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Influence of Inoculant Application Methods on the Physiological Quality of Common Bean Seeds

Lucas Oliveira de Castro[1], Itamar Rosa Teixeira[1], Gisele Carneiro da Silva Teixeira[1], Nathan Mickael de Bessa Cunha[1], Gessiele Pinheiro da Conceição Alves[2], Derblai Casaroli[2] & Alessandro Guerra da Silva[3]

[1] Instituto de Ciências Agrárias, Universidade Estadual de Goiás, Anápolis-GO, Brazil

[2] Departamento de Agronomia, Universidade Federal de Goiás, Goiânia-GO, Brazil

[3] Departamento de Agronomia, Universidade de Rio Verde, Rio Verde-GO, Brazil

Correspondence: Itamar Rosa Teixeira, Instituto de Ciências Agrárias, Universidade Estadual de Goiás, Anápolis-GO, BR 153, Campus Henrique Santillo, Brazil. Tel: 55-62-3328-1177. E-mail: itamar.texeira@ueg.br

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Abstract

Common bean (*Phaseolus vulgaris* L.) is one of Brazil's main crops, however, its productivity remains low. Biological nitrogen fixation (BNF) is a sustainable alternative to mineral nitrogen fertilization, reducing costs and environmental impacts. This study aimed to evaluate the physiological quality of BRS Estilo bean seeds under different inoculation strategies with *Rhizobium tropici*. The experiment was conducted during the 2022/2023 growing season in Anápolis-GO, using six treatments: seed inoculation, furrow inoculation, topdressing reinoculation at the V4 stage, their combinations, as well as a mineral nitrogen fertilization treatment and a control without nitrogen. The harvested seeds were subjected to germination, vigor, accelerated aging, seedling length, and dry mass tests. The results indicated that furrow inoculation combined with topdressing reinoculation at the V4 stage produced higher-quality seeds, with germination and vigor comparable to those of mineral nitrogen fertilization. Conversely, single seed inoculation was insufficient to ensure high quality seeds. It was concluded that furrow inoculation followed by topdressing reinoculation can partially or fully replace mineral nitrogen fertilization, improving nitrogen use efficiency in common bean and contributing to a more sustainable production system.

Keywords: biological nitrogen fixation, *Rhizobium tropici*, seed vigor, sustainable fertilization, bioinputs.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the main components of the Brazilian diet, alongside rice, and Brazil stands out as the world's largest producer of this legume. In the 2020/2021 growing season, the cultivated area was approximately 2.9 million hectares, resulting in a production of 3.2 million tons and an average yield of only 1,101 kg ha^{-1} (Conab, 2023). One of the factors contributing to this low productivity is the limited use of high-quality seeds, estimated at only 15%.

The physiological quality of seeds is directly related to plant nutrition, as proper nutrition is essential for obtaining seed lots with high vigor and germination rates (Marcos Filho, 2015). Among essential nutrients, nitrogen (N) is the most demanded by grain crops such as common bean, which can absorb up to 140 kg ha^{-1} of this element (Soratto et al., 2013). Nitrogen supply can occur through soil uptake, nitrogen fertilization, or the biological nitrogen fixation (BNF) process via inoculation with beneficial bacteria.

The increasing adoption of bio-inputs in agriculture, including biological inoculants, has been encouraged as a sustainable and economically viable alternative to mineral nitrogen fertilization. The use of inoculants reduces dependence on synthetic fertilizers and minimizes environmental impacts, such as water resource contamination resulting from nitrogen leaching in the soil (Garé, 2020).

Currently, commercial inoculants for common bean are formulated with *Rhizobium tropici*, a species highly adapted to tropical conditions due to its resistance to high soil temperatures, acidity, and greater competitiveness against native rhizobial populations (Brito et al., 2015). These characteristics have made BNF a viable alternative for meeting the nitrogen demand of common bean under field conditions, provided that proper inoculation practices are employed (Oliveira et al., 2018; Sousa et al., 2021; Teixeira et al., 2022; Sousa et al., 2022).

