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Bradyrhizobium spp. inoculation associated with nitrogen application enhances the quality of soybean seeds

Inoculação de *Bradyrhizobium* spp. associada à aplicação de nitrogênio melhora a qualidade das sementes de soja

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ABSTRACT

The physiological seed quality can be altered by nutritional management of the soybean crop, especially by the fertilization with nitrogen (N), which is a structural component of several organic compounds, including proteins, and has an important catalytic role in the activation of several enzymes. The aim of this research was to investigate the effects of sources and application rates of mineral N fertilizer associated with inoculation of *Bradyrhizobium* spp. on physiological quality of soybean seeds. The treatments were arranged in a completely randomized block design, in a 3 × 5 factorial arrangement [three N fertilizer sources (*Bradyrhizobium* spp. inoculation; *Bradyrhizobium* spp. inoculation + urea application; and, *Bradyrhizobium* spp. inoculation + ammonium sulfate application) and five N application rates (0, 50, 100, 150 and 200 kg N ha^{-1})], with four replicates. Nitrogen fertilizer was applied in topdressing at R_2 stage when soybean plants were in full flowering. The quality parameters evaluated in soybean seeds were: 1,000-seed mass, seed protein content, germination, first germination count test, seedling emergence, emergence speed index, accelerated aging, electrical conductivity, vigor and viability of seeds by tetrazolium test. The application of 50 kg N ha^{-1} of mineral fertilizer (ammonium sulfate or urea) associated with the *Bradyrhizobium* spp. inoculation enhanced the physiological quality of soybean seeds, resulting in higher seed germination percentage and higher emergence and seedling emergence speed index. The application of ammonium sulfate or urea (50 kg N ha^{-1}) at full flowering of soybean plants inoculated with *Bradyrhizobium* spp. can improve the physiological quality of soybean seeds.

Index terms: *Glycine max* (L.) Merrill.; ammonium sulfate; urea.

RESUMO

A qualidade fisiológica das sementes pode ser alterada pelo manejo nutricional da cultura da soja, principalmente pela adubação com nitrogênio (N), que é componente estrutural de diversos compostos orgânicos, incluindo as proteínas, e possui importante função catalítica na ativação de diversas enzimas. O objetivo desta pesquisa foi avaliar os efeitos da aplicação de fontes e doses de fertilizante nitrogenado mineral associado à inoculação de *Bradyrhizobium* spp. sobre a qualidade fisiológica das sementes de soja foram investigados nesta pesquisa. Os tratamentos foram dispostos no delineamento de blocos casualizados, em esquema fatorial 3 × 5 [três fontes de fertilizante de N (inoculação de *Bradyrhizobium* spp.; inoculação de *Bradyrhizobium* spp. + aplicação ureia e inoculação de *Bradyrhizobium* spp. + aplicação de sulfato de amônio] e da aplicação de cinco doses de N (0, 50, 100, 150 e 200 kg N ha^{-1})], com quatro repetições. O fertilizante nitrogenado foi aplicado em cobertura no estádio fenológico R_2, quando as plantas de soja estavam em plena floração. Os seguintes parâmetros de qualidade das sementes de soja foram avaliados: massa de 1.000 sementes, teor de proteína bruta, germinação, teste de primeira contagem de germinação, emergência de plântulas, índice de velocidade de emergência, envelhecimento acelerado, condutividade elétrica, vigor e viabilidade das sementes pelo teste de tetrazólio. A aplicação de 50 kg N ha^{-1} de fertilizante mineral (sulfato de amônio ou ureia) associada a inoculação de *Bradyrhizobium* spp. melhorou a qualidade fisiológica das sementes de soja, resultando na maior porcentagem de germinação, emergência e índice de velocidade de emergência de plântulas. A aplicação de sulfato de amônio ou ureia (50 kg N ha^{-1}) durante o estádio de pleno florescimento de plantas de soja inoculadas com *Bradyrhizobium* spp. pode melhorar a qualidade fisiológica das sementes de soja.

