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CROP PRODUCTION

Soybean production in SEALBA: inoculation, co-inoculation, and starter nitrogen fertilization

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ABSTRACT. The grain production area in the Brazilian agricultural region known as SEALBA, especially in Alagoas State, has seen significant growth in recent years. This study aims to contribute to sustainable regional development by evaluating the effects of inoculation, co-inoculation, and the application of starter mineral nitrogen (N), either individually or in combination, on soybean growth and yield. Eight field experiments were conducted from 2018 to 2021 in two municipalities within Alagoas, covering soybean cultivation areas ranging from the first to the fourth year. The experiments followed a randomized complete block design with four replications, employing a factorial arrangement of treatments. The first factor encompassed five levels related to inoculation technologies and N management: 1) Inoculation with *Bradyrhizobium*; 2) Inoculation with *Bradyrhizobium* combined with N basal fertilization; 3) Co-inoculation of *Bradyrhizobium* + *Azospirillum*; 4) Co-inoculation of *Bradyrhizobium* + *Azospirillum* + N fertilization at the base; and 5) Control treatment without microbiological inputs or nitrogen fertilizers. The second factor involved the evaluation of different soybean cultivars, with two materials assessed in 2018 and three cultivars in 2019, 2020, and 2021. Results demonstrated that seed inoculation with *Bradyrhizobium* led to increased soybean grain yield in first-year cultivation areas. However, N basal fertilization, commonly known as starter fertilization, did not result in yield improvements compared to the use of microbiological inputs alone. Soybean cultivars BRS 9383 IPRO and FTR 3191 IPRO exhibited greater responsiveness to seed inoculation with *Bradyrhizobium*.

Keywords: *Glycine max*; *Bradyrhizobium*; *Azospirillum*; nitrogen; cultivar.

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Introduction

Several municipalities in Alagoas State are part of the emerging agricultural region known as SEALBA, which encompasses the Brazilian states of Sergipe, Alagoas, and Bahia (Silva et al., 2022). Alagoas, historically a major sugarcane producer in the Northeast region of Brazil, has faced economic challenges in the sugar and alcohol sector, resulting in some sugarcane mills suspending operations, and previously cultivated sugarcane areas lying fallow. To address this, farmers in the area of *Tabuleiros Costeiros* of Alagoas (Coastal Tablelands of Alagoas) have turned to alternative crops, such as soybeans, to diversify their production.

Brazil currently leads global soybean production, with an estimated cultivated area of 43.53 million hectares in the 2022/2023 season and an average yield of 3,479 kg ha^{-1} (Companhia Nacional de Abastecimento [CONAB], 2023). To sustain its position as the world's largest soybean producer, increasing grain yield and incorporating new cultivation areas are crucial. In 2016, the Ministry of Agriculture, Livestock, and Supply issued the first agricultural zoning ordinance for soybean cultivation in Alagoas State, Brazil, encouraging farmers to start soybean cultivation on previously unused land.

One of the main challenges in first-year soybean cultivation areas is implementing balanced fertilization programs, particularly for nitrogen (N), which is the most heavily demanded nutrient by soybeans, requiring approximately 83 kg of N per ton of grain produced (Freitas, Cerezini, Hungria, & Nogueira, 2022). The most environmentally and economically sustainable method for supplying N to soybeans is biological atmospheric nitrogen fixation (BNF). Symbiotic bacteria of the *Bradyrhizobium* genus associate with soybean plants, forming specialized root structures called nodules, where BNF occurs (Hungria, Campo, & Mendes, 2001).

To emphasize the economic significance of this symbiotic process in soybeans, research has pointed out that BNF contributes around 60% of the N accumulated by plants (Salvagiotti et al., 2008). In Brazil, these figures are even higher due to widespread seed inoculation by farmers (Mendes, Hungria, & Vargas, 2003). The remaining nitrogen can be supplied from soil organic matter, plant residues, or chemical residues from previous crops (Garcia, Ceccon, & Kurihara, 2015). Despite the importance of BNF, occasional insufficient nodule formation in first-year soybean areas, even with inoculation, can result in inadequate N supply for grain production (Zuffo, Steiner, Busch, & Zoz, 2018). This is one reason some farmers employ starter N fertilization, which involves applying N fertilizers during the initial stage (Mandić et al., 2020). However, this practice raises agricultural and environmental concerns, potentially jeopardizing BNF sustainability.

To address these challenges and enhance N supply, practices such as re-inoculation or using other diazotrophic bacteria species have been employed in first-year soybean cultivation areas. A study on annual re-inoculation in soybeans observed an average grain yield increase of 4.7% (Hungria et al., 2006), highlighting the importance of annual re-inoculation in soybean production areas, even in soils with a long history of soybean cultivation and high native populations of *Bradyrhizobium*.

Moreover, to further boost biological N supply, the use of associative bacteria from the *Azospirillum* genus in soybeans is a viable strategy. These bacteria promote plant growth through production of growth hormones, disease resistance, environmental stress tolerance induction, phosphate solubilization, and BNF (Braccini, Mariucci, Suzukawa, Lima, & Piccinin, 2016). This strategy has given rise to co-inoculation technology in Brazil, which is adding more than one plant-benefiting microorganism (Hungria & Nogueira, 2014), combining the traditional application of *Bradyrhizobium* with *Azospirillum* (Barbosa et al., 2023).

However, it is crucial to validate these promising technologies regionally before recommending them to the agricultural sector, as their effectiveness can be influenced by local edaphoclimatic conditions. Given that inoculation and co-inoculation involve living microorganisms, their interaction with environmental factors requires assessment for an adequate response in soybean development and BNF process.

Therefore, this study aimed to assess the effects of inoculation, co-inoculation, and starter mineral N application, individually or in combination, on the development and yield of soybeans grown in SEALBA, Alagoas State, Brazil.

Material and methods

Over a span of four years, eight field experiments were conducted in cities within the state of Alagoas, Brazil, with one experiment taking place in Campo Alegre (166 m altitude) and the remainder in Jundiá (97 m altitude).

The climate in these cities falls under the type *As* category according to the Köppen classification, characterized by dry summers and rainy autumns/winters. Average annual temperature and rainfall in Jundiá and Campo Alegre are approximately 24.0°C and 1,470 mm, and 23.8°C and 1,121 mm, respectively (Climate-Date, 2023). Figure 1 displays the rainfall data between sowing and harvesting for each location and agricultural year. The total rainfall for each experiment and season was as follows: Campo Alegre (2018): 292 mm; Jundiá (2018): 305.5 mm; Jundiá (2019, 1^{st} and 2^{nd} year areas): 1,065 mm; Jundiá (2020, 1st year area): 998 mm; Jundiá (2020, 3^{rd} year area): 703 mm; and Jundiá (2021, 3^{rd} and 4^{th} year areas): 668 mm.

