of two agricultural production environments [UFMS 1 (medium-fertility soil) and UFMS 2 (high-fertility soil)]; two soybean cultivars (TMG 7067 IPRO – semideterminate growth type, a cycle of 110-115 days, maturation Group 7.2; and BMX Bônus IPRO – indeterminate growth type, a cycle of 115-120 days, maturityGroup 7.9); and five late N application rates (0, 50, 100, 150 and 200 kg N ha^{-1}). Nitrogen fertilizer topdressing was carried out at the R5.3 stage when the soybean pods had 25 to 50% grain filling. Urea (45% N) was used as the N source. The experimental units consisted of seven 5.0-m long rows, with 0.45 m between rows. The useful area comprised the three central rows of each plot, disregarding 0.5 m of each edge.

Experiment implementation and management

Soybeans were mechanically sown on October 17th, 2019, at a depth of 3.0 cm in Rows 0.45 m apart at a density of 13 seeds per meter to reach a final stand of 260,000 to 280,000 plants per hectare. The base fertilization consisted of 78 kg P_2O_5 ha^{-1} and 16 kg N ha^{-1} [monoammonium phosphate (MAP)] at the sowing furrow. Soybean fertilization was carried out according to the crop requirements (Embrapa, 2020) and the soil analysis (Table 1). At 30 days after sowing [V_4 stage - four fully expanded leaves (fourth trifoliolate)], 80 kg K_2O ha^{-1} (KCl) was applied in topdressing. At 42 days after sowing [R_1 stage – plants exhibiting at least one flower on any node (beginning flowering)], foliar fertilization was applied using 1 L ha^{-1} Actilase ZM® (5.0% Zn, 4.2% S, and 3.0% Mn) and 120 mL ha^{-1} of Racine® (11% Mo, 1.1% Co and 12.4% of organic carbon).

All soybean seeds used in the experiments were previously treated with pyraclostrobin + methyl thiophanate + fipronil (Standak Top®) at a rate of 2 mL c.p. kg^{-1} of seed and then inoculated with efficient *Bradyrhizobium* spp. strains. The commercial liquid inoculant Simbiose Nod Soja® (Simbiose: Biological Agrotechnology), containing the *Bradyrhizobium japonicum* strains [CPAC-15 (SEMIA 5079)] and *Bradyrhizobium diazoefficiens* strains [CPAC-7 (SEMIA

Table 1: Soil chemical properties of the two agricultural experimental areas.

Agricultural area	pH	OM	$P_{Mehlich-1}$	H+Al	Al^{3+}	Ca^{2+}	Mg^{2+}	K^+	CEC	V
	$CaCl_2$	g dm^{-3}	mg dm^{-3}	------------------- cmol$_c$dm^{-3} ---------------------						%
UFMS 1	5.3	27.3	14.1	3.70	0.20	2.70	0.80	0.30	7.50	51
UFMS 2	5.7	35.3	41.6	3.90	0.05	4.20	1.60	0.45	10.15	62

OM: Organic matter. CEC: cation exchange capacity at pH 7.0. V: Soil base saturation.

5080)] (minimum concentration of 7.2×10^9 viable cells per mL) was used at a rate of 3.0 mL kg^{-1} seeds as recommended by the manufacturer. The amount of inoculant used was added to a solution containing 2.0 mL kg^{-1} of additive for inoculant Protege® TS (Total Biotechnology), and then both products (inoculant + additive) were applied to the seeds. The inoculant additive consists of active metabolites of bacteria, sugar complexes and encapsulating biopolymers and accomplishes the purpose of improving the protection and viability of the bacteria on the seeds. The seeds were also treated with micronutrients, especially molybdenum, to potentiate soybean nodulation. The commercial seed fertilizer Nódulus® Premium 125 (Biosoja), containing 10% Mo, 1% Co, 1% S, 1% Ca, and 0.2% Fe, was used. In this study, all treatments were inoculated with elite strains of *Bradyrhizobium* spp., since this is a well-established and effective practice for soybean production in tropical soils (Embrapa, 2020).