Termos para indexação: *Glycine max* (L.) Merrill.; sulfato de amônio; ureia.

INTRODUCTION

Soybean is the main oilseed crop in Brazil and the world, especially on account of wide socioeconomic importance for the world agricultural sector. Therefore, the use of high-quality seeds is essential for the proper establishment of soybean agricultural fields (Zuffo et al., 2017). The proper plant establishment and development depends on balanced mineral nutrition management and this factor is essential for the commercial production of seeds with high vigor and high physiological quality (Carvalho; Nakagawa, 2012). Mineral fertilizers are utilized in the commercial fields to improve the quality of seeds (Toledo et al., 2009). The nitrogen (N) fertilization in supplementation to inoculation of *Bradyrhizobium* spp. has recently been investigated for this purpose (Zuffo et al., 2018b).

Nitrogen stands out among the essential nutrients for plants, especially on account of its crucial role in the physiological and biochemical metabolism of plants and its participation as a structural component of chlorophylls, proteins, and organic compounds (Leghari et al., 2016). Thus, the N fertilization can raise the development and productivity of the soybean crop, counting enhance the size and the quality of seeds (Marcos-Filho, 2015). Endosperm and embryo formation and accumulation of reserve compounds is dependent an appropriate supply of nutrients (Carvalho; Nakagawa, 2012).

Large N requirement of soybean crops has been supplied predominantly by biological nitrogen fixation (BNF) through the symbiosis process that plant roots establish with strains of *Bradyrhizobium japonicum* and *B. elkanii* (Domingos; Silva Lima; Braccini, 2015). Maximum rate of N_2 fixation by soybean plants occurs between the R_3 (beginning pod) and R_5 (beginning seed development) growth stage (Zapata et al., 1987). Furthermore, in the R_2 (full flowering) growth stage there is a rapid accumulation of dry biomass and nutrients in soybean plants (Werner et al., 2016), indicating that the supply of nutrients, especially N, is crucial for photoassimilate synthesis and seed development. Thus, any deficit between the crop N demand and amount of N supplied by the symbiotic process must be compensated for the absorption of N from other sources (McCoy et al., 2018). Sources of N supply can be constituted from the residual N of the soil (Zuffo et al., 2018a) and the application of mineral N fertilizer (Zuffo et al., 2020).

Mineral N fertilization is used by soybean seed producers in an effort to obtain high quality seeds. Indeed, N fertilizer application has been more common in commercial seed production fields when compared to grain production fields (Zuffo et al., 2018b). Therefore, the use of mineral N in supplementation to the symbiotic process (soybean-*Bradyrhizobium* spp.) can be an alternative to improve the physiological quality of soybean seeds.

Recent research has reported that the application of mineral N fertilizer has no significant effect on soybean grain yield (Korber et al., 2017; Saturno et al., 2017; Zuffo et al., 2018a; Zuffo et al., 2020, Zuffo et al., 2021). In turn, a study conducted by Zuffo et al. (2018b), revealed that seed quality was not influenced by the application of urea (N fertilizer) in supplementation to the inoculation of *B. japonicum* strains, but the incidence of pathogens reduced dramatically. However, Medeiros et al. (2021) showed that the best physiological quality of soybean seeds was obtained by fertilization with urea. But these authors mentioned that other studies are needed to reach a more correct conclusion about the adequate management of N nutrition in soybean fields.

According to Carvalho and Nakagawa (2012), N can have a beneficial effect on the physiological quality of crop seeds, but these effects remain dependent on the environmental conditions of production, the growth stage of the crops and timing of N fertilizer application. but these effects are dependent on the production environment conditions, crop growth stage and time of N fertilizer application. Therefore, the use of N fertilizer in supplementation to the biological N fixation process can optimize grain filling and quality of soybean seeds.

Therefore, the objective of this research was to evaluate the physiological quality and protein level of soybean seeds as affected by use of different sources and application rates of N fertilizer associated with inoculation of *Bradyrhizobiu*m spp.

