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Nitrogen supplementation improves yield and quality of soybean seeds

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ABSTRACT. Nitrogen use in soybeans is still not fully taken advantage of. Increased use of nitrogen fertilizer couldnot only increase agricultural productivity, but also strengthen food security in the context of the increasing global need for food. The objective of this study was to evaluate the performance and seed quality of soybeans supplemented with nitrogen. The research was carried out in the municipality of Los Cedrales, Alto Paraná, Paraguay. The trial was conducted in an experimental design of randomized blocks, organized in factorial scheme 2 (genotypes) x 4 (fertilization management). The evaluated genotypes were cultivar DM 5958 RSF Ipro and cultivar 6410 Ipro, both registered, protected and indicated for cultivation in the southern region of Brazil and in Paraguay. Nitrogen fertilization treatments were defined considering the ease of application of doses at soybean development stages. In 10 plants per experimental unit, the following variables were determined: plant height, number of nodes, pods and seeds per plant, and the weight of one thousand seeds. The grain yield (GY, kg ha^{-1}) was determined by harvesting the useful area of each experimental unit, corrected to 13% humidity. The physiological quality of seeds was evaluated through the following variables: germination and vigor of seeds and emergence of seedlings in beds. Nitrogen supplementation significantly increased the number of nodes, the weight of a thousand seeds and the number of soybean pods. Compared to no N application (0R2/0R5), treatments with application of 120 kg ha^{1} of N in R2 (120R2/0R5); and 60 kg ha^{1} of N in R2 and R5 (60R2/60R5) of soybeans, caused increases in seed productivity of 7 and 8%, and in the physiological quality of seeds with increases of up to 7 and 5% in vigor and seedling emergence, respectively. Based on soil and climate conditions, genotypes, management, and analyses performed, the split application of 120 kg ha^{-1} of N between stages R2 and R5 resulted in higher productivity and greater physiological quality of the soybean seeds.

Key words: *Glycine max*; Grain yield; Nitrogen; Plant nutrition; Seed vigor

INTRODUCTION

In recent decades, the production of agricultural commodities, especially soybean, corn and wheat are among the economic activities with the greatest increases in Brazil and Paraguay. The agribusiness chain has an important role in the generation of wealth in these countries, and in 2019 it represented 21.4 and 28.9% of the composition of the gross domestic production for Brazil (CEPEA, 2019) and Paraguay (BCP, 2020), respectively. Due to this, the production chains are well structured, and increasing their production in a sustainable way, through increases in productivity, is of fundamental importance for the advancement and economic and social development of these countries.

The productivity of a crop is determined by several factors; among them, the correct fulfillment of the demand for mineral nutrients stands out (Treter et al., 2022). In this sense, special emphasis goes to nitrogen, being the nutrient required in greater amounts by plants, since it is part of the constitution of chlorophyll, amino acids, proteins, and nucleic acids. Thus, the even greater importance of this element is evident for the soybean culture, whose main biochemical trait is the high concentration of proteins in the seeds (Wang et al., 2021). Loro et al. (2021) identified greater nitrogen content in soybean grains than any other nutrients, evidencing the importance of its availability for the synthesis of amino acids and proteins in this crop.

Proteins participate directly in specific metabolic functions, acting directly on enzymatic activities for the solubilization and transport of reserves to the embryonic axis during the germination process. Therefore, the availability of nitrogen to the plant can interfere with the concentration of proteins in the seeds and affect their physiological quality, since high vigor seeds have greater contents of soluble proteins and sugars, reflecting a greater and faster remobilization of these reserves, resulting in better germination and initial performance of the seedlings (Loro et al., 2021).

Several studies have evaluated the effects of nitrogen fertilization, both base and in coverage, and of biological nitrogen fixation (BNF) in the soybean culture (Hardarson et al., 1984; Barbosa et al., 2021; Chiluwal et al., 2021; Ferreira et al., 2022). For some researchers, the nitrogen demand is completely met by BNF, and no significant yield gains are observed with the use of nitrogen fertilizers, which, in some situations, are even negative to the BNF process and negatively affect grain yield, in addition to burdening production costs (Barbosa et al., 2021). On the other hand, some more recent studies carried out with more modern genotypes have demonstrated positive effects of nitrogen fertilization in combination with BNF, with compensatory productivity gains in the soybean culture (Chiluwal et al., 2021). These contrasting results may be explained, in part, by differences between N sources and doses used within the soybean development stages, associated with a diversity of factors, such as genotype response, BNF efficiency, nutrient mobility, soil type and management, and climatic factors of environments (Peter et al., 2012).

In the grain production system, farmers seek to raise crop productivity, while in the seed production system, in addition to productivity, it is necessary to ensure the supply of seeds with high germination rates and vigor, and both systems are based on the balance of the economic viability. Thus, when seeking higher and higher increments in the levels of productivity and quality, there is a demand for updated research that seeks to understand these relationships, being conducted with different genotypes in different production environments (Loro et al., 2023). Thus, the objective of this study was to evaluate the morphological and productive performance and seed quality of soybeans supplemented with nitrogen.