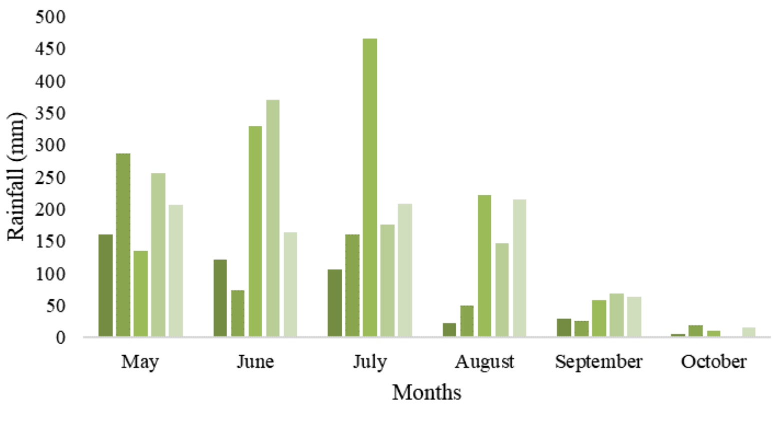

Figure 1. Accumulated rainfall per ten-day period (mm) during the soybean crop development cycle in different locations and agricultural years. CA = Campo Alegre; J = Jundiá.

The soil in all eight experimental areas was classified as *Argissolo Vermelho-Amarelo* (Red-Yellow Argisol) (Santos et al., 2018). Detailed information regarding the location, season, history of soybean cultivation, and the physical and chemical characteristics of the soils in the experimental areas is presented in Table 1.

Table 1. Area description and soil chemical and physical properties (layer 0-20 cm) in the locations where experiments were performed.

City/season	Year[1/]	pH	Ca	Mg	H+Al	Al	K	P	MO	Clay	Silt	Sand
		H_2O	------- $cmol_c\ dm^{-3}$ -------				$mg\ dm^{-3}$		$g\ dm^{-3}$	--------- $g\ kg^{-1}$---------		
Campo Alegre/2018	1st	6.3	1.5	1.1	2.3	0.00	25	27	14.7	166	222	612
Jundiá/2018	4th	5.6	0.7	0.5	3.7	0.02	65	12	19.4	166	235	599
Jundiá/2019	1st	5.1	1.8	0.6	6.5	0.49	30	18	30.0	165	270	565
Jundiá/2019	2nd	5.1	0.5	0.3	5.9	0.26	34	24	18.2	155	286	559
Jundiá/2020	1st	4.3	1.1	1.6	6.6	0.54	30	15	27.0	166	241	593
Jundiá/2020	3rd	4.1	0.8	0.6	5.7	0.71	64	14	19.2	185	302	513
Jundiá/2021	3rd	5.4	1.9	1.8	3.3	0.10	28	5	20.6	166	300	534
Jundiá/2021	4th	4.8	1.3	1.2	4.2	0.45	44	8	15.7	166	147	687

[1/]History of seasons grown with soybean.

A randomized complete block design was employed, following a 5 × 2 factorial scheme (in the 2018 experiments) and 5 × 3 (in the 2019, 2020, and 2021 experiments), with four replications. The treatments comprised five levels related to N inoculation/management technologies (Table 2) and either two soybean cultivars in 2018 or three soybean cultivars in 2019, 2020, and 2021 experiments (Table 3). Each plot consisted of four rows spaced 0.5 m apart and measuring 5 m in length, covering a total area of 10 m^2. The useful area for evaluation was represented by the two central rows, excluding 0.5 m from the initial and final plot edges (useful area = 4 m^2).

Table 2. Treatments involving inoculation technologies and N management in soybean cultivated in the SEALBA region, Alagoas State, Brazil.

Treatment	Description
Control	Without inoculation and nitrogen fertilization.
Inoculation via seed treatment (ST)	*Bradyrhizobium* based product.
Inoculation via ST + N fertilization	*Bradyrhizobium* based product + 22 kg ha^{-1} of N.
Co-inoculation via ST	*Bradyrhizobium* based product + *Azospirillum brasilense* based product.
Co-inoculation via ST + N fertilization	*Bradyrhizobium* based product + *Azospirillum brasilense* based product + 22 kg ha^{-1} of N.

Table 3. Cultivars evaluated in the experiment involving inoculation technologies and N management in soybean cultivated in the SEALBA region, Alagoas State, Brazil.

City/season	Year[1/]	Sowing date	Cultivars	Growth habit	Relative maturity group
Campo Alegre/2018	1st	30/05/2018	BRS 9180 IPRO	Determined	9.1
			BRS 9383 IPRO	Determined	9.3
Jundiá/2018	4th	04/06/2018	BRS 9180 IPRO	Determined	9.1
			BRS 9383 IPRO	Determined	9.3
Jundiá/2019	1st	05/06/2019	BRS 9180 IPRO	Determined	9.1
			BRS 9383 IPRO	Determined	9.3
			FTR 3191 IPRO	Indeterminate	9.1
Jundiá/2019	2nd	06/06/2019	BRS 9180 IPRO	Determined	9.1
			BRS 9383 IPRO	Determined	9.3
			FTR 3191 IPRO	Indeterminate	9.1
Jundiá/2020	1st	07/05/2020	BRS 9180 IPRO	Determined	9.1
			BRS 9383 IPRO	Determined	9.3
			FTR 1192 IPRO	Determined	9.2
Jundiá/2020	3rd	10/06/2020	BRS 9383 IPRO	Determined	9.3
			FTR 3185 IPRO	Indeterminate Determined	8.5
			FTR 1192 IPRO		9.2
Jundiá/2021	3rd	01/06/2021	BRS 9180 IPRO	Determined	9.1
			PP 90 RR	Semi-determined	9.2
			PP 80 RR	Semi-determined	9.4
Jundiá/2021	4th	02/06/2021	BRS 9180 IPRO	Determined	9.1
			PP 90 RR	Semi-determined	9.2
			PP 80 RR	Semi-determined	9.4

[1/]History of seasons grown with soybean.

For seed inoculation via ST (seed treatment), an inoculant based on *Bradyrhizobium* (Semia 5079 and 5080) was used at a rate of five times the recommended dose. For co-inoculation, an inoculant based on *Azospirillum brasilense* (AbV5 and AbV6) was adopted, with three times the recommended dose in 2018 and 2019 and the recommended dose in 2020 and 2021. Except for the 2021 experiments, which used liquid-based inoculants, all other experiments used products in peat formulation. In initial nitrogen fertilization, ammonium nitrate was applied at sowing furrow.