MATERIAL AND METHODS

Study site description

The field experiment was conducted in a deep sandy clay soil, classified as Latossolo Vermelho distrófico (Santos et al., 2018) or Rhodic Ferralsol (WRB, 2015), with 51–53% sand and 42–44% clay, in Chapadão do Sul, MS, Brazil (18°46′18″ S, 52°37′25″ W and altitude of 540 m), during the 2018/2019 growing season. The municipality of Chapadão do Sul plays an important role in agricultural production in the northern region of the state of Mato Grosso do Sul. The regional climate, according to the Köppen classification, is Aw, characterized as tropical, with hot summers and tendency toward high rainfall levels and dry winters, with a dry season between May and September. The mean annual temperature is 24.0 °C, with a minimum of 15.7 °C (July) and a maximum of 28.9 °C (January). Mean annual rainfall is 1,260 mm. The environmental conditions during the experiment included a mean air temperature of 26.8 °C (± 2.1 °C), mean air relative humidity of 72% (± 8%) and a total rainfall of 1,087 mm.

Before conducting this experiment, the experimental field area had been used for grain cropping under the no-till system for 12 years. Before starting the experiment, in September 2018, soil samples were collected at depth of 0.0–0.20 m using a hole auger in five points per plot. The results of the soil chemical analysis did not show significant variability between the plots regarding soil chemical characteristics of the experimental area. The average values of the soil chemical properties are shown in Table 1.

Experimental design and treatments

The experiment was arranged in a complete randomized block design, in a 3 × 5 factorial scheme, with four replicates. Treatments consisted of the application of three N supply sources (*Bradyrhizobium* spp. inoculation; *Bradyrhizobium* spp. inoculation + ureia (45% N) application; and, *Bradyrhizobium* spp. inoculation + ammonium sulfate (21% N) application] and five N application rates (0, 50, 100, 150 and 200 kg N ha^{-1}). Nitrogen fertilizer was applied in topdressing at R_2 growing stage when the soybean plants were in full flowering, according to the phenological scale of Fehr and Caviness (1977). 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 1.0 m of each edge.

Plant material, inoculation, fertilization, and crop management

Soybeans was mechanically sown on October 4th, 2018, at a depth of 3.0 cm, in rows 0.45 m apart at a density of 13 seeds per linear meter to reach a final stand of 260,000 to 280,000 plants per hectare. Base fertilization was carried out by applying 80 kg P_2O_5 ha^{-1} and 18 kg N ha^{-1} [monoammonium phosphate (MAP)] at the sowing furrow. At 30 days after sowing [V_4 stage - four fully expanded leaves (fourth trifoliolate)], 100 kg K_2O ha^{-1} (KCl) was applied in topdressing. At 40 days after sowing [R_1 growing stage – plants have at least one flower on any node (beginning flowering)], foliar fertilization was applied using 1.0 L ha^{-1} of 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% organic carbon). The soybean cultivar used in experiment was BMX Bônus IPRO, which has an indeterminate growth habit, maturity group 7.9, and an average cycle ranging from 114 to 121 day. This soybean cultivar was used for having high seed production potential, high stability, and wide adaptation region, and is one of the main soybean cultivars sown in the Brazilian Cerrado region.

All soybean seeds used in the experiment were previously treated with pyraclostrobin + methyl thiophanate + fipronil (Standak Top®) at the rate of 2 mL c.p. kg^{-1} of seed and then inoculated with efficient *Bradyrhizobium japonicum* 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 5080)] (with minimum concentration of 7.2 × 10^9 viable cells per mL) at a rate of 3.0 mL kg^{-1} of seeds, was used as recommended by the manufacturer. To potentiate the soybean nodulation, the seeds were treated with some micronutrients, mainly molybdenum. The commercial seed fertilizer Nódulus® Premium 125 (Biosoja), containing 10% of Mo, 1% of Co, 1% of S, 1% of Ca and 0.2% of Fe, was used.

