REMUNOM
ISSN 2178-6925

Received: 27/09/2026 - **Accepted:** 13/10/2026
Vol: 13.21
DOI: 10.66104/smjdtt60
Pages: 1-18

DOES INOCULATION METHOD AFFECT SOYBEAN PERFORMANCE? COMPARING SEED AND FURROW APPLICATION ACROSS FIELD CONDITIONS

O MÉTODO DE INOCULAÇÃO AFETA O DESEMPENHO DA SOJA? COMPARAÇÃO ENTRE A APLICAÇÃO NA SEMENTE E NO SULCO EM CONDIÇÕES DE CAMPO

¿AFECTA EL MÉTODO DE INOCULACIÓN AL RENDIMIENTO DE LA SOJA? COMPARACIÓN DE LA APLICACIÓN EN SEMILLA Y EN SURCO BAJO DIVERSAS CONDICIONES DE CAMPO

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REMUNOM
ISSN 2178-6925

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Resumo

A inoculação de sementes é uma prática amplamente utilizada na cultura da soja, porém a aplicação de inoculantes no sulco de semeadura constitui uma alternativa de manejo que pode reduzir a exposição dos microrganismos aos produtos utilizados no tratamento de sementes. Este estudo teve como objetivo avaliar os efeitos da inoculação via semente e via sulco de semeadura sobre os componentes de rendimento e a produtividade da soja em diferentes ambientes e safras de cultivo. Foram conduzidos dois experimentos de campo nas safras 2020/21 e 2021/22, avaliando-se características de crescimento, componentes de rendimento e produtividade de grãos. No Experimento 1, na safra 2020/21, a inoculação via sulco promoveu maior número de vagens por planta em comparação à inoculação via semente. No Experimento 2, não foram observadas diferenças significativas entre os métodos de inoculação para as características avaliadas em ambos os experimentos. A produtividade de grãos não diferiu significativamente entre os métodos de inoculação em nenhuma das safras ou experimentos. Os resultados indicam que os efeitos da forma de aplicação foram pontuais e variáveis entre as condições de cultivo, sem evidência de diferença consistente no desempenho produtivo da soja. Assim, nas condições avaliadas, a escolha entre a inoculação via semente e via sulco pode considerar aspectos operacionais e de manejo, além da resposta agronômica.
Palavras-chave: *Glycine max*; inoculação; inoculante; sulco de semeadura; produtividade.

Abstract

Seed inoculation is a widely used practice in soybean production; however, applying inoculant in the sowing furrow is a management alternative that may reduce microbial exposure to seed-treatment products. This study aimed to evaluate the effects of seed and furrow inoculation on soybean yield components across different environments and growing seasons. Two field experiments were conducted during the 2020/21 and 2021/22 growing seasons, evaluating growth traits, yield components, and grain yield. In Experiment 1, during the 2020/21 season, furrow inoculation resulted in a higher number of pods per plant and 1000-grain weight compared with seed inoculation. In Experiment 2, no significant differences between inoculation methods were observed for the evaluated traits in either experiment. Grain yield did not differ significantly between inoculation methods in any season or experiment. The results indicate that the effects of inoculation method were specific and varied across growing conditions, with no consistent difference in soybean productive performance. Thus, under the conditions evaluated, the choice between seed and furrow inoculation may consider operational and management aspects in addition to agronomic responses.
Keywords: *Glycine max*; inoculation; inoculant; sowing furrow; grain yield.

Resumen

La inoculación de semillas es una práctica ampliamente utilizada en el cultivo de soja; sin embargo, la aplicación de inoculantes en el surco de siembra constituye una alternativa de manejo que puede

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reducir la exposición de los microorganismos a los productos utilizados en el tratamiento de semillas. Este estudio tuvo como objetivo evaluar los efectos de la inoculación vía semilla y vía surco de siembra sobre los componentes del rendimiento y la productividad de la soja en diferentes ambientes y campañas agrícolas. Se realizaron dos experimentos de campo durante las campañas 2020/21 y 2021/22, evaluándose características de crecimiento, componentes del rendimiento y productividad de granos. En el Experimento 1, durante la campaña 2020/21, la inoculación vía surco promovió un mayor número de vainas por planta en comparación con la inoculación vía semilla. En el Experimento 2, no se observaron diferencias significativas entre los métodos de inoculación para las características evaluadas en ninguno de los experimentos. La productividad de granos no difirió significativamente entre los métodos de inoculación en ninguna de las campañas o experimentos. Los resultados indican que los efectos del método de inoculación fueron puntuales y variables entre las condiciones de cultivo, sin evidencia de una diferencia consistente en el desempeño productivo de la soja. Por lo tanto, en las condiciones evaluadas, la elección entre la inoculación vía semilla y vía surco puede considerar aspectos operativos y de manejo, además de la respuesta agronómica.
Palabras clave: *Glycine max*; inoculación; inoculante; surco de siembra; productividad de granos.