All experiments employed conventional tillage practices, including plowing and harrowing. After soil preparation, the areas were furrowed and mechanically fertilized, with manual sowing. An interrow spacing of 0.5 m was maintained in all experiments. Basal fertilization across all experiments consisted of 500 kg ha^{-1} of simple superphosphate and 120 kg ha^{-1} of potassium chloride. Soybean seeds in all experiments were treated with cobalt and molybdenum (2.4 g Co + 23.4 g Mo per 50 kg of seeds). A total of fifty seeds were planted per meter at a depth of 4 cm, irrespective of the experiment or soybean cultivar under evaluation. Plant thinning was carried out between 8 and 10 days after emergence (DAE), resulting in 18 plants per meter for experiments in 2018 and 2021, and 13 plants per meter in experiments conducted in 2019 and 2020.

In all experiments, top-dressing fertilization involved 120 kg ha^{-1} of potassium chloride, in addition to a foliar application of 100 g ha^{-1} of manganese between 15 and 20 DAE. Throughout the soybean growth cycle, cultural practices were executed in accordance with recommended guidelines, including weed, pest, and disease control, ensuring they did not adversely affect crop development (Seixas, Neumaier, Balbinot Júnior, Krzyzanowski, & Leite, 2020).

Initial assessments of soybean plant height were conducted at 18 and 21 DAE in the Jundiá (2018) and Campo Alegre (2018) experiments, respectively. At harvest in all experiments, the following evaluations were performed: plant height (cm) and first-pod insertion height (cm), with random sampling of 10 plants from the useful area of the plots; 100-grain weight (g) and grain yield (kg ha^{-1}), followed by standardization of grain moisture to 13%. In the experiments conducted in 2018 and 2021, plant lodging was also evaluated at harvest, with visual scores ranging from 1 (no lodging) to 5 (all plants lodged) (Bernard, Chamberlain, & Lawrence, 1965).

Initially, separate statistical analyses were conducted for each experiment using the SISVAR statistical software (Ferreira, 2019). The data underwent analysis of variance by the F-test ($p \leq 0.05$), and when a significant effect was detected among the tested factors, the Tukey's test ($p \leq 0.05$) was applied to compare means.

Multivariate tree regression (MRT) models (Breiman, Friedman, Olshen, & Stone, 1984) were employed to identify patterns of soybean response to different inoculation, co-inoculation, and starter N fertilization practices, considering explanatory variables such as cultivars, the number of seasons cultivated with soybeans (historic), year/season, and the city of the experiment. These relationships were established for the response variables plant height at harvest, first pod height, and grain yield, with separate analyses for each variable. Relationships included: i) inoculation via seed treatment versus control treatment; ii) inoculation via seed treatment + starter nitrogen fertilization versus inoculation via seed treatment; iii) co-inoculation via seed treatment versus inoculation via seed treatment; and iv) co-inoculation via seed treatment + starter nitrogen fertilization versus co-inoculation via seed treatment. Consequently, these relationships express the variation in each analyzed variable in response to the respective management practices, with values equal to 1 indicating no response to the considered practice. Relationships were established among the values of a given response variable obtained from the plots of treatments within the same block. Data from all eight experiments were included in the analysis.

These analyses were conducted using the TreeSplus library, implemented in the S-Plus 2000 statistical software. The analysis sequentially selects each explanatory variable that best explains the variability in the response-variable data, generating bifurcations containing two subsets of samples that exhibit the greatest between-group difference and the greatest within-group homogeneity among all factors and combinations of their respective classes. The explanatory variable associated with each bifurcation, along with their respective separation classes, is indicated for each bifurcation. Each new subgroup is sequentially and individually evaluated concerning the factors associated with variability in the response variable between the existing samples in each subgroup. The selection of the tree size (number of terminal nodes) to be analyzed utilized the modal tree with the minimum prediction error, following a cross-validation procedure. For the analysis of response variables, adjustments to the TreeSplus library parameters were made as follows: "Split measure: squared deviation", "cross-validation criteria: multiple CVs: 20", "CV groups: 10-fold", "min group size: 10", and "select CP: 0.001".

Results and discussion

The experiments conducted in 2018 aimed to assess potential variations in the initial height of soybean plants resulting from the application of inoculation, co-inoculation, and starter N fertilization. These experiments yielded interesting insights.

In the Campo Alegre experiment of the fourth year, conducted in a field with four years of soybean cultivation, we observed an interaction between the inoculation technology/N management factors and soybean cultivars. However, such interaction was absent in the Jundiá experiment, also in its fourth year of soybean cultivation, where the factors being studied did not appear to influence the initial growth of soybean plants (Table 4).

In the Campo Alegre experiment of 2018, the combination between co-inoculation and starter N fertilization resulted in taller plants for cultivars BRS 9180 IPRO and BRS 9383 IPRO when assessed at 21 days after emergence (DAE), compared to the control (Table 4). Nonetheless, the treatment combining co-inoculation with starter N fertilization did not differ significantly from treatments involving only inoculation, inoculation combined with N at the base, or co-inoculation when applied to the BRS 9383 IPRO cultivar. In contrast, the Jundiá experiment of 2018 did not exhibit any noticeable effects of the N inoculation/management technologies on the initial growth of soybean plants, whether for cultivars BRS 9180 IPRO or BRS 9383 IPRO.

Table 4. Initial height of soybean plants as a function of the use of inoculation, co-inoculation, and starting N fertilization.

Cultivar	Control	Inoculation	Inoculation + N	Co-inoculation	Co-inoculation + N	Average
	Plant height (cm) – Campo Alegre (2018) – 4th year of cultivation					
BRS 9180 IPRO	25.8 A b	27.4 A ab	27.7 A ab	25.9 A ab	27.9 A a	26.9
BRS 9383 IPRO	25.7 A b	26.3 A ab	27.4 A ab	25.8 A b	27.9 A a	26.6
Average	25.8	26.9	27.5	25.8	27.9	
CV (%)	3.67					
	Plant height (cm) – Jundiá (2018) – 4th year of cultivation					
BRS 9180 IPRO	24.1	25.1	24.6	24.8	25.0	24.7
BRS 9383 IPRO	23.5	23.9	24.9	24.3	25.4	24.4
Average	23.8	24.5	24.7	24.6	25.2	
CV (%)	4.20					

Means followed by equal letters, lowercase in the row and uppercase in the column, do not differ from each other by the Tukey's test ($p \leq 0.05$).

These findings suggest that applying low basal N doses does not consistently lead to increased initial growth of soybean plants, which contradicts a commonly held belief used to justify the practice of chemical N fertilization in soybean crops. This observation aligns with a study by Aratani, Lazarini, Marques, and Backes (2008), which found no significant increase in the initial height of soybean plants at 25 DAE due to the use of mineral N during sowing.

Regarding the height of soybean plants at harvest, no interactions were observed in any of the eight experiments between the evaluated factors (inoculation technology/N management) and soybean cultivars. Instead, only isolated effects were observed (Table 5). In almost all experiments, with the exception of the one in Jundiá in 2020, which was in its first year of soybean cultivation, there were significant differences in final plant height between cultivars, regardless of the inoculation or N management technique used. For instance, in the 2018 experiments, plants of the BRS 9383 IPRO cultivar were taller at harvest compared to the BRS 9180 IPRO cultivar.