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

Soybean harvest and seed preparation

The soybean harvest was performed manually in the R_8 phenological stage (when 95% of the pods have reached their full mature color). After harvest, plants were air-dried at room temperature for 48 h, and then the seeds were extracted by hand. After cleaning the seeds, the 1,000-seed mass (Brasil, 2009) and crude protein content (Detmann; Queiroz; Cabral, 2012) were determined. Subsequently, the seeds were sieved through round hole sieves with diameters of 6.00 mm, homogenized, and then the samples for the analyzes were subjected to the mechanical method with the aid of the centrifugal divider. A sample of about 600 g was separated for each field plot, packed in Kraf-type paper bags, and then kept in a laboratory environment with temperature of 25 °C ± 0.8 °C. Afterwards, the physiological quality of the seeds was determined using the following tests described below.

Table 1: Main chemical properties of the soil collected in the 0.0–0.20 m layer.

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} ------------------------						%
4.8	23.2	8.6	3.50	0.02	3.10	1.80	0.30	8.70	59.8

OM: organic matter. CEC: cation exchange capacity at pH 7.0. V: soil base saturation.

Measurement of the seed physiological quality

Germination test: four subsamples were used for each field replication with 50 seeds in three sheets of a paper towel moistened with distilled water, which had 2.5 times the mass of the non-hydrated paper, and seeds were allowed to germinate at a constant temperature of 25 ± 2 °C and under 12/12 h photoperiod (light/darkness). Counting was performed in 5 days (first count of germination test) and 8 days (total germination) (Brazil, 2009).

Accelerated aging: seeds were placed on a copper wire mesh inside plastic boxes (11.0 × 11.0 × 3.0 cm) containing 40 mL of distilled water. The plastic boxes were kept in a BOD incubator at 41 °C, for 48 h (Krzyzanowski et al., 1999). After this period, 200 seeds from each treatment were divided into four replicates of 50 seeds and germinated as previously described (germination test). The evaluation was assessed at the 5th day after sowing, and the percentage of normal seedlings was recorded (Brasil, 2009).

Electrical conductivity: four 50-seed replicates of each treatment were placed in 300 mL plastic cups and weighed on an analytical scale (0.001 g accuracy). Then, 75 mL of distilled water were added to each container. The containers were placed in a BOD incubator at a constant temperature of 25 °C. After this period, the containers were removed and the seeds were gently agitated for homogenization of the solution, and the electrical conductivity was measured with a conductivity meter (Krzyzanowski et al., 1999).

Tetrazolium test: the test was conducted with two sub-samples of 50 seeds per treatment. The seeds were placed on moistened germitest paper and kept for 24 hours at 25 °C. After this period, the seeds were placed fully submerged solution of 2,3,5-triphenyl tetrazolium in plastic containers, remaining in a dark for three hours at 35 °C (França-Neto; Krzyzanowski (2018). After this period, the seeds were washed in running water and each seed was individually examined. Seeds were classified according to vigor and viability, classifying each seed in one of the eight groups described by França-Neto and Krzyzanowski (2018). Seeds were classified into eight classes based on viability and vigor: 1- viable and very high vigor seeds, 2- viable and high vigor seeds, 3- viable and medium vigor seeds, 4- viable and low vigor seeds, 5- viable and very low vigor seeds, 6- unviable seeds, 7- unviable seeds and 8- dead seed. Subsequently, based on the values obtained in this first evaluation, the seed vigor level was calculated by the sum of the percentage values of the seeds from classes 1 to 3 [$TT_{(1-3)}$] and the seed viability was calculated by the sum of the classified percentage values in classes 1 to 5 of the tetrazolium test [$TT_{(1-5)}$], as reported by França-Neto and Krzyzanowski (2018).