MATERIAL AND METHODS

The work was carried out in the municipality of Los Cedrales, Alto Paraná, Paraguay, with the geographical coordinates of 25°39'07.2"s and 54°43'00.6"W, and 230m altitude. The soil of the site has a clay texture, being classified as Oxysol according to the American classification (Soil Taxonomy) adopted in Paraguay. The current Brazilian soil classification places it in the eutroferric red latsol class. The predominant climate in this region is classified humid subtropical climate (mesothermal). Rain occurs most often in spring / summer, with no defined dry season. The average annual rainfall is 1,500 mm, and the average monthly temperature ranges from 17 to 27°C.

The experimental design used was randomized complete blocks organized in factorial scheme 2 x 4, being two genotypes and four types of nitrogen supplementation, totaling eight treatments with four replications. The evaluated genotypes were cultivar DM 5958 RSF Ipro and cultivar 6410 Ipro, both registered, protected and indicated for cultivation in the Southern Region of Brazil and in Paraguay. The types of nitrogen supplementation in coverage studied were: 0.0 kg N ha^{-1} (0R2/0R5), 120 kg N ha^{-1} applied in the R2 stage (120R2/0R5), 120 kg N ha^{-1} in the R5 stage (0R2/120R5) and 60 kg N ha^{-1} in R2 + 60 kg N ha^{-1} in R5 (60R2/60R5). Nitrogen fertilization treatments were defined considering the ease of application of doses in the soybean development stages.

The trial was installed in a site with a direct planting system consolidated over twenty years of cultivation, using, as soil cover, the straw of black oat (*Avena strigosa*) previously dried 40 days before sowing. The sowing took place on September 25 within the recommended time for the genotypes, being carried out mechanically, with adequate soil moisture, and with 3 cm depth of seed deposition.

The base chemical fertilization occurred together with sowing using 250 kg ha^{-1} of formulated fertilizer N:P2O5:K2O in the ratio of 04-24-08, totaling 10-60-20 kg ha^{-1} of N:P2O5:K2O, respectively, according to the need indicated by the research and based on the interpretation of the chemical analysis of the soil. In order to supplement the potassium nutrient, a cover application was performed with the use of the potassium chloride-based fertilizer (60% K2O), at a dose of 80 kg ha^{-1} in all experimental plots. The application of mineral nitrogen in coverage was carried out manually, with the urea-based solid fertilizer (Super N® trademark) containing 45% N.

The seeds were treated with fungicide based on pyraclostrobin (25 g L^{-1}) and methyl thiophanate (225 g L^{-1}), and insecticide based on fipronil (250 g L^{-1}) at a dose of 2.0 mL per 100 kg-1 of seeds of commercial product Standak Top®; as a source of nutrients, commercial product Fertiactyl® (0.5% cobalt and 2.7% molybdenum) was used at a dose of 2.0 mL kg seeds-1. The seeds were also inoculated with bacteria *Bradyrhizobium japonicum*, strains SEMIA 5079 and SEMIA 5080 at a concentration of 7.0,109 UFC mL^{-1}; at a dose of 2.5 mL of commercial product Rizoliq LLI® per kg^{-1} of seeds. This operation was performed manually just before sowing, with the use of plastic bags in order to standardize the distribution of the products.

Twenty seeds were distributed per linear meter and at 30 days after sowing the plots underwent manual thinning with the aid of a graduated ruler, unifying the between the plants and thus obtaining a population of 280 thousand plants ha^{-1} in all the plots. The thinning criterion was the location of the plant within the sowing line, regardless of its size, and the established population is the one that breeders recommend for these genotypes, within the growing season adopted in the trial. The culture treatments followed the technical recommendations for soybean production in the region, keeping the plants protected against the attack of pests and diseases that could interfere with the good development of the crop. Each experimental unit was formed by seven sowing lines 5.0 m long, 0.45 m apart. In order to avoid a border effect, the useful area of the plot was composed of five central lines, discarding 0.50 m from its ends, totaling 9.0 m^2 per experimental unit.

In 10 plants per experimental unit, the following variables were determined: plant height at reproductive stages R2, R5, and R9 (AR2, AR5, and AR9, cm), number of productive nodes (NPN, unit), number of pods per plant (NLP), number of seeds per plant (NSP) and mass of one thousand seeds (MTS, g). The grain yield (GY, kg ha^{-1}) was determined by harvesting the useful area of each experimental unit, corrected to 13% humidity. The germination test (G, %), first germination count (FC, unit), seedling classification (VSC), viability of seeds (VIA), vigor by tetrazolium (TZ), accelerated aging (AA, %), emergence test in beds (EB, %), oil content (OL, %) and protein (PT, %) in the seeds.