In the 2019 experiments, the cultivar FTR 3191 IPRO displayed the tallest plants, except for the Jundiá experiment in its first year of soybean cultivation, where it was not significantly different from cultivar BRS 9383 IPRO (Table 5). In the 2020 experiments, the BRS 9180 IPRO, BRS 9383 IPRO, and FTR 1192 IPRO cultivars did not show significant differences in final plant height in the Jundiá experiment in its first year of soybean cultivation. However, in the same location and year, but in an area with three years of soybean cultivation, the cultivars FTR 3185 IPRO and BRS 9383 IPRO exhibited taller plants than the cultivar FTR 1192 IPRO. Moving to the 2021 experiments, the PP 80 RR cultivar had the tallest plants at harvest, surpassing the PP 90 RR and BRS 9180 IPRO cultivars.

In half of the experiments, we did observe the effects of inoculation/N management technologies on the final height of soybean plants, regardless of the associated cultivar (Table 5). For instance, in the Campo Alegre experiment of 2018, which was in its fourth year of soybean cultivation, the co-inoculation treatment combined with N at the base resulted in significantly taller soybean plants compared to the control. However, it did not differ significantly from treatments involving only inoculation, inoculation with basal N fertilization, or co-inoculation (Table 5). In the two experiments carried out in areas in their first year of soybean cultivation (2019 and 2020), the control treatment had shorter plants at the time of harvest compared to the other treatments.

Table 5. Height of soybean plants at harvest as a function of the use of inoculation, co-inoculation and starter N fertilization.

Cultivar	Control	Inoculation	Inoculation + N	Co-inoculation	Co-inoculation + N	Average
	Plant height (cm) – Campo Alegre (2018) – 4th year of cultivation					
BRS 9180 IPRO	69.2	71.6	73.5	71.8	77.5	72.7 B
BRS 9383 IPRO	74.9	79.6	77.4	78.2	82.4	78.5 A
Average	72.0 b	75.6 ab	75.4 ab	75.0 ab	79.9 a	
CV (%)	5.26					
	Plant height (cm) – Jundiá (2018) – 4th year of cultivation					
BRS 9180 IPRO	77.9	79.1	81.0	83.7	81.6	80.6 B
BRS 9383 IPRO	86.8	88.2	85.8	84.4	87.2	86.5 A
Average	82.4	83.7	83.4	84.0	84.4	
CV (%)	4.60					
	Plant height (cm) – Jundiá (2019) – 1st year of cultivation					
BRS 9180 IPRO	39.3	55.2	60.7	55.0	60.1	54.0 B
BRS 9383 IPRO	46.6	57.0	60.3	59.5	59.8	56.6 AB
FTR 3191 IPRO	39.8	60.6	65.9	59.6	71.5	59.5 A
Average	41.9 b	57.6 a	62.3 a	58.0 a	63.8 a	
CV (%)	10.07					
	Plant height (cm) – Jundiá (2019) – 2nd year of cultivation					
BRS 9180 IPRO	54.0	48.0	63.6	60.9	61.7	57.6 B
BRS 9383 IPRO	56.4	61.8	58.9	65.8	64.5	61.4 B
FTR 3191 IPRO	91.7	83.0	85.1	90.4	88.5	87.7 A
Average	67.3	64.2	69.2	72.3	71.6	
CV (%)	10.35					
	Plant height (cm) – Jundiá (2020) - 1st year of cultivation					
BRS 9180 IPRO	40.6	49.1	51.4	51.9	49.6	48.5
BRS 9383 IPRO	40.5	48.6	53.6	48.1	53.5	48.8
FTR 1192 IPRO	42.1	55.7	54.9	50.2	51.5	50.9
Average	41.1 b	51.1 a	53.3 a	50.1 a	51.5 a	
CV (%)	8.16					
	Plant height (cm) – Jundiá (2020) – 3rd year of cultivation					
FTR 3185 IPRO	64.5	69.5	68.3	67.0	69.0	67.7 A
BRS 9383 IPRO	62.4	67.1	66.3	64.2	59.7	64.0 A
FTR 1192 IPRO	56.3	56.3	57.5	54.2	52.8	55.4 B
Average	61.1	64.3	64.0	61.8	60.5	
CV (%)	8.92					
	Plant height (cm) – Jundiá (2021) – 3rd year of cultivation					
PP 90 RR	61.8	80.2	80.4	73.9	77.9	74.8 B
BRS 9180 IPRO	59.9	70.0	75.6	70.3	68.9	68.9 B
PP 80 RR	102.0	108.9	117.5	113.4	102.8	108.9 A
Average	74.6 b	86.4 ab	91.2 a	85.9 ab	83.2 ab	
CV (%)	13.48					
	Plant height (cm) – Jundiá (2021) – 4th year of cultivation					
PP 90 RR	81.9	85.0	81.6	83.3	86.5	83.6 B
BRS 9180 IPRO	73.4	73.9	72.1	72.8	75.6	73.6 C
PP 80 RR	102.0	118.3	113.2	111.5	103.0	109.6 A
Average	85.8	92.4	89.0	89.2	88.3	
CV (%)	11.31					

Means followed by equal letters, lowercase in the row and uppercase in the column, do not differ from each other by the Tukey's test ($p \leq 0.05$).

Combining inoculation and basal N fertilization also produced taller soybean plants compared to the control in the Jundiá experiment of 2021, which was in its third year of soybean cultivation, but it did not significantly differ from the other treatments (Table 5). A study investigating the effects of *Bradyrhizobium* inoculation and N fertilization at sowing in soybeans of first and second year found that inoculation did not impact the height of soybean plants at harvest. However, N fertilization stimulated soybean growth, up to a dose of 20 kg ha^{-1} of N, but only in areas with two years of cultivation (Silva et al., 2011).

The multivariate tree analysis provides a comprehensive assessment of the response to inoculation technologies/N management for the parameters under consideration. Specifically, in the case of final plant height, the analysis showed significance only in the comparison between the inoculation (exclusive use of *Bradyrhizobium*) and control treatments (Figure 2). The selected model explained 33% of the variability in the response data for plant height at harvest concerning the use of inoculation compared to the control treatment. The number of seasons cultivated with soybean in the area explained 100% of the observed response. According to the model, two subpopulations of samples emerged, one with 64 and the other with 24 samples, differentiated by the number of seasons of soybean cultivation in the area. This distinction separated samples from the first year of cultivation (number < 1.5 years) and those from subsequent years (number > 1.5 years), revealing an average increase in final plant height in response to *Bradyrhizobium* inoculation of 33% in the first year and 6% in subsequent years. The model did not discern further differences in the response of samples among areas cultivated for two to four years.