Emergence test: seedling emergence was carried out in greenhouse conditions, using four 50-seed replicates of each treatment. The seeds were placed to germinate in plastic trays (46 × 32 × 8 cm) containing soil and sand, at the proportion of 1:1 (v/v). The moisture content of the substrate was maintained at 70% of the field capacity with daily irrigations. The trays were maintained under greenhouse conditions at a temperature of 25 °C (±3 °C). Seedling counts were performed daily, and the emergence speed index was calculated according to Maguire (1962), at the end of the 10th day.

Statistical analysis

The data were previously submitted to tests of Kolmogorov–Smirnov and Levene to analyses the adjustment of residuals to the normal distribution and homogeneity of variances, respectively, both at the 5% significance level. Canonical correlation analysis (CCA) was used to study the interrelationships between sets (vectors) of independent (sources and rates of N application) and dependent (seed physiological quality) variables. These analyzes were performed using the Rbio software version 140 for Windows (Bhering, 2017).

RESULTS AND DISCUSSION

Canonical correlation analysis was used to verify the contribution of each dependent variable measured in the physiological quality tests of soybean seeds as a function of N application sources (Figure 1) and N fertilizer rates (Figure 2). For scores to be represented in a two-dimensional graph, the percentage of retained variance must be higher than 80% (Mingoti, 2005). In this study, variances accumulated in the two main canonical variables were 100% and 89.4%, respectively, for each graph (Figures 1 and 2), allowing an accurate interpretation.

When N was supplied only by the *Bradyrhizobium* spp. inoculation, the soybean plants had seed production with a higher viability percentage by the tetrazolium test (TT_{1-5}) (Figure 1). These higher percentage of viable seeds from plants inoculated only with *Bradyrhizobium* spp. may indicate that all the soybean N supply was fully supplied by the biological N_2 fixation process and by the small rate of N fertilizer applied at sowing (18 kg N ha^{-1} as monoammonium phosphate), which ensured the appropriate N nutrition of plants and formation of viable seeds. Therefore, these results show the importance of seed inoculation with bacteria of the genus *Bradyrhizobium* for the N supply in soybean crop in tropical climate conditions, which results in the production of seeds with high viability. However, contrary results were reported by Zuffo et al. (2018b), who showed that there was no significant effect of the inoculation with *B. japonicum* alone or combined with urea application on the seed viability percentage in the tetrazolium test (TT_{1-5}) and seedling emergence. These divergent results may be related to the N application rates (0, 20, 40 and 60 kg N ha^{-1}) and to the soil textural class (sandy soil with 10% clay and 85% sand) used in the study by Zuffo et al. (2018b). In this study, higher rates (0, 50, 100, 150 and 200 kg N ha^{-1}) of N fertilizer were applied, and the soil had sandy clay texture (43% clay and 52% sand).

The use of mineral N fertilizer associated with the inoculation of *Bradyrhizobium* spp. enhanced the physiological quality of soybean seeds (Figure 1). Soybean plants inoculated with *Bradyrhizobium* spp. and fertilized with urea resulted in seeds with higher values of emergence (E), emergence speed index (ESI), accelerated aging (AA), vigor percentage by the tetrazolium test [$TT_{(1-3)}$] and crude protein content (CP) of soybean seeds. In turn, plants inoculated with *Bradyrhizobium* spp. and fertilized with ammonium sulfate resulted in seeds with higher thousand seed mass (1,000-M), electrical conductivity (EC), germination (G) and first count of the germination test (FGC), as seen by the distance of the vectors (Figure 1). Toledo et al. (2009) also showed that fertilization with mineral N in common beans resulted in the seed production with a higher germination rate.