The data obtained were analyzed for the normality of errors by the Shapiro Wilk test and the homogeneity of residual variances by the Bartlett test; having met the assumptions, the analysis of variance at 5% significance by the F test was performed. When significant, the means of the treatments were compared by the Tukey test at 5% significance. In order to understand the relationships between the variables, the linear correlation analysis was performed at 5% significance by the Pearson t-test. In addition, the principal component analysis was used for the relationships between nitrogen fertilization management and the evaluated response variables that were represented by a Biplot graph. Subsequently, the MGIDI selection index (Olivoto and Nardino 2021) was used to select the most efficient treatments. All analyses were performed using R software.

RESULTS AND DISCUSSION

The variance analysis revealed a significant interaction between genotypes and nitrogen management for oil and protein content in seeds (Table 1). For the nitrogen management factor, at the level of 5% probability, a significant effect was observed on variables plant height in the R9 stage, number of productive nodes, number of pods per plant, mass of one thousand seeds, grain yield, germination, vigor by the test of first germination count, vigor by the test of seedling classification, viability, vigor by the tetrazolium test and emergence of seedlings in beds. For the main effect of the genotypes, a significant response was observed on plant height in stages R2, R5 and R9, number of nodes per plant, number of pods per plant, mass of one thousand seeds, classification of seedlings and emergence in beds, demonstrating different effects from those observed for management. The coefficients of variation presented an amplitude from 0.93 to 10.13%, being mostly considered low (CV < 10%), according to the classification, demonstrating high precision of the experimental results.

Table 1. Significance of the analysis of variance for the effects of soybean genotypes (Gen) and nitrogen management (Man) on variables plant height in stages R2, R5 and R9 (AR2, AR5 and AR9), number of productive nodes (NPN), number of pods (NLP) and number of seeds per plant (NSP), mass of one thousand seeds (MTS), grain yield (GY), oil content (OL), germination (G), first germination count (FC), seedling classification (VSC), accelerated aging (AA), viability (VIA), vigor by tetrazolium test (TZ) and emergence in beds (EB) and protein (PT). Los Cedrales, Paraguay, 2021.

SV	**DF**	**AR2**	**AR5**	**AR9**	**NPN**	**NLP**	**NSP**	**MTS**	**GY**	**OL**
						Pr>Fc				
Block	3	0.47	0.58	0.34	0.31	0.95	0.46	0.39	0.21	0.01
Man.	3	0.14	0.1	0.01	0.01	0.01	0.2	0.01	0.01	0.01
Gen.	1	0.01	0.01	0.01	0.01	0.01	0.59	0.01	0.27	0.01
N Man. X Gen.	3	0.9	0.99	0.35	0.83	0.07	0.76	0.05	0.3	0.01
Residue	21									
Total	31									
CV (%)		4.85	4.29	3.66	5.83	7.01	10.13	1.16	4.07	0.96
SV	**DF**	**G**	**FC**	**VSC**	**AA**	**VIA**	**TZ**	**EB**	**PT**	
						Pr>Fc				
Block	3	0.11	0.16	0.23	0.82	0.22	0.46	0.95	0.04	
N Man.	3	0.01	0.01	0.01	0.33	0.01	0.01	0.01	0.01	
Gen.	1	0.69	0.17	0.04	0.18	0.11	0.77	0.03	0.04	
Man. X Gen.	3	0.36	0.7	0.11	0.38	0.12	0.52	0.97	0.01	
Residue	21									
Total	31									
CV (%)		2.35	3.24	3.76	1.67	1.44	2.7	2.14	0.93	

*significant at 5% probability by the F test. SV: sources of variation; DF: degrees of freedom. N Man: nitrogen management. Gen: genotype. CV (%): coefficient of variation.

For genotype DM 5958 RSF IPRO (Table 2), the highest potential of protein content in seeds was observed when subjected to split nitrogen management, of 60 kg ha^{-1} in the R2 stage and 60 kg ha^{-1} in the R5 stage. A similar result is evident for genotype 6410 Ipro, where the protein content was maximized in the split management of D60R260R5 and in the single application management of D0R2120R5 in the R5 stage. With this, it can be inferred that higher doses of nitrogen near the stage of the beginning of seed filling can enhance protein contents. As for management, it is evident that the response of the D0R20R5 and D60R260R5 treatments was equal in both genotypes used. When using the management with a greater application of nitrogen in the R2 stage, it is noticed that genotype DM 5958 RSF Ipro promoted the greatest increase of protein in the seeds. The opposite occurred when the largest fraction of nitrogen was used in the R5 stage in which the 6410 Ipro genotype showed superiority in protein contents. The variability in the response of genotypes to nitrogen applications may be due to genetic capacity. Hardarson et al. (1984) revealed that soybean cultivars differed in their ability to fix N at different levels of inorganic N.

Table 2. Dismemberment of the simple effects of the interaction between genotypes and nitrogen management. Los Cedrales, Paraguay, 2021.