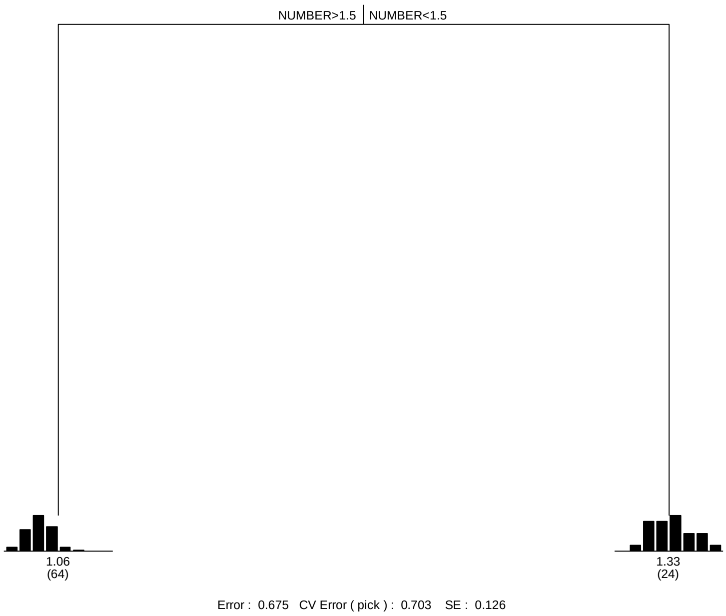

Figure 2. Average final plant height gains in soybeans in response to *Bradyrhizobium* inoculation (comparison between inoculation treatment *versus* control treatment).

As verified for the soybean plant height at harvest, in all the experiments conducted, there was no evidence of interaction between the assessed factors of inoculation technology/N management and soybean cultivars concerning the height of the first pod insertion, with only isolated effects observed (Table 6). The examined soybean cultivars exhibited notable distinctions in terms of the first-pod insertion height in the experiments conducted in 2020 and 2021, regardless of the inoculation/N management technique employed. In the 2020 Jundiá experiment, situated in a first-year soybean cultivation area, the FTR 1192 IPRO cultivar displayed a greater first-pod insertion height than the BRS 9180 IPRO and BRS 9383 IPRO cultivars. Conversely, in another Jundiá experiment conducted in 2020, but in an area undergoing its third year of soybean cultivation, the FTR 1192 IPRO cultivar featured the lowest first-pod insertion height compared to the FTR 3185 IPRO and BRS 9383 IPRO cultivars. In the two experiments carried out in 2021, one in an area of the third year of cultivation and the other in an area of the fourth year, the PP 80 RR cultivar exhibited a greater first-pod insertion height relative to the PP 90 RR and BRS 9180 IPRO cultivars.

Significant impacts of the inoculation technology/N management factor on the height of the first pod insertion in soybean plants were solely detected in experiments conducted within areas undergoing their initial year of soybean cultivation (Table 6). In the Jundiá experiment of 2019, situated in a first-year cultivation area, it was evident that soybean plants within the control treatment exhibited a lower first-pod insertion height in comparison to the other treatments, irrespective of the cultivar under evaluation.

In 2020, within a first-year cultivation area, the combination of inoculation treatment with basal N fertilization led to soybean plants with an increased first-pod insertion height compared to the control treatment. However, this increase was not statistically different from the other treatments that were assessed.

A study that investigated the use of starter N fertilization (20 kg ha^{-1} at sowing) in soybean cultivation within a previously cultivated area found that combining N with inoculation of *Bradyrhizobium* positively impacted first-pod insertion height (Barbosa et al., 2023).

Table 6. First-pod insertion height in soybean plants as a function of the use of inoculation, co-inoculation, and starter N fertilization.

Cultivar	Control	Inoculation	Inoculation + N	Co-inoculation	Co-inoculation + N	Average
	Height of first pod (cm) – Campo Alegre (2018) – 4th year of cultivation					
BRS 9180 IPRO	14.2	16.9	16.3	14.3	15.7	15.5
BRS 9383 IPRO	15.6	15.4	15.8	13.7	15.2	15.1
Average	14.9	16.1	16.1	14.0	15.4	
CV (%)	12.9					
	Height of first pod (cm) – Jundiá (2018) – 4th year of cultivation					
BRS 9180 IPRO	12.1	12.3	12.6	12.9	12.1	12.4
BRS 9383 IPRO	13.0	12.0	13.1	12.3	12.2	12.5
Average	12.5	12.1	12.8	12.6	12.1	
CV (%)	8.57					
	Height of first pod (cm) – Jundiá (2019) – 1st year of cultivation					
BRS 9180 IPRO	7.3	10.7	11.6	10.6	10.5	10.1
BRS 9383 IPRO	8.3	11.6	11.4	10.1	11.8	10.6
FTR 3191 IPRO	10.4	11.1	10.6	11.3	11.3	10.9
Average	8.7 b	11.1 a	11.2 a	10.6 a	11.2 a	
CV (%)	14.03					
	Height of first pod (cm) – Jundiá (2019) – 2nd year of cultivation					
BRS 9180 IPRO	11.8	11.0	12.8	12.7	11.7	12.0
BRS 9383 IPRO	12.7	12.1	10.9	12.7	11.8	12.0
FTR 3191 IPRO	11.2	11.4	11.2	11.7	11.8	11.4
Average	11.9	11.5	11.6	12.4	11.7	
CV (%)	8.35					
	Height of first pod (cm) – Jundiá (2020) – 1st year of cultivation					
BRS 9180 IPRO	9.3	10.1	10.9	10.2	9.9	10.1 B
BRS 9383 IPRO	9.5	10.1	10.4	9.9	10.6	10.1 B
FTR 1192 IPRO	10.2	10.9	11.0	10.6	10.5	10.6 A
Average	9.7 b	10.4 ab	10.8 a	10.2 ab	10.3 ab	
CV (%)	6.51					
	Height of first pod (cm) – Jundiá (2020) – 3rd year of cultivation					
FTR 3185 IPRO	10.3	11.1	10.5	10.3	10.9	10.7 A
BRS 9383 IPRO	10.4	10.3	10.8	10.9	10.0	10.5 A
FTR 1192 IPRO	9.6	9.2	9.5	9.2	8.6	9.2 B
Average	10.1	10.2	10.3	10.1	9.8	
CV (%)	8.95					
	Height of first pod (cm) – Jundiá (2021) – 3rd year of cultivation					
PP 90 RR	11.0	11.7	11.9	11.3	11.5	11.5 B
BRS 9180 IPRO	11.6	12.0	13.6	12.7	11.3	12.2 B
PP 80 RR	14.3	16.3	17.3	16.9	16.3	16.2 A
Average	12.3	13.3	14.3	13.6	13.0	
CV (%)	17.72					
	Height of first pod (cm) – Jundiá (2021) – 4th year of cultivation					
PP 90 RR	10.8	11.8	11.1	12.0	11.3	11.4 B
BRS 9180 IPRO	11.8	11.3	11.7	11.6	12.5	11.8 B
PP 80 RR	16.2	16.4	18.1	18.8	16.4	17.2 A
Average	12.9	13.2	13.6	14.1	13.4	
CV (%)	17.62					

Means followed by equal letters, lowercase in the row and uppercase in the column, do not differ from each other by the Tukey's test ($p \leq 0.05$).