1. Introduction

Biological nitrogen fixation (BNF), which results from the symbiotic association between bacteria of the genus Bradyrhizobium and soybean crops, is one of the key components of the sustainability and competitiveness of soybean production (Hungria *et al*., 2024). The association between these microorganisms and soybeans significantly meets the crop's nitrogen demand, reducing the need for mineral nitrogen sources and helping to lower production costs (Pandit *et al.*, 2026). Furthermore, the activity of root-associated microorganisms is essential for achieving high crop yields (Galindo *et al*., 2018).

Bradyrhizobium inoculation is traditionally carried out using the seed treatment method, in which the inoculant comes into direct contact with the seeds before planting. However, the effectiveness of this strategy can be compromised by the bacteria's exposure to products used in seed treatment, including fungicides, insecticides, and micronutrients (Campo *et al.,* 2010).

Incompatibility between inoculants and plant protection products poses a significant challenge to maintaining bacterial viability and establishment during crop establishment (Braccini *et al.*, 2025). In this context, applying inoculants directly into the seed furrow has been proposed as an alternative to reduce contact between the microorganisms and the products applied to the seeds.

Furrow inoculation involves applying the inoculant directly to the soil, near the area where the roots will establish themselves. This strategy can promote contact

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between the microorganisms and the developing root system and reduce the microorganisms' exposure to seed treatment products. Studies conducted under field conditions have indicated that furrow inoculation can reduce the negative effects of fungicides and micronutrients on nodulation and biological nitrogen fixation and is considered an alternative to conventional seed inoculation (Pereira *et al*., 2016a). However, the performance of each method may vary depending on edaphoclimatic conditions, soil characteristics, and growing conditions; thus, a potential advantage of furrow application does not necessarily translate into improved agronomic performance of the crop.

Despite the widespread use of seed inoculation and the growing adoption of furrow application, it is still necessary to understand whether changing the inoculation method results in consistent responses regarding the agronomic performance of soybeans under different field conditions. Comparisons between these methods across contrasting environments and growing seasons can help determine whether any differences in the establishment of microorganisms are reflected in the yield components and productivity of the crop.

Therefore, this study aims to assess the effects of seed and furrow inoculation on soybean yield components and productivity under field conditions, across different environments and growing seasons.

2. Methodology

Location and experimental conditions

The experiments were conducted at the experimental site of the Federal Institute of Farroupilha, São Vicente do Sul Campus, located in the Central Depression of the state of Rio Grande do Sul (Latitude: 29° 41' 30''S, Longitude: 54° 40' 46 W, Altitude: 129 m). The climate is classified as humid subtropical (Cfa) according to Köppen and Geiger (1928).

The experiments were conducted during the 2020/21 and 2021/22 growing seasons under two distinct conditions: Experiment 1 was conducted under high-input conditions, with investment in soil fertility and supplemental irrigation via a center-pivot sprinkler system. Experiment 2 - under conditions typical of soybean–rice rotation

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fields. The soil in Experiment 1 is classified as Argissolo, and in Experiment 2, as Planossolo. Experiment 1 was conducted in an area with a long-term history of ten years of continuous soybean cultivation as the primary summer crop. Experiment 2 was established in a lowland field managed under a soybean–rice crop rotation system, featuring a two-year interval between soybean crops and flooded rice. Prior to the establishment of this study, both experimental fields had received regular inoculations with commercial *Bradyrhizobium* inoculants during previous soybean growing seasons.

An automated spraying system coupled to the seeding rows was used to apply the inoculants to the furrow.

Experimental design and treatments

The experiments were conducted using a randomized block design with four replicates. The treatments included two inoculation methods: (1) inoculation seed and (2) inoculation furrow.

Each experimental unit consisted of seven 5-meter-long rows spaced 0.45 meters apart. A total of 16 experimental units were used.