Traditionally, common bean inoculation is performed via seed treatment; however, this technique can compromise bacterial viability when combined with the application of chemical protectants such as fungicides, insecticides, growth regulators, and micronutrients, potentially causing seed damage and reducing BNF efficiency (Campo et al., 2010). Given this, applying inoculants in the planting furrow has been a viable alternative, especially for soybean (Vieira Neto et al., 2008a, b), although limited information is available regarding its efficiency in common bean cultivation.

Another promising strategy is topdressing reinoculation, complementing seed inoculation. This practice can enhance BNF efficiency during the stages of highest nitrogen demand in common bean, particularly during flowering and grain filling. At this stage, early formed nodules become less functional, reducing the contribution of BNF to crop nutrition (Sousa et al., 2021; Teixeira et al., 2022).

Studies on soybean have demonstrated that reinoculation during early growth stages can promote nodulation and crop recovery when initial nodulation fails, as well as increase productivity even when initial nodulation is successful (Zilli et al., 2008; Hungria et al., 2015). Additionally, supplemental inoculation up to stage R6 can significantly increase nodulation, suggesting that the plant can override autoregulation of nodulation and form new functional nodules to meet the increased nitrogen demand during pod filling (Moretti et al., 2018).

Although studies have addressed BNF and its contribution to nitrogen supply in common bean, there is still a knowledge gap regarding the relationship between inoculation and the physiological quality of the produced seeds. Therefore, it is essential to investigate the most efficient method of applying *R. tropici* inoculant to obtain high quality seeds.

Given this context, the present study aimed to evaluate the physiological quality of BRS Estilo common bean seeds produced under different inoculation strategies with *R. tropici*, either individually or in combination.

2. Materials and Methods

2.1 Seeds Used in the Study and Experimental Conditions

The seeds used in the study were produced during the 2022/2023 winter season at the experimental area of the Emater Experimental Station in Anápolis-GO, in partnership with Anápolis Campus of Exact and Technological Sciences – Henrique Santillo (CCET), of the State University of Goiás (UEG). The area is located at the following geographical coordinates: latitude 16°20'12.13" S, longitude 48°53'15.96" W, with an average altitude of 1,058 m and an annual mean temperature of 25°C (Google Earth, 2019). The experiment evaluated the following treatments: seed inoculation (SI), furrow inoculation (FI), topdressing inoculation (TI), combinations of SI + TI and FI + TI, in addition to mineral nitrogen fertilization (MNF) and a control treatment (CT).

The *Rhizobium tropici* source used was a commercial liquid inoculant containing the SEMIA 4077, SEMIA 4080, and SEMIA 4088 strains, with a concentration of 3.0×10^9 viable cells per mL, exceeding the legal minimum of approximately 1.0×10^9 viable cells mL^{-1} for inoculants (Brazil, 2011), at a dose of 150 mL per 50 kg of seed, and was triplet in the application to soil and topdressing in R5 phenological phases using a spray volume of 200 L ha^{-1}.

2.2 Soil Preparation and Crop Management

Soil preparation followed the conventional tillage system, consisting of one plowing followed by two harrowings. Base fertilization involved applying 200 kg ha^{-1} of the 05-52-00 fertilizer, supplying 10 kg ha^{-1} of nitrogen to support early seedling growth, as the inoculated bacterial community would not yet be fully functional. The seeding density was 12 plants per linear meter of the BRS Estilo cultivar, adjusted after thinning.

In the MNF treatment, topdressing fertilization was applied at 25 days after emergence (DAE) using urea (60 kg ha^{-1} of N) and potassium chloride (60 kg ha^{-1} of K). Weed, pest, and disease management were carried out according to the crop's needs.

2.3 Harvesting and Seed Processing

Harvesting took place at the R9 growth stage, with all plants from the useful plot area being collected. The seeds were manually threshed and dried until reaching a moisture content of approximately 12%. Subsequently, 0.5 kg samples were packed in labeled paper bags and sent to the CCET/UEG Plant Analysis Laboratory (LABAV), for physiological quality analysis using the following tests:

Germination: Four replicates of 50 seeds were placed on germitest paper moistened with deionized water (2.5 times the dry paper weight) and maintained in a germinator (Biomatic TIC-175) at 25°C (Brazil, 2009). Normal seedlings were evaluated on the eighth day, with results expressed as a percentage.