Similar to the findings regarding final plant height, the analysis using tree regression models only demonstrated significance when comparing the inoculation treatments (exclusive use of *Bradyrhizobium*) with the control treatment concerning the evaluation of the first-pod insertion height. The selected model (Figure 3) accounted for 12% of the variability in the first-pod insertion height response data attributed to the use of inoculation in contrast to the control treatment. The explanatory variable, the number of years of soybean cultivation in the area, explained 100% of the observed response. According to the model, two sample subpopulations emerged, one with 64 samples and another with 24 samples, distinguished by the number of years of soybean cultivation in the area. This division was based on samples from the first year of cultivation (number < 1.5 years) and those from subsequent years of cultivation (number > 1.5 years). The model revealed an average increase in first-pod insertion height in response to *Bradyrhizobium* inoculation of 27% in the first year and 3% in subsequent years. Moreover, the model did not reveal further differentiation in response among samples with varying years of cultivation, ranging from two to four, which was also observed in the context of plant height at harvest.

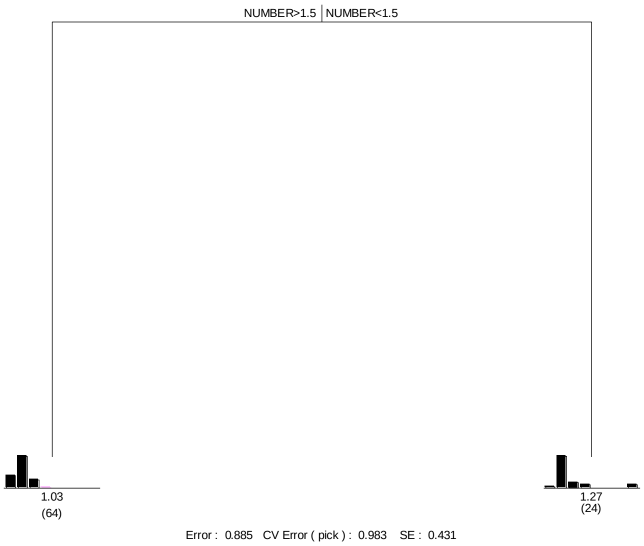

Figure 3. Average first-pod insertion height gains in soybean plants in response to *Bradyrhizobium* inoculation (comparison between inoculation treatment *versus* control treatment).

Lodging in soybean plants is characterized by their bending or collapsing due to stem weakness or insufficient root anchorage (Hwang & Lee, 2019). In the evaluations related to soybean plant lodging, the observed variations were solely attributed to the characteristics of the cultivars, with no discernible influence from the inoculation/N management technologies examined in this context. In the experiments conducted in 2018, there was a lower incidence of lodging observed in the BRS 9180 IPRO cultivar compared to the BRS 9383 IPRO (Table 7). This trend was also evident in the experiments conducted in 2021, where the BRS 9180 IPRO cultivar exhibited a reduced lodging rate compared to the PP 90 RR and PP 80 RR cultivars, solidifying its reputation as the soybean cultivar with the least susceptibility to lodging.

Table 7. Lodging in soybean plants at harvest as a function of the use of inoculation, co-inoculation and starter N fertilization.

Cultivar	Control	Inoculation	Inoculation + N	Co-inoculation	Co-inoculation + N	Average
	Lodging – Campo Alegre (2018) – 4th year of cultivation					
BRS 9180 IPRO	1	1	1	1	1	1
BRS 9383 IPRO	3	3	3	3	3	3
Average	2	2	2	2	2	
	Lodging – Jundiá (2018) – 4th year of cultivation					
BRS 9180 IPRO	2	2	2	2	2	2
BRS 9383 IPRO	4	4	4	4	4	4
Average	3	3	3	3	3	
	Lodging – Jundiá (2021) – 3rd year of cultivation					
PP 90 RR	2	2	2	2	2	2
BRS 9180 IPRO	1	1	1	1	1	1
PP 80 RR	4	4	4	4	4	4
Average	2	2	2	2	2	
	Lodging – Jundiá (2021) – 4th year of cultivation					
PP 90 RR	3	3	3	3	3	3
BRS 9180 IPRO	2	2	2	2	2	2
PP 80 RR	4	4	4	4	4	4
Average	3	3	3	3	3	

Regarding 100-grain mass, only individual effects were observed for inoculation technology/N management and cultivar, with no discernible interaction between these factors (Table 8). Among the experiments conducted, only one did not reveal significant differences in 100-grain mass among the tested soybean cultivars (Jundiá in 2018, a location in the fourth year of cultivation). In specific instances, the BRS 9180 IPRO cultivar exhibited a greater 100-grain mass in experiments conducted in Campo Alegre (2018), an area with four years of soybean cultivation, as well as in Jundiá (2020), a region in its first year of soybean cultivation, where it was on par with the BRS 9383 IPRO cultivar. In Jundiá (2021), an area with three years of soybean cultivation, BRS 9180 IPRO also showed a comparable 100-grain mass to the PP 80 RR cultivar. In the same year, in a location with four years of soybean cultivation, BRS 9180 IPRO again exhibited a higher 100-grain mass compared to other cultivars. Conversely, in the experiments conducted in 2019, the FTR 3191 IPRO cultivar stood out in terms of 100-grain mass, while in the Jundiá (2020) experiment, which took place in an area with three years of soybean cultivation, the FTR 3185 IPRO and FTR 1192 IPRO cultivars displayed the highest grain mass.

Table 8. 100-grain mass in soybean plants as a function of the use of inoculation, co-inoculation, and starter N fertilization.