Crop establishment and management

In the 2020/21 growing season, the preceding crop in Experiment 1 was oats, while ryegrass was used as a cover crop in Experiment 2. During the winter period of the 2021/22 growing season, ryegrass was used as a cover crop in both experiments.

In the 2020/21 growing season, the soybean cultivar Brasmax Garra 63i64 RSF IPRO, belonging to maturity group 6.3, was used. In the 2021/22 growing season, the cultivar Brasmax Compacta 65i65 RSF IPRO, belonging to maturity group 6.5, was used. Both cultivars have a growth cycle considered average. The seeds were treated with the chemical fungicides Metalaxyl-M (20 g/L – 2.0% w/v), Thiabendazole (150 g/L – 15.0% w/v), and Fludioxonil (25 g/L – 2.5% w/v), as well as the chemical insecticides Abamectin (500.0 g/L) and Thiamethoxam (350 g/L).

Seeding was carried out within the period recommended by the Agricultural Climate Risk Zoning for the region (microregion 101), on November 3 for the 2020/21

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crop and on November 9 for the 2021/22 crop. The plant population was set at 250,000 plants ha^{-1}. For seed inoculation, the inoculant was applied to the seeds approximately 6 h before sowing, and the inoculated seeds were subsequently used for sowing. The inoculant used was *Bradyrhizobium elkanii*, strains SEMIA 587 and SEMIA 5019, at a concentration of 1×10^{9} viable cells per mL. The inoculant doses followed the recommendations of Hungria and Nogueira (2020), with 100 mL of inoculant per 50 kg of seeds in 1×10^{11} cells/ha^{-1} concentration, and 2.5×10^{11} cells/ha^{-1}, corresponding to 250 mL in 40 liters for the furrow-sowing method.

Prior to the present study, both experimental fields received annual re-inoculation with commercial *Bradyrhizobium* inoculants during previous soybean cropping seasons. This practice aligns with regional technical recommendations for continuous biological nitrogen fixation management in Southern Brazil.

Fertilizer was applied to the seed furrow at a rate of 350 kg ha^{-1} of 5-20-20 fertilizer, based on the soil's chemical characteristics. In Experiment 1, supplemental irrigation was carried out as needed by the crop during periods characterized by the absence of precipitation for several consecutive days. The other management practices were carried out in accordance with the technical guidelines for soybean cultivation in the states of Rio Grande do Sul and Santa Catarina (Ceraffa *et al.*, 2018).

Agronomic evaluations

The agronomic performance of soybeans was evaluated at the R8 growth stage by determining the crop's yield components and productivity.

The yield components evaluated were plant height, height of the first pod insertion, number of stems per plant, number of productive nodes, number of unproductive nodes, number of pods per plant, and number of seeds per pod.

To determine productivity, the plots were harvested and weighed, and the weight of 1,000 seeds was determined. The values were corrected for 13% moisture content. The harvest area consisted of five 2-meter-long rows, with the outer rows excluded to minimize edge effects.

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Statistical analysis

Data was evaluated using a randomized complete block design with four replications. Prior to analysis of variance (ANOVA), statistical assumptions were verified: residual normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was tested using Levene's test.

Data transformations were applied to the number of pods per plant (2020/21 growing season) and the number of stems per plant (2021/22 growing season) prior to analysis to meet ANOVA assumptions. Mean comparisons between inoculation methods were performed using Tukey's HSD test at a 5% significance level ($p < 0.05$). Treatment means are reported accompanied by their respective standard errors of the mean (Mean $\pm$ SE), exact p-values, and coefficients of variation (CV %). All statistical analyses were conducted in R software (version 4.4.1) using the AgroR package.

3. Results and discussion

Climatic conditions varied between the growing seasons evaluated (Figure 1). During the growing periods, differences were observed in the distribution of precipitation, mean air temperature, and solar radiation, particularly between the 2020/21 and 2021/22 growing seasons. These variations resulted in different environmental conditions for crop development and were considered when interpreting the responses to the inoculation methods.