Genotypes	Nitrogen Management			
	D0R20R5	**D120R20R5**	**D0R2120R5**	**D60R260R5**
		Protein content		
DM 5958	43.77 aB	44.25 aB	44.57 bB	45.83 aA
6410 Ipro	43.72 aB	42.93 bB	45.23 aA	45.26 aA
		Oil content		
DM 5958	19.11 aC	19.75 bA	19.56 aAB	19.25 aBC
6410 Ipro	19.13 aB	20.47 aA	18.33 bC	18.98 bB

*Means followed by the same lowercase letter in the column and uppercase in the row do not differ from each other by Tukey's test at 5% probability.

Genotypes and managements that enhanced the protein contents in the grains tend to present lower oil indices. Thus, it is possible to reveal an increase in the oil contents of the DM 5958 RSF iPro genotype when subjected to the nitrogen management with a greater application in the R2 stage, where 120 kg of N ha^{-1} was applied, not statistically differing from the management with the application of 120 kg of N in R5. A similar result is observed for genotype 6410 Ipro, however, the highest expression of oil content occurred when the application of 120 kg of N was carried out in the R2 stage. Thus, it can be inferred that the use of nitrogen in flowering stages promotes a greater expression of oil contents in soybean seeds. As for management, there is an inversion of the treatments that enhance the oil contents if compared to those of protein; this conforms the tendency in the expression of these components. When using the management with a greater application of nitrogen in the R2 stage, it is noticed that genotype 6410 Ipro promoted the greatest increase of protein in the seeds. The opposite occurred when the largest fraction of nitrogen was used in the R5 stage in which genotype DM 5958 RSF Ipro presented superiority in protein contents. However, just like for proteins and oil, the response of the D0R20R5 and D60R260R5 treatments was the same in both genotypes used.

Plant height demonstrated a significant response to the different nitrogen fertilization methods only at the harvest point (AR9). In this stage, the split nitrogen fertilization (60R2/60R5) resulted in significantly higher height, of 7%, when compared to the treatment without N in coverage (0R2/0R5) (Table 3). The height of soybean plants demonstrates the ability of plants to produce more nodes and, consequently, a greater number of flowers, and potentially, of pods and seeds (Basal and Szabó 2018). According to Zimmer (2012), the application of N after soybean flowering may not have effects on the morphological traits of plants since it will normally be channeled directly to seed formation and grain filling. Bagateli et al. (2022) reported that the application of N to wheat did not change the physiological quality of the seeds, but it promoted an 11% increase in grain yield. Ferreira et al. (2022) observed that the application of foliar nitrogen fertilizers did not influence the yield performance of soybean.

Table 3. Dismemberment of the main effects of nitrogen management. Los Cedrales-PY, 2021.

N Man.	Variables					
	AR9	**NPN**	**NLP**	**MTS**	**GY**	**G**
D0R20R5	97.6 b	13.1 b	85.2 c	151.5 c	4.507.2 b	92.1 b[1]
D120R20R5	100.9 ab	14.0 ab	92.2 bc	152.1 bc	4.338.6 b	92.7 b
D0R2120R5	102.8 ab	14.5 a	104.6 a	154.5 ab	4.821.5 a	94.4 ab
D60R260R5	104.0 a	14.8 a	98.5 ab	154.6 a	4.857.6 a	96.1 a
N Man.	**EB**	**TZ**	**FC**	**VSC**	**VIA**	
D0R20R5	92.1 b	86.9 b	85.4 b	78.6 b	94.2 c	
D120R20R5	96.6 a	90.9 a	86.5 b	82.2 ab	95 bc	
D0R2120R5	96.4 a	92.7 a	88.0 ab	83.2 a	97.1 a	
D60R260R5	96.9 a	91.5 a	91.0 a	84.4 a	96.5 ab	

*Means followed by the same lowercase letter in the column do not differ from each other by Tukey's test at 5% probability. Nitrogen managements (N Man.) Plant height at stage R9 (AR9), number of productive nodes (NPN), number of pods per plant (NLP), mass of one thousand seeds (MTS), grain yield (GY), germination (G), emergence in beds (EB), vigor by tetrazolium test (TZ), first germination count (FC), seedling classification (VSC) and viability (VIA).

For the number of nodes per plant, the different management of N resulted in a positive effect with an increase in the number of nodes compared to the treatment without N in coverage, except for the total application of N in R2 (120R2/0R5), which showed no difference. These responses may be related to the fact that N is an element involved in the synthesis of chlorophylls and protein compounds, presenting a potential to increase the ability of plants to produce reproductive buds (Zimmer, 2012). For the variables related to the yield components, the different nitrogen fertilization methods influenced all the variables analyzed, except for the number of seeds per plant. This result corroborates that one found by Pereira et al. (2018), who, when evaluating different forms and stages of application of N in the soybean culture, observed a significant effect of treatments on the number of pods per plant, number of seeds per legume, mass of one thousand seeds and seed productivity.