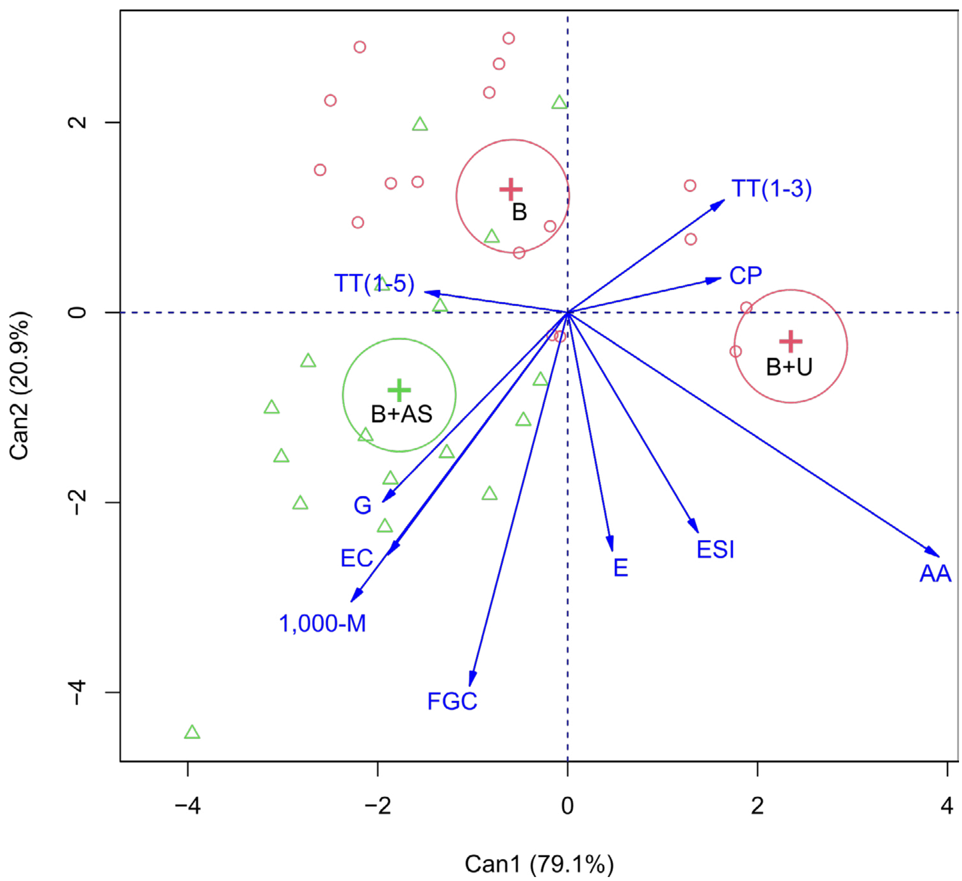

Figure 1: Canonical correlation analysis (CCA) between the physiological quality traits of soybean seeds and nitrogen fertilizer sources. The blue lines show the canonical correlation between centroids of the first pair of canonical variates and lineal tendency line.
Abbreviations: G: germination; FGC: first germination count test; E: seedling emergence; ESI: emergence speed index; AA: accelerated aging. EC: electrical conductivity, $TT_{(1-3)}$: vigor percentage by the tetrazolium test; $TT_{(1-5)}$: viability percentage by the tetrazolium test; CP: crude protein; 1,000-M: thousand-seed mass; B: *Bradyrhizobium* spp. inoculation; B+U: *Bradyrhizobium* spp. inoculation + ureia application; B+AS: *Bradyrhizobium* spp. inoculation + ammonium sulfate application.