During plant development, the management of weeds, pests, and diseases was carried out according to the requirements and technical recommendations of the soybean crop (Embrapa, 2020). The following products were used: glyphosate, haloxyfop-p-methyl, pyraclostrobin + epoxiconazole, picoxystrobin + benzovindiflupir, mancozeb, azoxystrobin + cyproconazole, teflubenzuron, chlorpyrifos, and imidacloprid + β-cyfluthrin. The application of these products was carried out according to the recommendations for the registered commercial products.

Measurement of agronomic traits and grain yield

At full maturity (R_8 stage), the plant height, first pod height, grain yield, grain protein concentration, and production components (number of pods per plant, number of grains per pod, and 1,000-grain mass) were measured. The plant and first pod height were determined from the soil surface to the last leaf or first pod insertion, respectively, using a millimeter ruler. Grain yield was determined at the end of the crop cycle from a central portion of each plot of 1.35 m × 4.00 m (4.05 m^2). Grains were cleaned and weighed, and the grain yield was estimated after correcting grain weights to 13% moisture. The average number of pods per plant and the average number of grains per pod were determined in a random sample of five plants in the useful area of the plot, and the mass of 1,000 grains was determined by averaging four measurements of 100 grains taken at random. The grain protein concentration was determined using the semimicro Kjeldahl method as reported by Detmann, Queiroz and Cabral (2012).

Statistical Analysis

The data were previously submitted to the statistical hypothesis verification tests of homoscedasticity of variances (Levene test; $p > 0.05$) and normality of residues (Shapiro–Wilk test; $p > 0.05$). Then, the data were subjected to joint analysis of variance according to the model and statistical procedure presented by Ramalho et al. (2012). The means of the qualitative factors (agricultural production environments or soybean cultivars) were compared by the Fisher-Snedecor F test at the 0.05 level of confidence. Regression analysis was used for the N fertilizer application rates (quantitative factor), and significance equations (F test, $p \leq 0.05$) were adjusted according to the highest coefficients of determination. These analyses were performed using Sisvar® software, version 5.6 for Windows (Statistical Analysis Software, UFLA, Lavras, MG, BRA).

Canonical correlation analysis (CCA) was used to study the interrelationships between sets (vectors) of independent (agricultural production environments, soybean cultivars, and N application rates) and dependent (soybean agronomic traits) variables. This analysis was performed using RBio software version 140 for Windows (RBio Software, UFV, Viçosa, MG, BRA) (Bhering, 2017).

RESULTS AND DISCUSSION

Analysis of variance indicated that the effect of the agricultural production environment was significant with respect to plant height, first pod height, number of pods per plant, and 1,000-grain mass, while the effect of cultivars was significant to all soybean agronomic traits, except for grain protein concentration. The impact of N application rates was not significant ($p > 0.05$) for any of the soybean agronomic traits (Table 2). The interaction between the production environment and soybean cultivar showed a significant effect on the 1,000-grain mass, grain yield, and grain protein concentration. The triple interaction between production environment, cultivar, and N application rates and the interaction between production environment and N application rates or the interaction between cultivar and N application rates did not show significant effects ($p> 0.05$) for any of the soybean agronomic traits (Table 2). A significant interaction between the production environment and soybean cultivar was expected due to the production environment interfering with the development of soybean plants. Silva et al. (2015) also reported a significant effect of production environments on soybean yield potential due to genotype and environment interaction (G × E). On the other hand, the nonsignificant interaction between environments, cultivar, and nitrogen rates on soybean agronomic traits was also reported by Chiluwal et al. (2021).

The plant height and first pod height were significantly higher in the medium-fertility production environment (UFMS 1), whereas a greater number of pods per plant was obtained in the high-fertility production environment (UFMS 2) (Table 3). These results confirm those reported by Chiluwal et al. (2021), which showed that agricultural production environments interfere with plant development and soybean production components due to differences in the chemical properties of soil. In this study, the soil with the higher nutrient availability and higher organic matter content (UFMS 2) resulted in the lowest plant height and highest number of pods per plant, but did not affect the grain yield of the crop.