The highest values of number of pods per plant were obtained in the treatments with total application of N during the grain filling stage (0R2/120R5) and in the split application in R2 and R5 (60R2/60R5), which presented a higher number of pods per plant of 23 and 16%, respectively, when compared to the treatment without N in coverage (0R2/0R5). In trials conducted in two crop seasons by Silva et al. (2011), these researchers observed, in the second year of cultivation, a linear response of the number of pods per plant, with the increase of doses from 0 to 40 kg of N ha^{-1}. These results disagree with those found by Bahry et al. (2013a), who observed no effects of the application of different doses of N on soybean components yield and productivity in the reproductive stage. In this study, the total application of N in R2 showed no significant difference in the number of pods per plant, compared to the 0R2/0R5 treatment. The number of pods per plant is considered one of the main indicators of phenotypic plasticity in soybean genotypes, and therefore there is a direct relationship with the final productivity (Balbinot Junior et al., 2018). Also, in this sense, Bahry et al. (2013b) highlight that the number of pods per plant is an important yield component for soybean, being responsible for serving as a translocation channel of photoassimilates and also for protecting the grains during their formation and filling until harvest time (Zimmer, 2012).

The mass of one thousand seeds was positively influenced by the treatments with N in coverage, resulting in small increments in grain mass, especially in the 0R2/120R5 and

60R2/60R5 treatments, which differed from the treatment without N in coverage. Petter et al. (2012) found an increase in the mass of one thousand grains of different soybean cultivars with applications of 20 and 40 kg of N ha^{-1}, associating this increase in weight with a greater accumulation of proteins in the grains, due to a greater synthesis of amino acids caused by the application of N. In this sense, in a study conducted by Caliskan et al. (2008), researchers reported that productivity and protein content of soybean seeds can be increased with the association of BNF and complementary fertilization with N at the grain filling stage. Also in this sense, Bahry et al. (2013b) observed an increase in the mass of one thousand grains when applying N, regardless of the source and the stage of application of the soybean crop. In this study, the same way as what was observed for number of pods per plant, the single application of N in the R2 stage did not result in a difference in MTS, when compared to the 0R2/0R5 treatment.

Grain yield was influenced by the different kinds of management of N in coverage. A positive effect was observed for this variable when there was total application of N in the R5 stage (0R2/120r5), or split (60R2/60R5), with increases of up to 8% in crop yield. These results corroborate those found by Bahry et al. (2013b), who, working with increasing doses of N, applied in different reproductive stages of soybean, also found increased crop productivity. On the other hand, the total application of N in R2 and without nitrogen application showed no significant difference between them and showed the lowest grain yield. This result may be associated with the high dose of N used in this study (120 kg ha^{-1}), applied at a single time. The application of N in the R5.1 stage showed an increasing effect on productivity up to the dose of 60kg ha^{-1} and, after this dose, there was a gradual reduction on yield, indicating a probable detrimental effect of higher doses (Bahry et al., 2013b), which may be associated with the excessive presence of nitrate in rhizobia, which are sensitive to the most varied biotic and abiotic factors (Sinclair et al., 2007).

On the other hand, Gan et al. (2003) highlight that the application of N in the soybean culture is a positive and fundamental point for the increase of productivity, and that without the supply of N, productivity tends to be lower, even with the reinoculation of the soybean. The impact of the use of chemical fertilizers as a source of N on biological fixation (BNF) and on the productivity response of the soybean crop depends, among other factors, on the genotype, the *Bradyrhizobium* strain, the efficiency of symbiosis, the amount of N and the stage of application (Zuffo et al., 2018), which may explain the contradictory results regarding complementary fertilization with N in the soybean culture and the effects on grain yield. In the present study, since there was no interaction between the N management factors versus genotypes, it is inferred that the positive effect of nitrogen fertilization, especially for grain yield, does not depend on the genotype, corroborating the absence of interaction observed by Zuffo et al. (2018), when working with different doses and stages of application of N, in different soybean cultivars.

Of the total N required by the crop, 65 to 85% is supplied by BNF and the rest by the soil (Amado et al., 2010). Thus, the positive effects of nitrogen fertilization found in this research may be related to the complementary effect of BNF in the obtaining of the high yields. This statement corroborates the results obtained by Bahry *et al*. (2013a), who attributed the absence of a positive result regarding the application of N due to the low production levels in his study (2 to 2.5 ton ha^{-1}), and in these cases, possibly, the supply of N from the biological fixation itself can meet the demand of the crop.

The different kinds of management of N in coverage affect all the variables related to the physiological quality of the seeds produced, except for the vigor measured by the accelerated aging test. According to Zimmer (2012), N can influence the physiological quality of seeds; but the effects vary, depending on the environmental conditions and the development stage of the plant in which the fertilizer application occurs. The treatment with a split application of N (60R2/60R5) resulted in the highest percentage of germination and the highest vigor measured by the first germination count, with increments of up to 4.3 and 6.5%, respectively, compared to the absence of N in coverage (0R2/0R5), not differing from the treatment with total application of N in R5. The application of N only in R2 showed no difference on germination and first germination count, if compared to 0R2 / 0R5. Corroborating these results, in the research carried out by Zuffo et al. (2018), increases in soybean seed germination were observed with a quadratic response to different doses of N (0, 20, 40 and 60 kg ha^{-1}) and different times of application of N in the soybean culture (at sowing; at 30 and 50 days after emergence). In contrast, in the study carried out by Schmid *et al*. (2016), no effects of different doses of N, applied in the R5 stage, were found on the percentage of germination of the seeds produced.