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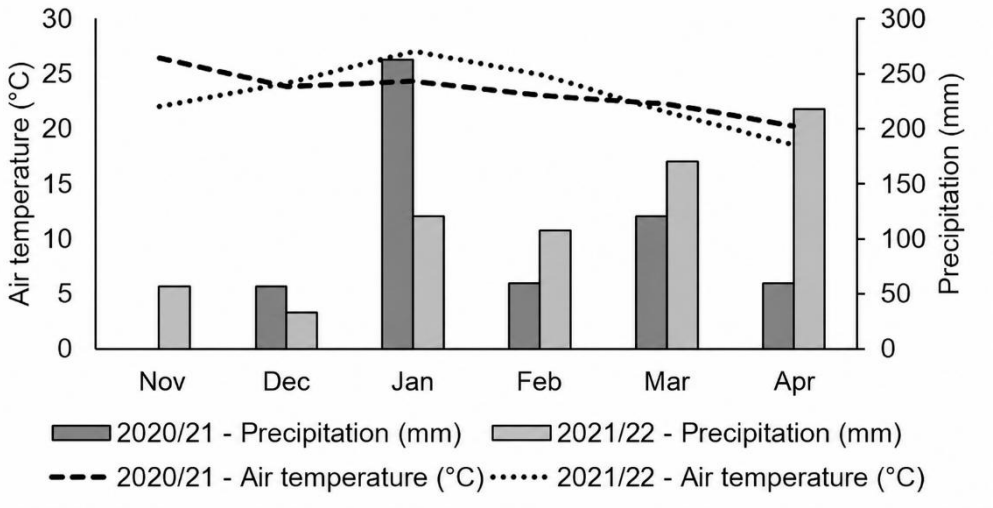
Figure 1. Precipitation (mm) and mean air temperature (°C) recorded between November 2020 and April 2022 in São Vicente do Sul, Rio Grande do Sul, Brazil.



The contrasting responses observed across crop seasons highlight the critical role of environmental conditions during the early stages of crop establishment and the reproductive stages. In the 2020/21 growing season, adequate rainfall distribution after sowing may have provided more favorable soil moisture conditions for early crop establishment; however, bacterial establishment and nodulation were not directly evaluated in this study.

In contrast, the 2021/22 growing season was characterized by periods of high air temperature and lower water availability, particularly during critical stages of crop development. Under lower water availability, physiological constraints on nitrogen fixation occur (King; Purcell, 2001; Sinclair *et al*., 2007), explaining the absence of significant responses to the treatments for all traits evaluated in 2021/22.

Experiment 1

In the 2020/21 growing season, the inoculation method influenced specific components of soybean yield (Table 1). Furrow inoculation significantly increased the number of pods per plant (NPP), reaching 80.9 pods per plant compared to 62.2 pods per plant with seed inoculation ($p = 0.003$), representing an increase of approximately 29.9%.

Although furrow inoculation increased NPP, this structural improvement was not

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reflected in significant gains in final grain yield (5,270.3 kg ha^{-1} for seed inoculation versus 5,144.3 kg ha^{-1} for furrow inoculation; p = 0.686). This apparent inconsistency is explained by the physiological compensation mechanisms typical of soybean yield components. Final grain yield is a complex trait resulting from the interaction among plant density, the number of pods per plant, the number of seeds per pod, and individual seed weight. Consequently, localized increases in a single yield component do not necessarily result in proportional increases in total crop productivity, as compensatory adjustments occur throughout the canopy (Board; Kahlon, 2011; Egli, 2015).

In addition, grain yield reflects the total mass of grain produced per unit area. Microspatial adjustments in the canopy and variability among plants often mitigate the benefits observed at the individual-plant level. Therefore, the higher NPP observed with furrow application was insufficient to generate significant yield gains on the field scale.

Table 1. Performance of soybean yield components under seed and furrow inoculation methods in Experiment 1 during the 2020/21 and 2021/22 growing seasons.

2020/21								
Inoculation Method	**PHT (cm)**	**FPH (cm)**	**NSP**	**NNP**	**NPN**	**NPP**	**NGP**	**TGW (g)**
Seed	128.1 ± 1.97 ns	32.2 ± 0.76	1.7 ± 0.55	24.2 ± 0.33	7.4 ± 0.33	62.2 ± 3.79 b	2.4 ± 0.03	184.5 ± 2.28
Furrow	129.2 ± 1.97	31.8 ± 0.76	2.2 ± 0.55	24.7 ± 0.33	7.2 ± 0.33	80.9 ± 3.79 a	2.4 ± 0.03	191.1 ± 2.28
p-value	0.686	0.689	0.542	0.696	0.696	0.003	0.676	0.055
CV (%)	5.3	8.2	44.5	7.4	15.9	18.3	4.6	4.2
2021/22								
Inoculation Method	**PHT (cm)**	**FPH (cm)**	**NSP**	**NNP**	**NPN**	**NPP**	**NGP**	**TGW (g)**
Seed	74.2 ± 1.45	22.2 ± 0.78	1.7 ± 0.22	20.6 ± 0.43	6.2 ± 0.22	48.7 ± 3.68	2.2 ± 0.04	189.9 ± 2.82
Furrow	75.3 ± 1.45	21.2 ± 0.78	1.2 ± 0.22	21.4 ± 0.43	6.7 ± 0.22	50.1 ± 3.68	2.2 ± 0.04	192.1 ± 2.82
p-value	0.623	0.383	0.166	0.205	0.14	0.795	0.408	0.608
CV (%)	6.7	12.4	51.6	7.1	11.8	25.8	6.8	5.1