The plant height, first pod height, and number of pods per plant were significantly higher in the cultivar BMX Bônus IPRO, while the highest number of grains per pod, 1,000-grain mass, and grain yield were obtained for cultivar TMG 7067 IPRO (Table 3 and Figure 1). The 1000-grain mass and grain yield of the cultivar BMX Bônus IPRO were significantly higher in the high-fertility production environment (UFMS 2), whereas the 1000-grain mass and grain yield of the soybean cultivar TMG 7067 IPRO were not changed by agricultural production environments (Figure 1A and 1B). The grain protein concentration of cultivar TMG 7067 IPRO was significantly higher in the medium-fertility production environment (UFMS 1). In the BMX Bônus IPRO cultivar, the grain protein concentration was higher in the high-fertility production environment (UFMS 2) (Figure 1C).

Table 2: Summary of analysis of variance for the measurements of grain yield and agronomic traits of soybean crops for the effects of the agricultural production environments, cultivars, and late N application rates during the 2019/2020 growing season in Chapadão do Sul, MS, Brazil.

Sources of variation	Probability > F						
	PH	FPH	NPP	NGP	1.000-G	GY	CP
Environmental (E)	<ins><0.01</ins>	<ins>0.047</ins>	<ins>0.028</ins>	0.281	0.067	0.070	0.219
Cultivar (C)	<ins><0.01</ins>	<ins><0.01</ins>	<ins><0.01</ins>	<ins><0.01</ins>	<ins><0.01</ins>	<ins><0.01</ins>	0.376
Nitrogen rates (N)	0.238	0.945	0.191	0.237	0.753	0.091	0.093
E × C	0.336	0.078	0.254	0.347	0.011	<ins><0.01</ins>	<ins><0.01</ins>
E × N	0.583	0.118	0.725	0.122	0.455	0.158	0.115
C × N	0.874	0.140	0.773	0.439	0.134	0.218	0.856
E × C × N	0.516	0.730	0.524	0.476	0.071	0.668	0.741
CV (%)	4.54	10.00	14.31	9.85	12.33	14.34	10.82

PH: plant height. FPH: first pod height. NPP: number of pods per plant. NGP: number of grains per pod. 1,000-G: 1,000-grain mass. GY: grain yield. CP: grain crude protein concentration. CV: coefficient of variation.

Table 3: Effects of agricultural production environments and cultivars on some of the agronomic traits of soybean plants grown during the 2019/2020 season in Chapadão do Sul, MS, Brazil.

Treatments	Plant height (cm)	First pod height (cm)	Pods per plant (N°)	Grains per pod (N°)
UFMS 1	105.8 a	19.8 a	41.1 b	2.27 a
UFMS 2	96.3 b	18.8 b	44.7 a	2.21 a
TMG 7067 IPRO	90.5 b	17.7 b	34.7 b	2.42 a
BMX Bônus IPRO	111.6 a	20.8 a	51.0 a	2.06 b

Means followed by distinct letters in the columns for the agricultural production environment or soybean cultivar show significant differences by the F test ($p \leq 0.05$).