As for vigor, evaluated through the seedling classification test, it was superior in all the treatments with N in coverage, except for treatment 120R2/0R5, which did not differ from the absence of N. The split application of 120 kg of N ha^{-1}, between stages R2 and R5, provided an increase of approximately 7.4% in seed vigor. The vigor evaluated through the tetrazolium test revealed a positive effect resulting from the application of N in all treatments, with increments of up to 6.7%, if compared to the absence of N in coverage. In this sense, the results of this research corroborate those found by Aisenberg et al. (2018), who, when evaluating the vigor by the seedling emergence velocity index, found out that seedlings from seeds under the influence of the application of N at the dose of 50 kg ha^{-1} reached higher values in 16.5, 11.4 and 19.1%, when compared to those under 0, 25 and 75 kg ha^{-1} of N, respectively.

The viability measured by the tetrazolium test demonstrated a positive effect when the seeds were originated from N applications in coverage, with superior results if compared to the 0R2/0R5 treatment, except for the total application of N in R2 (120R/20R5), which showed no difference. For the emergence of seedlings in beds, all kinds of management with the application of N in coverage, regardless of the application stage, presented higher emergency values than 0R2/0R5, with increments of up to 5.2%, when the application of N was split between stages R2 and R5 (60R2/60R5). These results were lower than those observed by Aisenberg et al. (2018), who observed increments of 12, 15 and 10% in the emergence of seedlings, for seeds from plants subjected to applications of 25, 50, and 75 kg of N ha^{-1}, respectively, and compared to the absence of N in coverage. In the same study, the emergence of seedlings presented a quadratic response to the different doses of N evaluated, with maximum emergence of seedlings at the estimated dose of 47.3 kg of N ha^{-1}, with a decreasing tendency for doses higher than this. Contradictory results were obtained in the study carried out by Schmid et al. (2016), in which there was no significant effect of different doses of N applied in the R5 stage of the soybean culture, on the percentage of emergence of seedlings in the field. However, in the same research, the authors observed a positive effect of nitrogen fertilization on some physiological parameters of seeds, such as germination speed index, electrical conductivity, and dry matter mass of seedlings.

When analyzing the main effect of the genotypes (Table 4), plant height, in all the evaluated stages, as well as the number of productive nodes, was higher in cultivar 6410 Ipro, demonstrating the predominance of the effects of the genotype for these variables. In this cultivar, in stages R2, R5 and R9, the height was higher by 18.9, 14.1 and 19.9 cm, respectively. Furthermore, genotype 6410 Ipro presented three productive nodes more per plant if compared to DM 5958 RSF Ipro. The number of pods per plant was higher in cultivar 6410 Ipro, while the mass of one thousand seeds was higher in cultivar DM 5958 RSF Ipro. For some authors, the main components of soybean yield are more influenced by how plants respond when exposed to different seeding densities (Ferreira et al., 2016).

For the variables related to the physiological quality of the seeds produced, there was a significant effect only for the classification of seedlings and emergence in beds. For these variables, cultivar 6410 Ipro demonstrated superiority over DM 5958 RSF Ipro. Similar results were obtained by Zuffo et al. (2018), working with two soybean cultivars and different doses of N and development stages in the soybean culture. The same authors attribute the difference in physiological quality to the genetic traits of each of the materials. Also, possible differences in the physiological quality between seeds of different cultivars may be associated with genetic potential, type of growth and other intrinsic traits of each cultivar (Soares et al., 2015), in addition to the soil and climate conditions between different production environments.

Table 4. Dismemberment of the main effects of genotypes. Los Cedrales, Paraguay, 2021.

Genotypes	**Variables**			
	AR2	**AR5**	**AR9**	**NPN**
DM5958	45.5 b	67.4 b	91.4 b	12.6 b
6410 Ipro	64.4 a	81.5 a	111.3 a	15.6 a
Genotypes	NLP	MTS (g)	VSC	EB
DM5958	89.0 b	163.0 a	80.9 b	94.6 b
6410 Ipro	101.3 a	143.4 b	83.3 a	96.4 a

*Means followed by the same lowercase letter in the column do not differ from each other by Tukey's test at 5% probability. Plant height at stage R2 (AR2), plant height at stage R5 (AR5), plant height at stage R9 (AR9), number of productive nodes (NPN), number of pods per plant (NLP), mass of one thousand seeds (MTS), seedling classification (VSC) and emergence in beds (EB).