*Values are expressed as mean ± standard error (SE). **PHT** = plant height; **FPH** = first pod height; **NSP** = number of stems per plant; **NNP** = number of nodes per plant; **NPN** = number of non-productive nodes per plant; **NPP** = number of pods per plant; **NGP** = number of grains per pod; **TGW** = thousand-grain weight; CV = coefficient of variation (%). None of the variables differed significantly between inoculation methods at the 5% significance level in either growing season (p > 0.05).

No significant differences between inoculation methods were observed for plant

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height, first pod insertion height, number of stems per plant, number of nodes per plant, number of unproductive nodes, or number of grains per pod in the 2020/21 growing season. In the 2021/22 growing season, none of the evaluated variables differed significantly between inoculation methods, indicating that the effects observed in the previous season were not consistent across growing cycles.

One possible explanation is the lower direct exposure of *Bradyrhizobium* cells to seed-treatment chemicals, although nodulation, biological nitrogen fixation, and bacterial survival were not directly evaluated in the present study. Furrow application may also reduce microbial exposure to products used in seed treatment and could potentially favor bacterial survival and establishment compared with seed application; however, this mechanism was not directly evaluated in the present study (Vieira Neto et al., 2008; Campo et al., 2010).

Despite the difference observed in the number of pods per plant, this effect was not accompanied by a significant difference in grain yield (Figure 2). This result indicates that an increase in specific yield components does not necessarily result in a proportional increase in grain yield, as final yield is determined by the interaction among different yield components and environmental conditions throughout the crop cycle.

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ISSN 2178-6925

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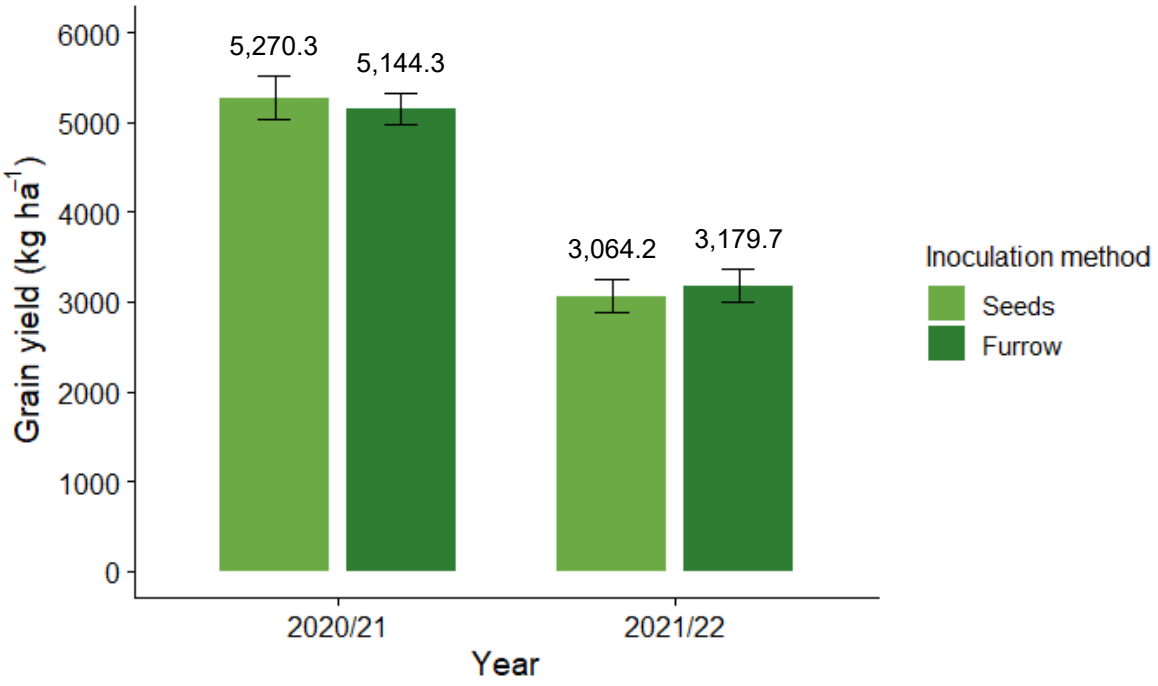
Figure 2. Mean grain yield, in kilograms per hectare, in Experiment 1. A: 2020/21 growing season; B: 2021/22 growing season.