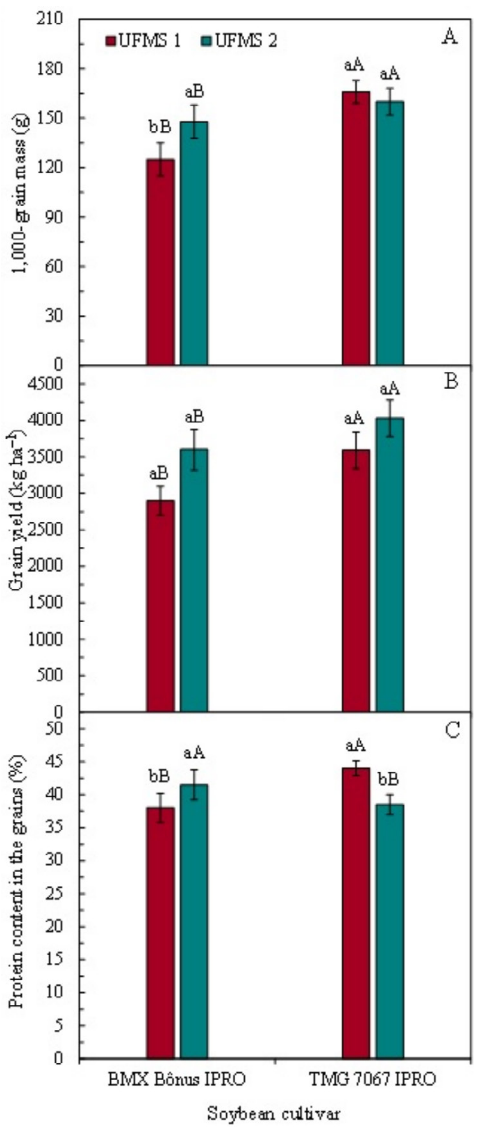

Figure 1: Effects of agricultural production environments on 1000-grain mass (A), grain yield (B), and grain crude protein concentration (C) of the two soybean cultivars grown in Chapadão do Sul, MS, Brazil, during the 2019/2020 growing season. Bars followed by the same lowercase letters, representing the production environments, or the same uppercase letters, for the soybean cultivars, are not significantly different by F test at the 0.05 level of confidence. Data refer to mean values (n = 4) ± standard error.

The changes in the 1000-grain mass, grain yield, and grain protein concentration of soybean cultivars are dependent on the agricultural production environment, which indicates that soybean cultivars exhibit different responses in each production environment. The G × An interaction is frequently reported in the literature for other self-pollinating crops (Azevedo et al., 2020) and represents an essential factor in the soybean crop (Silva et al., 2015).

The medium-fertility production environment (UFMS 1) resulted in an increase in plant height, first pod height, and number of grains per pod, while the high-fertility environment (UFMS 2) resulted in an increase in the number of pods per plant, 1,000-grain mass, grain yield and grain protein concentration (Figure 2). These different responses of soybean cultivars are related to the soil chemical properties in the production environments, especially the levels of P, Ca, Mg, and organic matter in the UFMS 2 production environment, which are superior when compared to the UFMS 1 production environment. The soil chemical characteristics enhanced traits related to the production components and the grain quality of soybeans.

Macronutrients such as Ca, Mg, and P directly affect the photosynthetic process of plants (Bang et al., 2021). Therefore, in the high-fertility production environment (UFMS 2), the highest levels of these nutrients in the soil increased the photosynthetic rate of soybean plants, resulting in greater production of photoassimilates for pod binding (number of pods) and grain filling (1000-grain mass), as well as in the higher yield and quality of soybeans (Figure 2).

Among soybean genotypes, the cultivar BMX Bonus IPRO resulted in an increase in plant height, first pod height, number of pods per plant, and grain protein concentration, while cultivar TMG 7067 IPRO increased the number of grains per pod, 1000-grain mass, and grain yield (Figure 3). Investigating the effect of N fertilization on other soybean cultivars, Zuffo et al. (2018) also reported different responses of soybean genotypes to production components (number of pods per plant, number of grains per pod, 1,000-grain mass, and grain yield). These results are related to the fact that the agronomic characteristics of soybean cultivars are controlled mainly by genetic factors and interactions with the production environment. The two cultivars used in this study have different growth and development potentials. These results were expected due to the genetic differences in the cultivars, growth habits, and maturation groups (Soares et al., 2015; Chiluwal et al., 2021), among other characteristics.

The late N application rates did not significantly affect ($p > 0.05$) the agronomic traits, grain protein concentration, or soybean yield (Table 4). Similar results have been reported by Kaschuk et al. (2016), Korber et al. (2017), and Zuffo et al. (2021), who showed that N fertilization had no significant effect on the agronomic traits of soybeans.