Cultivar	Control	Inoculation	Inoculation + N	Co-inoculation	Co-inoculation + N	Average
	Mass of 100 grains (g) – Campo Alegre (2018) – 4th year of cultivation					
BRS 9180 IPRO	15.4	15.3	15.4	15.5	15.2	15.4 A
BRS 9383 IPRO	15.0	14.6	14.3	14.9	14.4	14.7 B
Average	15.2	15.0	14.8	15.2	14.8	
CV (%)	4.77					
	Mass of 100 grains (g) – Jundiá (2018) – 4th year of cultivation					
BRS 9180 IPRO	17.1	17.0	17.6	17.8	17.0	17.3
BRS 9383 IPRO	17.2	17.3	17.1	17.2	17.1	17.1
Average	17.1	17.1	17.3	17.5	17.0	
CV (%)	3.80					
	Mass of 100 grains (g) – Jundiá (2019) – 1st year of cultivation					
BRS 9180 IPRO	18.8	17.0	16.7	18.6	18.3	17.9 B
BRS 9383 IPRO	17.8	16.3	16.7	16.3	16.0	16.6 C
FTR 3191 IPRO	19.9	18.3	18.8	18.2	18.9	18.8 A
Average	18.9 a	17.2 b	17.4 b	17.7 b	17.7 b	
CV (%)	5.06					
	Mass of 100 grains (g) – Jundiá (2019) – 2nd year of cultivation					
BRS 9180 IPRO	15.5	16.2	15.6	15.9	15.0	15.6 B
BRS 9383 IPRO	13.4	14.2	13.3	14.1	14.8	14.0 C
FTR 3191 IPRO	16.8	17.0	17.1	17.4	17.5	17.2 A
Average	15.2	15.8	15.3	15.8	15.7	
CV (%)	6.15					
	Mass of 100 grains (g) – Jundiá (2020) – 1st year of cultivation					
BRS 9180 IPRO	19.8	17.9	18.7	17.2	19.0	18.5 A
BRS 9383 IPRO	19.0	18.9	18.6	18.5	18.9	18.8 A
FTR 1192 IPRO	18.3	17.6	17.2	18.0	17.8	17.8 B
Average	19.1 a	18.1 b	18.2 ab	17.9 b	18.6 ab	
CV (%)	4.15					
	Mass of 100 grains (g) – Jundiá (2020) – 3rd year of cultivation					
FTR 3185 IPRO	22.4	21.8	22.2	21.4	21.9	21.9 A
BRS 9383 IPRO	19.6	20.6	20.3	20.2	20.8	20.3 B
FTR 1192 IPRO	20.6	21.6	20.7	21.9	21.3	21.2 A
Average	20.9	21.3	21.1	21.2	21.3	
CV (%)	5.33					
	Mass of 100 grains (g) – Jundiá (2021) – 3rd year of cultivation					
PP 90 RR	17.3	17.4	17.2	17.3	16.9	17.2 B
BRS 9180 IPRO	18.1	19.4	18.7	19.0	18.3	18.7 A
PP 80 RR	18.6	18.6	18.4	18.3	18.5	18.5 A
Average	18.0	18.5	18.1	18.2	17.9	
CV (%)	3.84					
	Mass of 100 grains (g) – Jundiá (2021) - 4th year of cultivation					
PP 90 RR	15.8	15.9	15.0	14.7	15.0	15.3 B
BRS 9180 IPRO	17.7	17.8	17.6	17.2	17.6	17.6 A
PP 80 RR	15.4	15.8	16.4	15.8	16.5	16.0 B
Average	16.3	16.5	16.4	15.9	16.3	
CV (%)	6.79					

Means followed by equal letters, lowercase in the row and uppercase in the column, do not differ from each other by the Tukey's test ($p \leq 0.05$).

As for first-pod insertion height, significant effects of inoculation technology/N management on 100-grain mass were only observed in first year cultivation area (Table 8). In the 2019 experiment, conducted in a first-year cultivation area, the control treatment, which received no inputs (including inoculation, co-inoculation, or mineral Basal N fertilization), showed greater 100-grain mass results regardless of the cultivar. In the 2020 experiment, also in a first-year cultivation area, the control treatment exhibited greater 100-grain mass, not significantly different from treatments involving inoculation with basal N fertilization and co-inoculation with basal N fertilization.

This phenomenon can be explained by fewer pods (data not shown) and consequently fewer grains in the soybean plants in the control treatment, resulting in fewer grains being filled. A study in the literature, spanning two agricultural seasons, found no significant differences in grain mass accumulation in soybeans when comparing treatments involving seed treatment inoculation (*Bradyrhizobium* spp.) and co-inoculation (*Bradyrhizobium* spp. and *A. brasilense*) with the control lacking any form of inoculation (Brignoli et al., 2023).

No interaction was observed between inoculation technology/N management and soybean cultivars for grain yield (Table 9). Only isolated effects were observed. In three out of eight experiments, no differences were detected among cultivars in terms of grain yield. Cultivar BRS 9383 IPRO had the highest grain yield in the 2018 (fourth year of cultivation) and 2020 (third year of cultivation) experiments in Jundiá, with average yields of 3,145 and 3,581 kg ha^{-1}, respectively. Cultivar BRS 9180 IPRO performed best in the 2021 experiment in Jundiá (fourth year of cultivation), achieving an average yield of 3,386 kg ha^{-1}. In the 2021 experiment in Jundiá (third year of cultivation), the PP 90 RR cultivar had an average yield of 3,231 kg ha^{-1}, outperforming other materials. Cultivar FTR 1192 IPRO achieved the highest average yield (3,842 kg ha^{-1}) in the 2020 experiment in Jundiá (first year of cultivation), the highest yield among all experiments.

The response to inoculation technology/N management in soybean yield was only observed in first-year cultivation areas (Table 9). In these experiments, treatments involving inoculation, inoculation with basal N fertilization, co-inoculation, and co-inoculation with basal N fertilization resulted in higher soybean yields compared to the control treatment. A study based on data from 11 experiments found no yield benefit from additional inoculation in soybean grown in soils with a history of soybean cultivation and under non-severe stress conditions (e.g., high early-season temperatures and/or saturated soils) (Carciochi et al., 2019). Similarly, a study on productive response of soybean to inoculation showed inconsistent results in 21 experiments in areas with a history of soybean cultivation in mid-southern Paraná State, regardless of the type of inoculant tested (solid or liquid) (Ambrosini et al., 2019).

Some studies have reported increased soybean yields with periodic re-inoculation (Brandão Júnior & Hungria, 2000; Leggett et al., 2017), a sustainable and cost-effective practice for nitrogen supply. In contrast, the average results from 25 field experiments conducted in various U.S. production environments using the same experimental design showed yield responses to co-inoculation in only two out of the 25 locations/years evaluated (Reis et al., 2022). However, other studies have demonstrated positive effects of co-inoculation on soybeans, even indicating significant yield improvements (Hungria, Nogueira, & Araújo, 2013; Galindo et al., 2018; Moretti et al., 2019), including under water-stressed conditions (Naoe, Peluzio, Campos, Naoe, & Silva, 2020). Further research involving the interaction of *Azospirillum* strains with SEALBA-adapted soybean cultivars is needed to enhance these plant-microorganism interactions and replicate the benefits observed in other locations with co-inoculation. Interestingly, a similar lack of response to co-inoculation was observed in Mozambique (Chibeba, Kyei-Boahen, Guimarães, Nogueira, & Hungria, 2020).

In all eight experiments conducted, treatments with mineral N fertilization did not show higher grain yields compared to treatments using only microbiological inputs (inoculation and/or co-inoculation). This aligns with Brar and Lawley (2020), who observed no effect of starter nitrogen fertilization on soybean yield in three seasons. Similarly, under Cerrado conditions, it was observed that adding small N doses at sowing did not boost soybean grain yields (Mendes et al., 2003). Another study in the literature also found that varying N doses applied at sowing (20, 40, and 60 kg ha^{-1}) did not increase the final soybean yield (Zuffo et al., 2018).