The Pearson linear correlation analysis evaluated 300 associations between the 25 variables analyzed and, among these, 90 pairs were significant at the level of 5% probability by the t test (Figure 1). This analysis is based on a correlation coefficient, which, according to Loro et al. (2021) range from -1 (negative correlation) to +1 (positive correlation). There was a strong positive correlation between plant height in the three evaluated stages (AR2, AR5 and AR9) with the number of nodes per plant, with values of 0.87, 0.93 and 0.91, respectively. On the other hand, for the mass of one thousand seeds, strong negative correlations were observed in the associations with plant height in stages R2 (-0.91), R5 (-0.84) and R9 (-0.86) and with the number of nodes per plant (-0.75). There was a positive correlation of high magnitude between the number of pods per plant and grain yield, a result that supports conclusions by Carvalho et al. (2017) and Carvalho et al. (2021a). Similarly, the number of seeds per plant demonstrates a positive correlation of a medium magnitude with grain yield. Carvalho et al. (2021b) and Ferreira et al. (2021) showed similar results, where number of seeds per plant and grain yield demonstrate a positive correlation. Thus, the indirect selection of superior genotypes for number of pods and seeds per plant can promote positive results for grain yield in soybean.

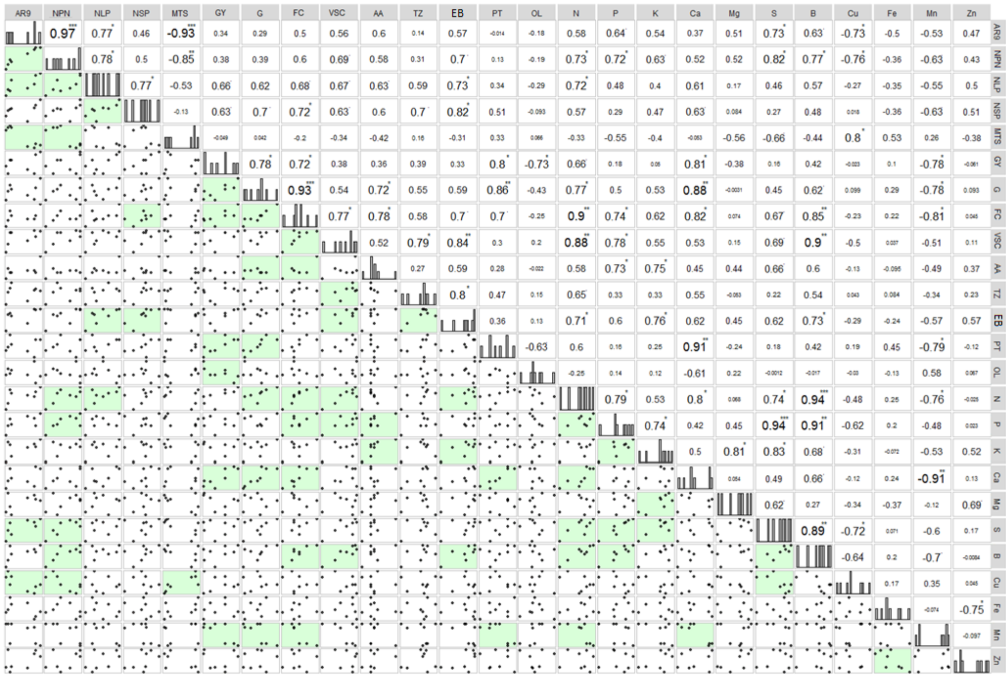

Figure 1. Pearson's linear correlation estimates between variables soybean plant height in stage R9 (AR9), number of productive nodes (NPN), number of pods (NLP) and number of seeds per plant (NSP), mass of one thousand seeds (MTS), grain yield (GY), germination (G), first germination count (FC), seedling classification (VSC), accelerated aging (AA), vigor by tetrazolium test (TZ) and emergence in beds (EB), oil content (OL), protein content (PT), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn). Los Cedrales, Paraguay, 2021. Significant linear correlation coefficients by the t-test at 5% probability.

The soil attributes demonstrated significant correlations with the productive and morphological traits of the plants, as well as with the seed quality variables. Sulfur showed a strong and positive correlation (0.73) with plant height in the R9 stage, that is, this nutrient, when available in the soil, tends to enhance trait plant height in this stage (Figure 1). However, Cu demonstrates a contrary tendency, where its presence in the soil reduces plant height, as well as the number of nodes per plant. Similarly, negative correlations are observed for manganese and grain yield; this can be explained, since calcium strongly enhances grain yield (0.81) and the higher the manganese contents the lower the calcium contents, all of this with a negative correlation of a high magnitude (0.91). Significant correlations of a strong magnitude with a positive sense are evidenced when one infers on soil attributes nitrogen, phosphorus, sulfur and boron with the number of nodes per plants. Similarly, macronutrients nitrogen and calcium show the same tendencies of associations for number of pods per plant (0.72) and grain yield (0.81), respectively (Figure 1).