*ANOVA F-test 2020/21 p= 0.68 CV (%): 14.4; 2021/22 p= 0.67 CV (%): 21.0. CV = coefficient of variation.

Regarding grain yield, no differences were observed between the inoculation methods in any of the crops evaluated. In the 2020/21 growing season, although furrow inoculation resulted in a higher numerical mean grain yield, the difference was not sufficient to demonstrate a significant effect of the inoculation method. Souza *et al*. (2020) observed a greater increase when the inoculant was deposited directly onto the seed compared with furrow inoculation, whereas Braccini *et al*. (2016) highlighted seed application as an alternative capable of optimizing inoculation costs. Thus, under the conditions evaluated, no significant differences in grain yield were detected between the two inoculation methods, despite specific differences in some yield components during the 2020/21 growing season.

Under conditions of lower water availability, furrow inoculation may offer advantages associated with the greater proximity of microorganisms to the root zone and the availability of moisture at the time of application, potentially favoring their survival (Pereira *et al*., 2016b).

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However, in the present study, the occurrence of periods of lower water availability did not result in significant differences between the inoculation methods for yield components or grain yield. This result indicates that the potential advantage of furrow application under conditions of lower water availability was not sufficient to produce a consistent agronomic response during the 2021/22 growing season.

Experiment 2

In Experiment 2, conducted under lowland conditions, no differences were observed in the inoculation methods (Table 2), number of stems per plant, number of nodes per plant, number of non-productive nodes, number of pods per plant, or number of grains per pod during the 2020/21 growing season. In the 2021/22 growing season, none of the evaluated variables differed significantly between the inoculation methods.

Table 2. Performance of soybean yield components under seed and furrow inoculation methods in the lowland environment during the 2020/21 and 2021/22 growing seasons.

2020/21								
Inoculation Method	**PHT (cm)**	**FPH (cm)**	**NSP**	**NNP**	**NPN**	**NPP**	**NGP**	**TGW (g)**
Seed	129.2 ± 3.23 ns	33.8 ± 1.06	4.6 ± 0.24	25.0 ± 0.37	4.5 ± 0.45	93.7 ± 4.33	2.4 ± 0.02	175.2 ± 3.62
Furrow	138.1 ± 3.23	36.1 ± 1.06	4.1 ± 0.24	25.9 ± 0.37	5.3 ± 0.45	87.1 ± 4.33	2.4 ± 0.02	168.7 ± 3.62
p-value	0.068	0.152	0.11	0.13	0.228	0.292	0.068	0.224
CV (%)	8.4	10.5	19.4	5	31.9	16.6	2.7	7.3
2021/22								
Inoculation Method	**PHT (cm)**	**FPH (cm)**	**NSP**	**NNP**	**NPN**	**NPP**	**NGP**	**TGW (g)**
Seed	79.5 ± 1.95	22.9 ± 1.23	1.2 ± 0.19	20.9 ± 0.31	6.5 ± 0.17	40.5 ± 3.68	2.3 ± 0.04	177.3 ± 4.49
Furrow	80.5 ± 1.95	21.2 ± 1.23	1.1 ± 0.19	21.0 ± 0.31	6.6 ± 0.17	41.2 ± 3.68	2.2 ± 0.04	171.6 ± 4.49
p-value	0.717	0.323	0.708	0.721	0.67	0.795	0.134	0.379
CV (%)	8.4	19.4	56.9	5.2	9	25.8	5.5	8.9

*Values are expressed as mean ± standard error (SE). **PHT** = plant height; **FPH** = first pod height; **NSP** = number of stems per plant; **NNP** = number of nodes per plant; **NPN** = number of non-productive nodes per plant; **NPP** = number of pods per plant; **NGP** = number of grains per pod; **TGW** = thousand-grain weight; CV = coefficient of variation (%). None of the variables differed significantly between inoculation methods at the 5% significance level in either growing season ($p > 0.05$).