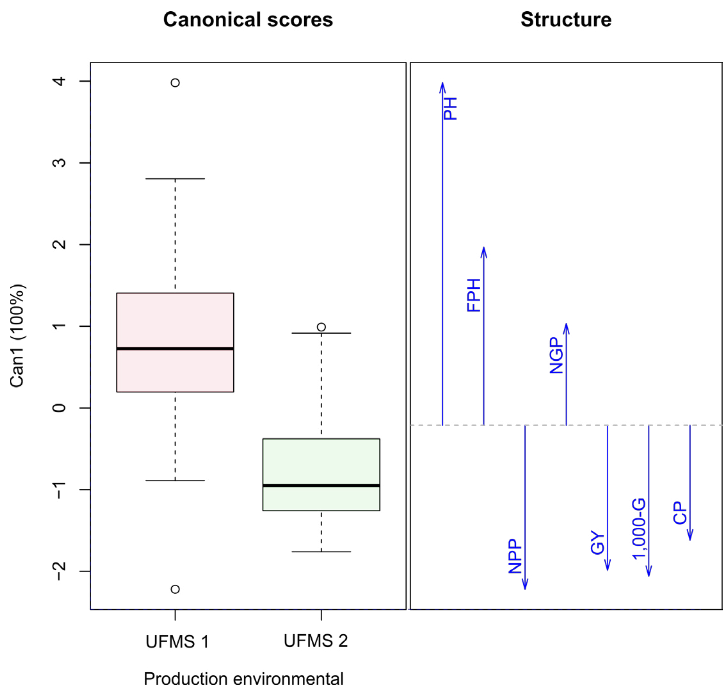

Figure 2: Canonical correlations between the agronomic traits and agricultural production environments.
Abbreviations: PH: plant height. FPH: first pod height. NPP: number of pods per plant. NGP: number of grains per pod. 1,000-G: 1,000-grain mass. GY: grain yield. CP: grain crude protein concentration.

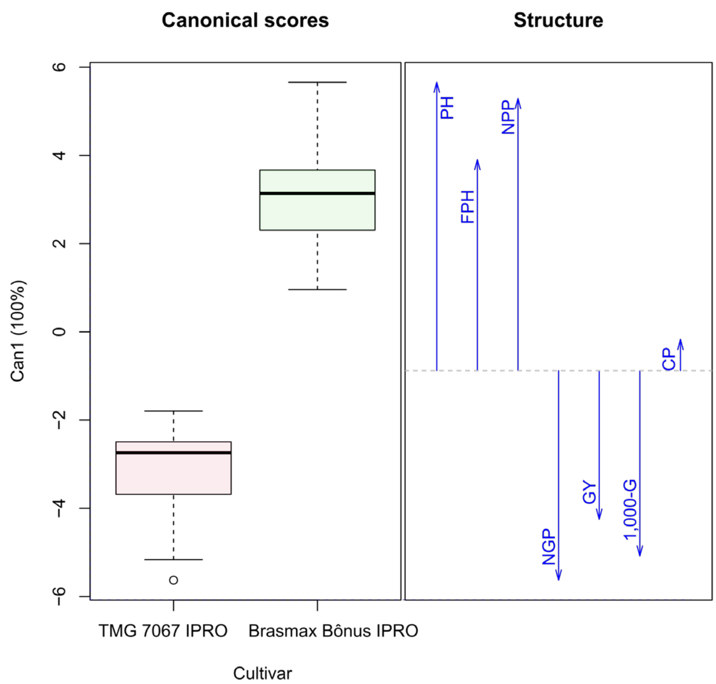

Figure 3. Canonical correlations between the agronomic traits and soybean cultivars.
Abbreviations: PH: plant height. FPH: first pod height. NPP: number of pods per plant. NGP: number of grains per pod. 1,000-G: 1,000-grain mass. GY: grain yield. CP: grain crude protein concentration.

Canonical correlation analysis was used to verify the contribution of each dependent variable measured in the soybean plants as affected by late N application rates (Figure 4). For scores to be represented in a two-dimensional graph, the percentage of retained variance must be higher than 80% (Saed-Moucheshi et al., 2013). In this study, variances accumulated in the two main canonical variables were 58.0 and 83.4%, respectively, for each graph (Figure 4), allowing an accurate interpretation. Late N application rates did not optimize the grain protein concentration or yield of soybeans (Figure 4). Some research has also shown that N fertilization has not improved the productive performance or the grain protein concentration of soybeans (Aratani et al., 2008; Fipke et al., 2016; Kaschuk et al., 2016; Korber et al., 2017; Zuffo et al., 2018, Zuffo et al., 2021).

Table 4: Effects of the late N application rates on some of the agronomic traits of soybean crops grown during the 2019/2020 season in Chapadão do Sul, MS, Brazil.

N application rates (kg ha^{-1})	PH (cm)	FPH (cm)	NPP	NGP	1,000-G (g)	GY (kg ha^{-1})	CP (%)
0	102.2	19.2	41.6	2.21	153	3.604	37
50	99.4	19.2	42.6	2.37	152	3.739	41
100	101.7	18.9	42.6	2.20	148	3.727	42
150	102.6	19.4	46.7	2.26	150	3.240	41
200	99.2	19.5	41.0	2.17	144	3.411	41
Linear regression	ns	ns	ns	ns	ns	ns	ns
Quadratic regression	ns	ns	ns	ns	ns	ns	ns

ns: not significant at 5% by the t test. PH: plant height. FPH: first pod height. NPP: number of pods per plant. NGP: number of grains per pod. 1,000-G: 1,000-grain mass. GY: grain yield. CP: grain crude protein concentration.