First count: Conducted alongside the germination test, considering normal seedlings on the fifth day of evaluation. Results were expressed as a percentage.

Accelerated aging: The methodology of Silva et al. (2010) was adopted. Seeds were distributed in a single layer on a metal screen inside gerbox containers with 40 mL of water at the bottom. The containers were sealed and kept at 42°C for 48 hours under 100% relative humidity. After this period, four subsamples of 50 seeds were tested using the germination method, with evaluation on the fifth day and results expressed as a percentage.

Seedling length: Four replicates of 10 seeds per treatment were placed on germitest paper moistened with distilled water at a ratio of 2.5 mL per gram of dry paper and maintained at 25°C for eight days (Nakagawa, 1994). The length of normal seedlings (Brazil, 2009) was measured with a millimeter ruler.

Seedling dry mass: Normal seedlings from the length test were dried in a forced-air oven at 80°C for 24 hours (Nakagawa, 1994). After cooling in a desiccator, the samples were weighed on a precision balance (0.001 g), with results expressed in mg seedling^{-1}.

2.4 Statistical Analysis

The data obtained were subjected to normality and homoscedasticity tests. Subsequently, analysis of variance (ANOVA) was performed, and when significant, Tukey's test at a 5% probability level was applied. Statistical analyses were conducted using Sisvar® 5.6 software (Ferreira, 2011).

3. Results and Discussion

The physiological quality of common bean seeds was influenced by the different inoculant application methods, as evidenced by the results obtained from all the tests conducted. Overall, good experimental precision was observed in data collection.

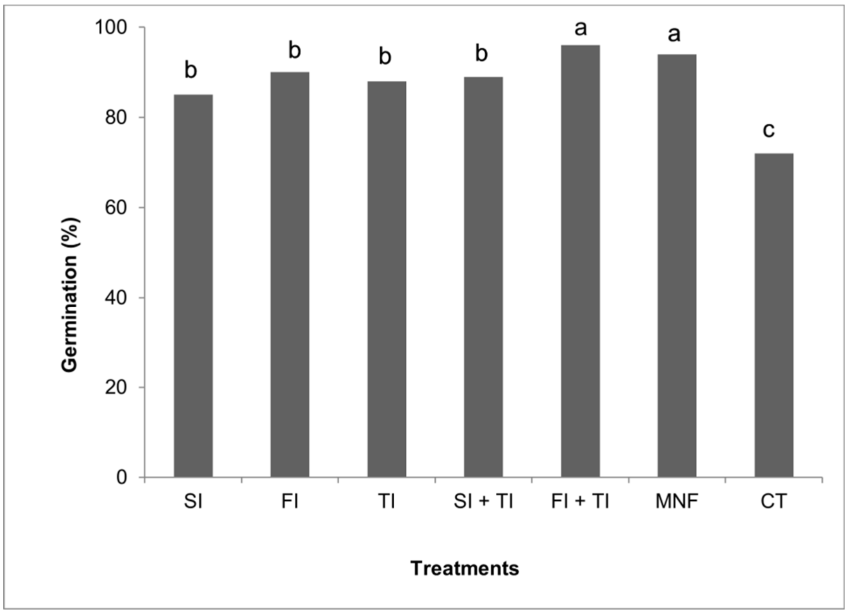
There are several recent studies addressing the negative effect of the application of pesticides, especially insecticides and fungicides, on the population of bacteria inoculated in the seeds of the most diverse grain crops, mainly soybean and beans (Sartori et al., 2023; Araújo et al., 2023; Camargo et al., 2022; Santos et al., 2021; Silva et al., 2019). Therefore, the only product applied to the seed or soil was the inoculant, and it is therefore not the objective of the study to investigate this interaction. It should be noted that if you intend to use chemical products such as insecticides and fungicides in seed treatments, it is recommended that these products be applied prior to planting, around 24 hours before applying the inoculant to the seeds, thus avoiding damage that could lead to the death of the inoculated bacteria.