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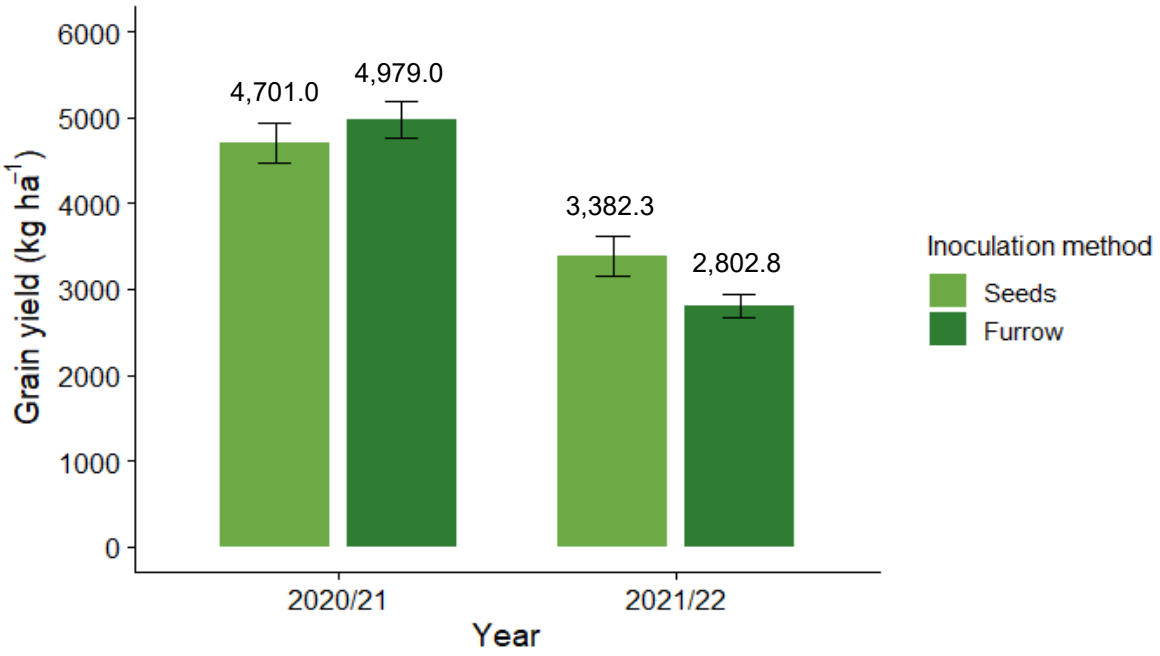
Some yield components showed relatively high coefficients of variation, particularly the number of stems per plant and the number of non-productive nodes. This variability may reflect the intrinsic variability of these plant-level traits and the influence of environmental and plant-to-plant differences within the experimental plots. Therefore, the results for these variables should be interpreted with caution, particularly when no significant differences between inoculation methods were detected.

Regarding grain yield, no differences between the inoculation methods were observed during the 2020/21 growing season (Figure 3). Although furrow inoculation resulted in a higher numerical mean, this difference was not sufficient to demonstrate an effect of the inoculation method on grain yield.

Figure 3. Mean grain yield, in kilograms per hectare, in Experiment 2. A: 2020/21 growing season; B: 2021/22 growing season.



*ANOVA F-test 2020/21 p= 0.40 CV (%): 16.4; 2021/22 p= 0.05 CV (%): 21.4. CV = coefficient of variation.

Under cultivation conditions in Planosols, furrow inoculation may represent an operational alternative due to the characteristics of these environments, which may affect microbial establishment and survival. In addition, aspects related to application efficiency may contribute to the adoption of this technology (Prando *et al*., 2019).

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In the 2021/22 growing season, no significant differences in grain yield were observed between the inoculation methods, although seed inoculation resulted in a higher numerical mean. The variation observed between growing seasons may be related to the different environmental conditions occurring throughout the crop cycle, as inoculation efficiency may be influenced by factors such as water availability and soil conditions.

Results reported by Voss (2002) show that the response to the inoculation method may vary under different water availability conditions. Voss (2002) observed that seed inoculation was superior to furrow inoculation under drought conditions during grain filling. In the present study, however, the numerically higher grain yield obtained with seed inoculation in the 2021/22 growing season did not differ significantly from that obtained with furrow application.