Regarding the multivariate analysis of grain yield, the selected model explained 40% of the variability in soybean grain yield response to isolated *Bradyrhizobium* inoculation (Figure 4). The variables "number of years of soybean cultivation" and "cultivar" contributed 81.5 and 18.5%, respectively, to the variability explained by the model. It distinguished two subpopulations based on the number of years of soybean cultivation, one with 64 samples and the other with 24 samples.

Table 9. Grain yield in soybean plants as a function of the use of inoculation, co-inoculation and starter N fertilization.

Cultivar	Control	Inoculation	Inoculation + N	Co-inoculation	Co-inoculation + N	Average
	Yield (kg ha^{-1}) – Campo Alegre (2018) – 4th year of cultivation					
BRS 9180 IPRO	1,664	1,923	1,604	1,543	1,691	1,541
BRS 9383 IPRO	1,705	1,559	1,635	1,432	1,378	1,685
Average	1,684	1,741	1,619	1,487	1,534	
CV (%)	15.43					
	Yield (kg ha^{-1}) – Jundiá (2018) – 4th year of cultivation					
BRS 9180 IPRO	2,599	2,660	2,272	2,589	2,639	2,552 B
BRS 9383 IPRO	2,984	3,295	2,834	3,286	3,326	3,145 A
Average	2,792	2,978	2,553	2,938	2,983	
CV (%)	12.71					
	Yield (kg ha^{-1}) – Jundiá (2019) – 1st year of cultivation					
BRS 9180 IPRO	1,877	2,692	2,583	2,768	3,054	2,595
BRS 9383 IPRO	1,954	2,781	2,765	2,398	2,828	2,545
FTR 3191 IPRO	1,741	2,671	2,719	2,431	2,491	2,411
Average	1,857 b	2,715 a	2,689 a	2,533 a	2,791 a	
CV (%)	16.98					
	Yield (kg ha^{-1}) – Jundiá (2019) – 2nd year of cultivation					
BRS 9180 IPRO	2,299	2,478	2,705	2,710	2,611	2,561
BRS 9383 IPRO	2,706	2,423	2,721	2,455	2,387	2,538
FTR 3191 IPRO	2,577	1,955	2,241	2,626	2,144	2,309
Average	2,527	2,285	2,556	2,597	2,381	
CV (%)	22.90					
	Yield (kg ha^{-1}) – Jundiá (2020) – 1st year of cultivation					
BRS 9180 IPRO	2,222	3,202	2,860	3,337	2,907	2,906 B
BRS 9383 IPRO	1,568	3,115	3,082	3,100	3,133	2,800 B
FTR 1192 IPRO	3,241	3,771	3,858	4,031	4,127	3,842 A
Average	2,404 b	3,363 a	3,267 a	3,490 a	3,389 a	
CV (%)	12.19					
	Yield (kg ha^{-1}) – Jundiá (2020) – 3rd year of cultivation					
FTR 3185 IPRO	2,875	3,245	2,864	2,975	2,632	2,918 B
BRS 9383 IPRO	3,658	3,733	3,805	3,591	3,118	3,581 A
FTR 1192 IPRO	3,129	2,711	2,836	2,463	2,562	2,740 B
Average	3,221	3,229	3,169	3,010	2,771	
CV (%)	21.12					
	Yield (kg ha^{-1}) – Jundiá (2021) – 3rd year of cultivation					
PP 90 RR	3,152	3,435	2,934	3,296	3,337	3,231 A
BRS 9180 IPRO	2,564	2,426	3,029	2,819	2,213	2,610 B
PP 80 RR	2,756	2,630	2,444	1,772	2,541	2,429 B
Average	2,824	2,830	2,803	2,629	2,697	
CV (%)	21.03					
	Yield (kg ha^{-1}) – Jundiá (2021) – 4th year of cultivation					
PP 90 RR	3,241	3,070	2,217	2,673	2,981	2,836 B
BRS 9180 IPRO	3,192	3,509	3,317	3,226	3,688	3,386 A
PP 80 RR	2,558	2,101	2,191	1,836	2,373	2,211 C
Average	2,997	2,893	2,575	2,578	3,014	
CV (%)	19.02					

Means followed by equal letters, lowercase in the row and uppercase in the column, do not differ from each other by the Tukey's test ($p \leq 0.05$).

In the analysis, we differentiated between samples from the first year of cultivation (number < 1.5 years) and those from subsequent years (number > 1.5 years). In response to inoculation with *Bradyrhizobium*, there was an average yield gain of 54% in the first year and 3% in the following years. However, the model did not reveal any further differentiation in sample response between years two to four of cultivation.

Furthermore, the model indicated differences in the response to inoculation among soybean cultivars during the first year of cultivation. On average, there was a 34% increase in grain yield for cultivars BRS 9180 IPRO and FTR 1192 IPRO, which were less responsive, and a 75% increase for BRS 9383 IPRO and FTR 3191 IPRO, which were more responsive. This variance in soybean cultivar response to inoculation is attributed to inherent genetic factors, as variations in cycle length, growth habits, and other characteristics can influence BNF process (Cigelske, Kandel, & Desutter, 2020).

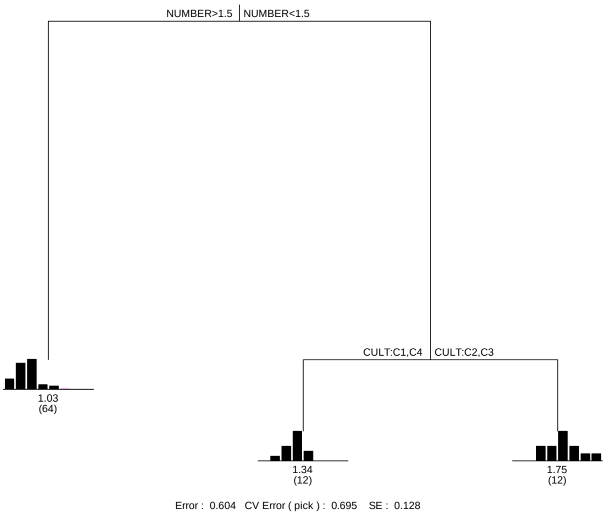

Figure 4. Average grain yield gain in soybean plants in response to inoculation with *Bradyrhizobium* (comparison between inoculation treatment *versus* control treatment).

Conclusion

Seed inoculation with *Bradyrhizobium* enhances soybean grain yield in areas during the first year of cultivation. The application of nitrogen fertilizer at the base (22 kg ha^{-1} of N) does not result in increased grain yield in soybean compared to using microbiological inputs alone (inoculation and co-inoculation). Among the soybean cultivars tested, BRS 9383 IPRO and FTR 3191 IPRO are the most responsive to seed inoculation with *Bradyrhizobium*.

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