Inoculation of *R. tropici* in the planting furrow, combined with topdressing reinoculation at the V4 stage, resulted in higher quality bean seeds, performing equivalently to the mineral nitrogen fertilization treatment (Figure 1A). The corresponding germination values were 96% and 94%, both exceeding the thresholds established by the Seed Analysis Rules - RAS (Brazil, 2009) for commercial seed sales, which range between 80% and 85%.

On the other hand, single seed inoculation a common practice among farmers was not sufficient to produce high quality seed lots, showing performance superior only to the control treatment. The latter, which did not receive any nitrogen source, resulted in the production of lower quality seeds. Additionally, although furrow inoculation yielded better results compared to seed inoculation, its performance was inferior to the combined strategy of furrow inoculation followed by topdressing reinoculation.

These results may be attributed to the formation of new nodules after reinoculation, ensuring a greater nitrogen supply during the crop's peak demand period (flowering and grain filling). These findings corroborate studies by Hungria et al. (2015) and Moretti et al. (2018), which indicate that a single inoculation, whether via seed or planting furrow, may be insufficient to meet the plant's nutritional needs.

(A)



(B)

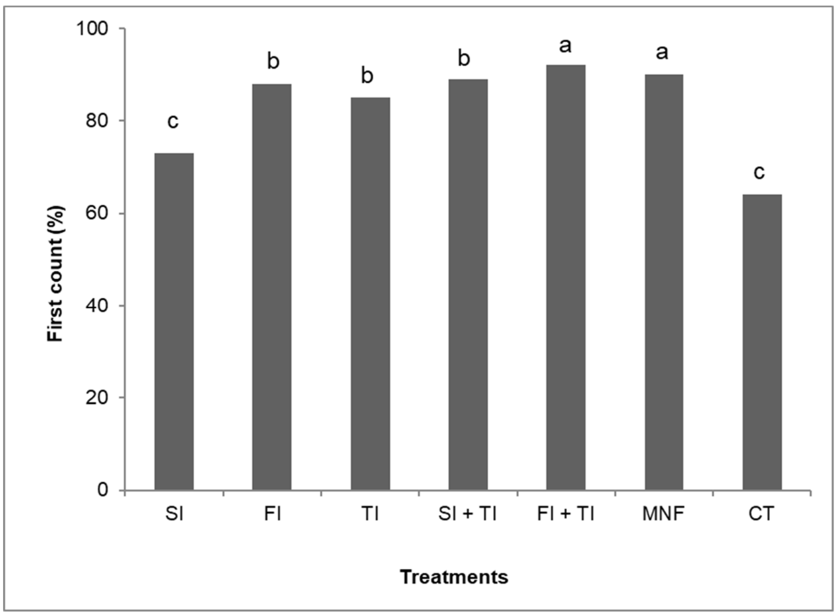


Figure 1. Common bean seed vigor as a function of inoculant application method, based on germination (A) and first count (B) test results

Seed vigor, assessed through the first count test, confirmed the results of the germination test. The treatment with furrow inoculation followed by topdressing reinoculation at the V4 stage stood out, with performance statistically equivalent to that of nitrogen fertilization. The observed vigor values in these treatments were 87% and 85%, respectively (Figure 1B). Conversely, treatments where inoculation was performed only once whether via seed, furrow, or topdressing resulted in lower quality seed lots, similar to the values observed in the control treatment.

Seedling vigor is directly related to seedling emergence capacity in the soil (Marcos Filho, 2015), typically at an average depth of 7 cm. This parameter is crucial as it influences seedling emergence speed, reducing competition with soilborne pests and diseases and ensuring greater uniformity in the initial stand, which can minimize weed problems.