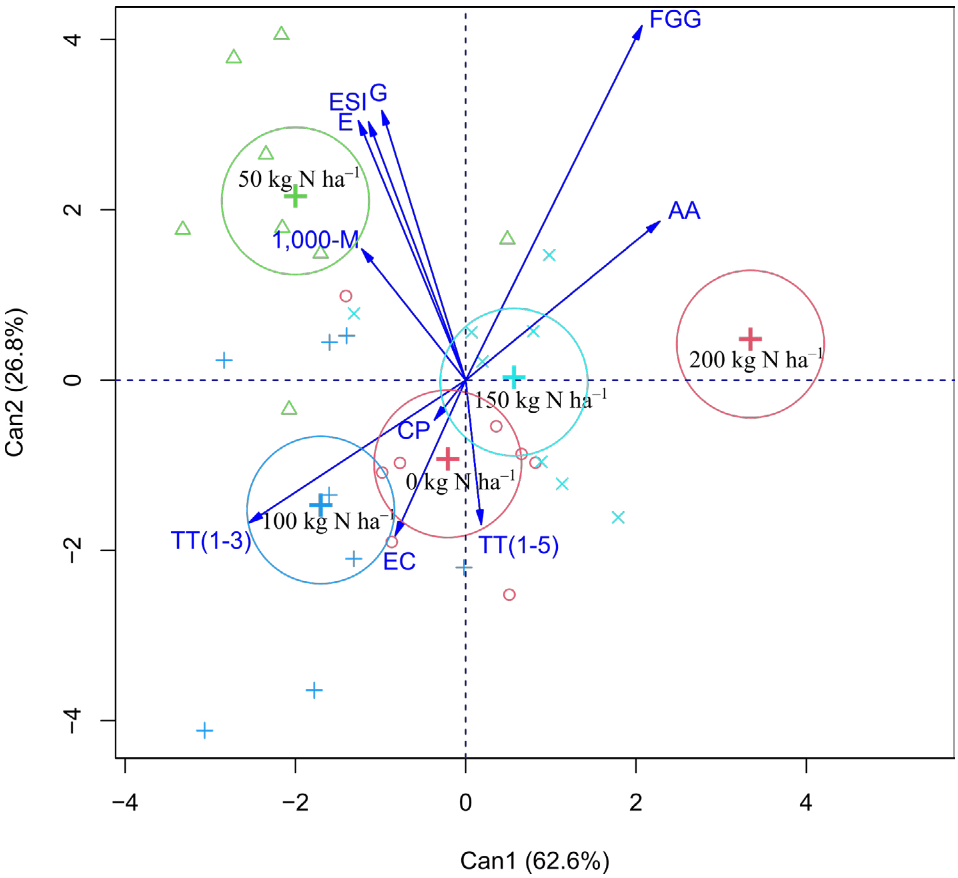

Figure 2: Canonical correlation analysis (CCA) between the physiological quality traits of soybean seeds and nitrogen application rates (0, 50, 100, 150 and 200 kg N ha^{-1}). The blue lines show the canonical correlation between centroids of the first pair of canonical variates and lineal tendency line.
Abbreviations: G: germination; FGC: first germination count test; E: seedling emergence; ESI: emergence speed index; AA: accelerated aging. EC: electrical conductivity, $TT_{(1-3)}$: vigor percentage by the tetrazolium test; $TT_{(1-5)}$: viability percentage by the tetrazolium test; CP: crude protein; 1,000-M: thousand-seed mass.

The soybean is a leguminous plant that has high protein content in its seeds, the N fertilizer sources applied increase the crude protein content and they resulted in increase in the thousand seed mass. Therefore, when the *Bradyrhizobium* spp. inoculation was supplemented with the ammonium sulfate application, there was a rapid release of N in the soil, which enhanced the uptake and assimilation of N by soybean plants, resulting in an increase in the photosynthetic rate of plants and, consequently, in the greater production of photoassimilates for seed filling.

Nitrogen is a component responsible for several physiological and biochemical processes in plants, being a structural constituent of chlorophyll molecules, proteins, enzymes, and nucleic acids (Nunes-Nesi et al., 2010; Taiz et al., 2017). Thus, plants with proper N nutrition have higher chlorophyll content, which can result in increased photosynthetic rate, photoassimilates production, filling, and chemical composition of seeds and, consequently, in improving the physiological quality of soybean seeds. According to Henning et al. (2010), high vigor soybean seeds have a higher content of soluble proteins, starch and soluble sugars, and greater capacity to mobilize reserves during the germination process, resulting in seedlings with better initial development. The chemical composition and mobilization of the reserve during the seed germination process has a direct effect on the formation of structural components and growth rate of soybean seedlings. In addition, larger seeds can give rise to seedlings with greater vigor (Carvalho; Nakagawa, 2012). Therefore, ammonium sulfate application associated with *Bradyrhizobium* spp.

inoculation resulted in larger seeds (greater thousand seed mass) and with higher crude protein content, which can result in seedlings with greater vigor (as shown by the first germination count test) and with higher germination percentage (Figure 1).