When inferences of soil nutrients are made with seed quality traits, soils with higher calcium levels tend to promote seeds with higher germination (0.88). Similarly, nitrogen, phosphorus, potassium, calcium, sulfur, and boron show positive correlations from a medium to high magnitude with all seed quality indicators (Figure 1). On the other hand, the presence of manganese at high levels, certainly because it promotes antagonism with other nutrients, shows negative correlations with all variables of seed quality. Soils with high calcium levels promote greater protein synthesis in soybean grains, this is evidenced by the high positive association between calcium and protein. However, it is observed that, in the opposite way, oil contents in the grains decrease when calcium levels are high. Obviously, calcium and manganese are strongly associated negatively, since it is possible to notice the high negative correlation between manganese and protein, that is, when the manganese contents are high, there is a tendency for the reduction of protein synthesis in soybean grains, since these manganese contents reduce those of calcium, which promotes protein synthesis. Consequently, oil levels rise in the presence of high manganese contents in the soil.

In this study, the two main components represented a total of 91.09% of the total variation between nitrogen fertilizer management and the variables evaluated, which suggests high confidence and clarity in the explanation and interpretation of the results (Figure 2A).

Grain yield, germination and first germination count were grouped in the same quadrant, and these variables were largely influenced by the 60R2/60R5 treatment. For the other management and traits evaluated, no interrelationships were observed. Based on the multi-trait selection (Figure 2B) and, considering the agronomic ideotype of reference, the management with split nitrogen fertilization in stages R2 and R5 (60R2/60R5) was shown to be the ideal one for the joint increment of the evaluated variables.

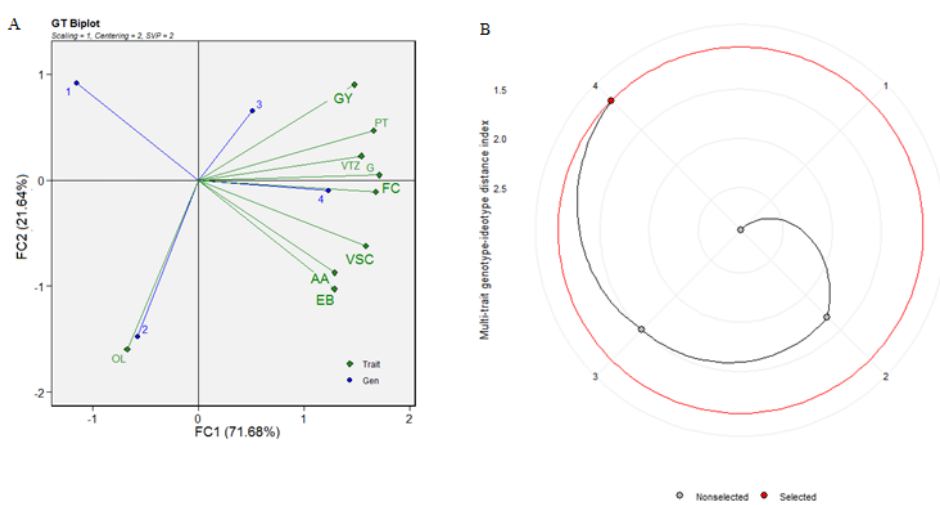

Figure 2. A. Biplot analysis of principal components for the relationships between nitrogen fertilizer management and the soybean response variables evaluated. No N in coverage (ABSENCE) (0R2/0R5), 120 Kg N ha^{-1} in stage R2 (120R2/0R5), 120 Kg N ha^{-1} in stage R5 (0R2/120R5) and split application of 120 kg Kg N ha^{-1} in stages R2 and R5 (60R2 / 0R5). Grain yield (GY), germination (G), first germination count (FC), accelerated aging (AA), seedling classification (VSC), vigor by tetrazolium test (TZ) and emergence in beds (EB). **B.** Biplot Multi-trait genotype-ideotype distance index based on the different nitrogen management (1: absence, 2: D120R2D0R5, 3: D0R2D120R5, 4: D60R2D60R5) and on variables grain yield (GY), germination (G), first germination count (FC), seedling classification (VSC), accelerated aging (AA), vigor by tetrazolium test (TZ) and site emergency in beds (EB). Los Cedrales, Paraguay, 2021.

CONCLUSIONS

Supplemental nitrogen fertilization positively influenced the number of productive nodes and pods of the plants and the mass of one thousand seeds. The applications of 120 kg of nitrogen per hectare, in a single dose in the R5 stage or split between the R2 and R5 stages, resulted in productivity increases of approximately 7 and 8%, respectively, compared to the absence of nitrogen in coverage in the soybean crop.

The physiological quality of the seeds produced was positively influenced by nitrogen fertilization, especially in single-dose nitrogen applications in stage R5 or split between stages R2 and R5. Increases of up to 7.4 and 5.2% were observed in seed vigor and emergence of soybean seedlings, respectively, due to the nitrogen application, when compared to the absence of nitrogen in coverage. Based on the soil and climate conditions, the cultivars, the different kinds of management and the analyses performed, the split application of 120 kg of nitrogen per hectare, between stages R2 (60 kg) and R5 (60 kg), reflected in higher productivity and physiological quality of the seeds produced.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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