The results of the accelerated aging test, though showing lower percentages of vigorous seedlings, also indicated superior performance for the treatments with furrow inoculation followed by topdressing reinoculation at the V4 stage. This treatment was statistically similar to the vigor observed in seeds from the mineral nitrogen fertilization treatment (Figure 2). The lower viability observed in this test may be attributed to the extreme conditions imposed (high temperature and humidity), which simulate field challenges, making it a highly realistic method for evaluating seed longevity.

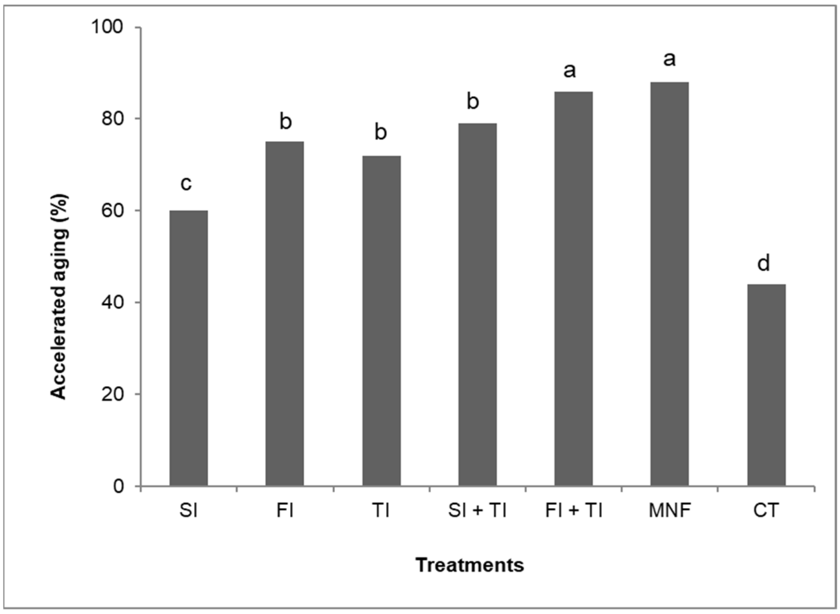


Figure 2. Common bean seed accelerated aging as a function of inoculant application method, based on accelerated aging test results

Treatments that received only one inoculant application, regardless of the application method, showed lower seedling vigor, statistically similar to the control treatment. These results reinforce the hypothesis that a single inoculant application is insufficient to meet the nitrogen demand of common bean, negatively impacting seed quality.

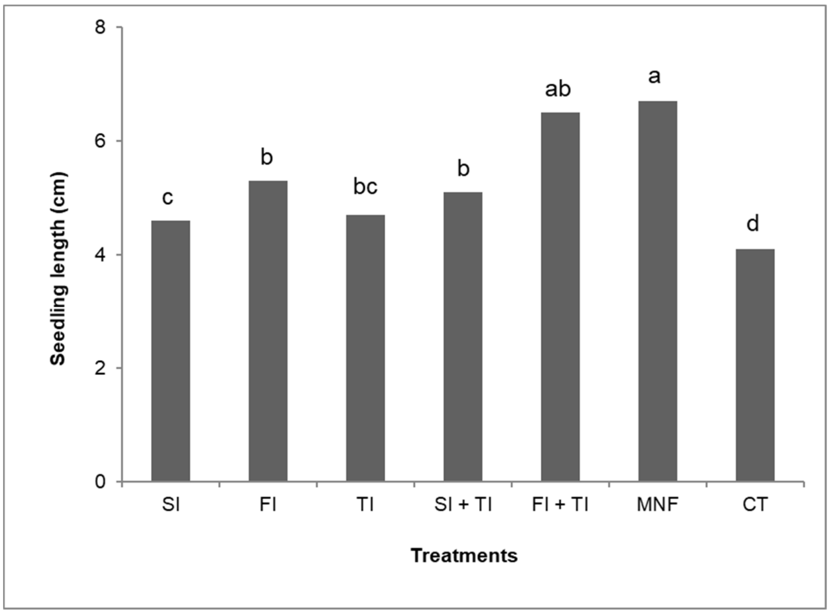
Despite some variations in statistical significance among treatments, the results of the seedling length test (Figure 3A) and seedling dry mass test (Figure 3B) were consistent with those obtained in previous tests. The highest average values for seedling length (6.8 cm) and seedling dry mass (0.08 g) were observed in the treatments with *R. tropici* inoculation in the planting furrow followed by topdressing reinoculation at the V4 stage, being statistically equivalent to the mineral nitrogen fertilization treatment. These values were significantly higher than those observed in the other treatments, especially in those with a single inoculation via seed, furrow, or topdressing.