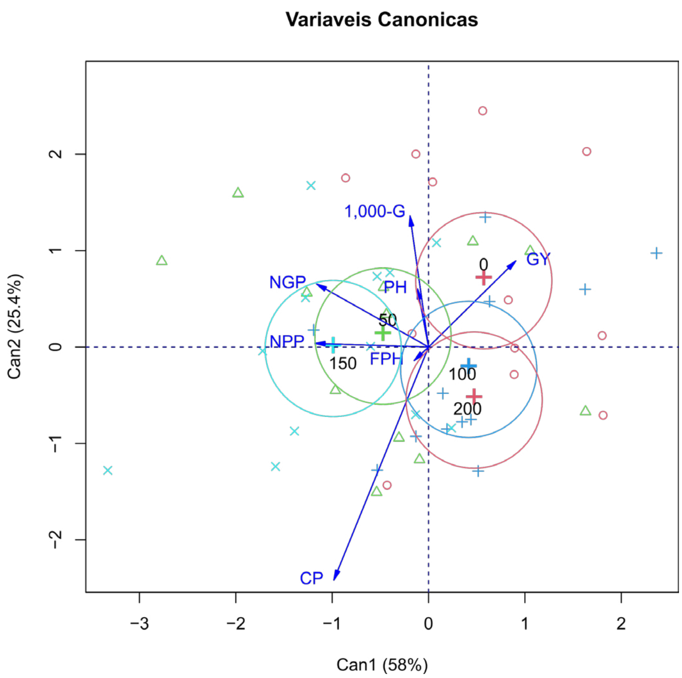

Figure 4: Canonical correlation analysis (CCA) between soybean agronomic traits and N fertilizer application rates. The blue lines show the canonical correlation between the centroids of the first pair of canonical variates and the linear tendency line.
Abbreviations: PH: plant height. FPH: first pod height. NPP: number of pods per plant. NGP: number of grains per pod. 1,000-G: 1,000-grain mass. GY: grain yield. CP: grain crude protein concentration.

The lack of soybean response to late N application may be related to the appropriate supply of this nutrient to plants, caused by the high rate of organic matter mineralization in tropical soils and the high efficiency of the symbiotic N fixation process by rhizobia. Therefore, our results show the importance of inoculating efficient *Bradyrhizobium* spp. strains for the appropriate supply of N for the soybean crop. In addition, the inoculation of *Bradyrhizobium* spp. has been the primary source of N used by Brazilian producers in soybean cropping since it is an efficient practice with a low economic cost compared to the use of mineral N fertilizer.

In general, the average grain yield of soybeans (Table 4) was slightly lower than the average yield observed for the state of Mato Grosso do Sul during the 2019/2020 growing season, which was 3767 kg ha^{-1} (Companhia Nacional de Abastecimento - CONAB, 2020). In turn, the average grain protein concentration in soybeans varies from 37 to 42%, and these levels are lower than the standard protein levels required for the international commercialization of soybeans (46 to 47%) (Assefa et al., 2019).

CONCLUSIONS

Late nitrogen applications had no effect on agronomic traits and did not increase the grain protein concentration or yield of the soybean cultivars, regardless of the fertility level of the agricultural area. Therefore, when efficient *Bradyrhizobium* spp. strains are used in soybean cropping in medium- and high-fertility Cerrado soils, there is no need to apply late nitrogen fertilizer rates.

AUTHOR CONTRIBUTION

Conceptual idea: Zuffo, A.M., Methodology design: Zuffo, A.M., Ratke, R.F., Data collection: Zuffo, A.M., Ratke, R.F., Aguilera, J.G., Data analysis and interpretation: Zuffo, A.M., Ratke, R.F., Aguilera, J.G., Steiner, F. and Writing and editing: Zuffo, A.M., Ratke, R.F., Aguilera, J.G., Steiner, F.

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