An analysis of the results shows that seed inoculation has the advantage of not requiring specialized spraying equipment on the seeder. However, it imposes restricted operational windows due to microbial sensitivity to chemical seed treatments and the reduction in cell survival within the seed coat over time (Campo et al., 2010; Braccini *et al*., 2025).

In contrast, furrow application reduces the direct contact between the inoculant and agrochemicals applied to the seeds.

Overall interpretation of inoculation responses across growing seasons and experimental conditions

When the results from the two experiments are considered descriptively, the effects of the inoculation method were specific and varied across growing seasons and experimental conditions, with no evidence of a consistent yield advantage for either seed or furrow application. In Experiment 1, furrow application increased the number of pods per plant and 1000-grain weight during the 2020/21 growing season, whereas in Experiment 2, the same method increased plant height during the same growing season. However, these effects were not observed in the 2021/22 growing season.

The absence of a non-inoculated control should also be considered when interpreting the results, as the experimental design allowed comparison between seed

REMUNOM
ISSN 2178-6925

Received: 27/09/2026 - **Accepted:** 13/10/2026
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DOI: 10.66104/smjdtt60
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and furrow inoculation methods but did not allow the effect of inoculation itself to be evaluated against a non-inoculated treatment.

The lack of consistent differences between the methods was even more evident for grain yield. In neither experiment were significant differences in grain yield observed between seed and furrow inoculation, although the direction of the numerical differences varied between experiments and growing seasons. This pattern suggests that specific changes in vegetative traits or yield components do not necessarily result in differences in final crop yield.

The variable response between growing seasons may be related to differences in the environmental conditions prevailing during crop development. Water availability, soil characteristics, and growing conditions may affect the survival, establishment, and activity of microorganisms associated with plants. It should also be noted that different soybean cultivars were used between growing seasons, which prevents the effects of growing season from being interpreted independently of cultivar. Therefore, differences between 2020/21 and 2021/22 should be interpreted as responses observed under different combinations of environmental and genetic conditions rather than as a pure growing-season effect.

Additionally, the cultivars used in the two growing seasons differed in maturity group (6.3 in 2020/21 and 6.5 in 2021/22), which should also be considered when interpreting the results. Differences in maturity group may influence crop development and the timing of reproductive stages, potentially affecting plant responses to environmental conditions and inoculation. However, because cultivar and growing season were not evaluated independently in the present study, the specific contribution of maturity group to the observed differences cannot be isolated.

The lack of marked yield differences between seed and in-furrow inoculation across growing seasons can also be explained by the presence of an established, naturalized *Bradyrhizobium* population in the soil. In agricultural fields with a history of soybean cultivation and annual re-inoculation in Southern Brazil, naturalized rhizobial populations often exceed 10^3 to 10^5 cells g^{-1} of soil (Hungria; Nogueira, 2020). This naturalized microbial pool supplies substantial nodulation capability throughout the root profile. Consequently, while localized in-furrow application may enhance early bacterial

REMUNOM
ISSN 2178-6925

Received: 27/09/2026 - **Accepted:** 13/10/2026
Vol: 13.21
DOI: 10.66104/smjdtt60
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survival near the root crown under specific conditions, the baseline nitrogen fixation driven by soil-resident strains buffers the overall crop performance, smoothing out potential yield disparities between application methods (Hungria *et al*., 2024)

Therefore, the results indicate that the choice of inoculation method should not be based exclusively on the expectation of increased grain yield, since both systems showed similar productive performance under the conditions evaluated. From an operational perspective, the choice between seed and furrow application may also depend on characteristics of the production system, application logistics, compatibility with products used for seed treatment, and the specific conditions of each environment. These aspects were not directly evaluated in the present study and should therefore be considered as practical implications rather than experimental outcomes.

4. Conclusion

No significant differences in grain yield were detected between seed and furrow inoculation under the conditions evaluated. Although differences were detected for some yield components in 2020/21, these responses were not consistent across growing seasons and did not result in differences in grain yield.

In Experiment 1, furrow inoculation resulted in a higher number of pods per plant in the 2020/21 growing season. These effects were not observed in experiment 2, indicating that responses of individual agronomic traits varied across growing conditions.

Overall, no significant differences in grain yield were detected between seed and furrow inoculation under the conditions evaluated, and no consistent agronomic advantage of one inoculation method over the other was detected.

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REMUNOM
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Vol: 13.21
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