The presence of sulfur (S) with the ammonium sulfate application may have optimized the thousand seed mass and physiological potential of soybean seeds, as observed in the first germination count test. These results confirm the observations reported by César et al. (2008), which showed that the application of S in common bean crops resulted in seeds with greater physiology quality and seedlings with greater vigor. Such inference is due to the fact that S is involved in several enzymatic processes and in numerous redox reactions. This is because this element is a constituent of amino acids (cystine, cysteine and methionine), coenzymes and vitamins, which represent about 90% of the total S in plants (Marschner, 1995; Malavolta et al., 1997; Taiz et al., 2017).

The main effect of N fertilizer (ureia and ammonium sulfate) rates applied was observed on thousand seed mass, seedling emergence, emergence speed index and germination percentage, and the application rate of 50 kg N ha^{-1} resulted in the highest value of these dependent variables (Figure 2). Similar results were reported by Zuffo et al. (2018b), which showed that the application rate of 60 kg N ha^{-1} resulted in the highest germination percentage of soybean seeds.

Although biological N_2 fixation has the ability to supply the largest proportion of the N requirement of the soybean crop, this symbiotic process has a high energy cost for plants (McCoy et al., 2018). Symbiotic microorganisms that fix atmospheric N require 16 moles of adenosine triphosphate (ATP) to reduce each mole of N, and these microorganisms obtain this energy from their host plants' rhizospheres (Hubbell; Kidder, 2009). According to Pate and Layzell (1990), the theoretical biological cost of N assimilation through the N_2 fixation process can be up to 36% higher than for the absorption of mineral N ($N\text{-}NO_3$) from the soil. Therefore, due to the potential limitations of biological N_2 fixation, the supplementation of 50 kg N ha^{-1} of mineral fertilizer is an alternative to enhance the size and quality of soybean seeds.

The germination percentage of soybean seeds was higher than 86%, regardless of the treatment. This value is higher than the required standard (80%) for the commercialization of soybean seeds in Brazil, according to marketing standards (Brasil, 2013). Evaluating the effect of N fertilization on the physiological quality of soybean seeds, Zuffo et al. (2018b) reported a low percentage of seed germination (≈60%). These differences in seed germination capacity may be due to the genetic characteristics of soybean cultivars, since each cultivar has seeds with different chemical composition (Sediyama, 2016). This difference in chemical composition can affect the germination capacity of seeds. Seeds with low germination potential result in seedlings with less competitive potential in the field, which can compromise the successful establishment of the plant stand (França-Neto et al., 2010).

According to Carvalho and Nakagawa (2012), seed quality and vigor can be influenced by soil fertility and crop nutritional management. This is because the seed chemical composition is dependent on plant metabolism. Initial seedling development is maintained by embryo reserves, and later seedling development is maintained through consumption of seed reserve tissue components, enzymatic activity, and flow of soluble components to the growing regions. Therefore, our results showed that the use of 50 kg N ha^{-1} of mineral N fertilizer (urea or ammonium sulfate) in supplementation to *Bradyrhizobium* spp. inoculation resulted in an increase in the thousand seed weight, crude protein, physiological quality, and vigor of soybean seeds.

CONCLUSIONS

The application of 50 kg N ha^{-1} of mineral fertilizer (ammonium sulfate or urea) in associated with inoculation of *Bradyrhizobium* spp. enhanced the physiological quality of soybean seeds, resulting in higher germination percentage, seedling emergence, emergence speed index and vigor percentage by the tetrazolium test. Mineral nitrogen fertilization increased the thousand seed mass and crude protein content of the seeds.

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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