The results confirm that the strategy of planting furrow inoculation, complemented by topdressing reinoculation, is capable of meeting the nitrogen demand of common bean, directly impacting the physiological quality of the produced seeds. Furthermore, it was found that this strategy can largely replace mineral nitrogen fertilization, making it a viable alternative for producing high quality seeds.

The cost of nitrogen fertilizer such as urea, the most used source in fertilizations was 220.58 USD per hectare in March 2021 (Osaki, 2022) in Brazil, while the cost of inoculant with *R. tropici* for beans is normally less than 1.70 USD per hectare. This price difference is extremely important, especially in cases where the chances of yield losses due to adverse factors are high, and clearly proves the lower cost for producing bean seeds using inoculants compared to the use of nitrogen fertilizers, in addition to improving seed quality as verified in this research.

It is also noteworthy that the use of inoculants in bean cultivation is an effective strategy to reduce costs with nitrogen fertilizers and make production more sustainable. This practice not only guarantees high yield and seed quality, but also contributes to more balanced and ecologically responsible agriculture, contributing to reducing greenhouse gas emissions, water contamination and improving soil health.

(A)



(B)

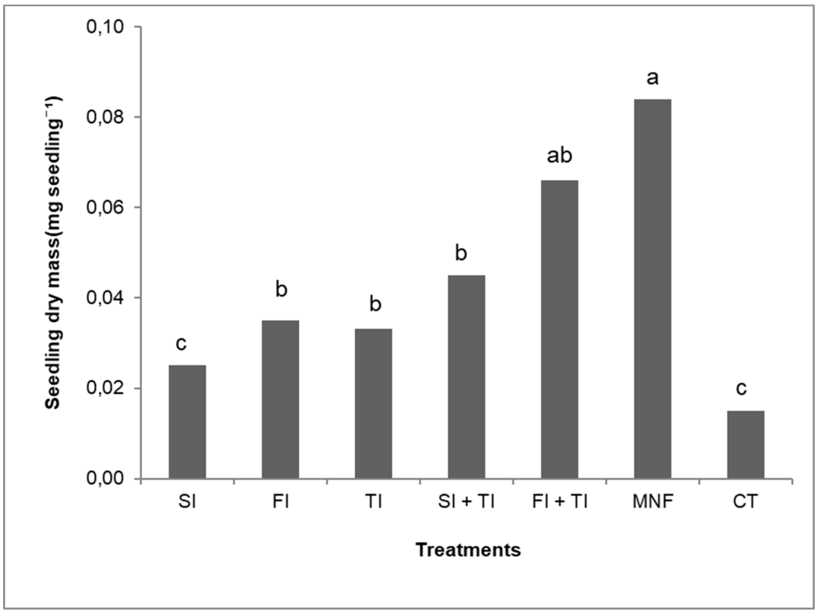


Figure 3. Common bean seed seedling lengh and seedling dry mass as a function of inoculant application method, based on seedling length (A) and seedling dry mass (B) test results

4. Conclusions

The physiological quality of bean seeds is influenced by the method of inoculant application in the crop.

Inoculation with *R. tropici* in the planting furrow, combined with reinoculation as a top dressing at the V4 stage, results in the production of superior quality bean seeds.

Furthermore, this strategy can partially or completely replace nitrogen fertilization, contributing to the efficiency of fertilization in bean cultivation.

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Competing interests

Not applicable.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

Not commissioned; externally double-blind peer reviewed.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.