Original Research Article

Effect of co-inoculations with growth-promoting bacteria on soybean crop

ABSTRACT

Aim: To evaluate the co-inoculation of soybean with *Pseudomonas fluorescens* and *P. fluorescens* + *Azospirillum brasilense*.
Study design: Randomized complete block design with five treatments and eight replications.
Place and Duration of Study: Ibiporã, Paraná State, Brazil, during the 2020/21 season.
Methodology: The treatments consisted of untreated control (Cont); mineral fertilizer (Min); mineral fertilizer + (*P. fluorescens* + *A. brasiliense*) (Min – Psf + Azb); mineral fertilizer + *P. fluorescens* at two doses (Min – Psf 100 and Min – Psf 200). The effect in the soybean was assessed by determining the effect on the seedling emergence speed, crop development and crop yield. Data were subject to ANOVA at $P = 0.05$. Treatments means were separated using the Duncan test at a 0.10 level of significance.
Results: The inoculant treatments did not affect the emergence speed index and crop stand. However, the co-inoculation with *P. fluorescens* at two doses resulted in the best plant vigor. In addition, the treatments with co-inoculation increased shoots and root biomass, with Min – Psf 100 inducing more nodules. Finally, Min – Psf + Azb and Min – Psf 200 resulted in the greatest soybean yield.
Conclusion: This study revealed that the co-inoculation treatments tested led to great soybean response, especially for *P. fluorescens* at a doubled dose and *P. fluorescens* + *A. brasiliense*.

Keywords: Pseudomonas fluorescens, Plant vigor, Nodulation, Crop yield.

1. INTRODUCTION

In Brazil, the world's largest soybean producer, a crop that was incipient became, in a relatively short period, one of the main products of the national economy with expressiveness in the export trade balance. Much of the advance of soybean in Brazil is due to the development of management techniques to increase productivity and the efforts of genetic improvement programs for the development of cultivars more adapted to new regions [1]. As a result, in the 2020/2021 season, there was a 4.3% growth in the planted area compared to the previous seasons, reaching 38.5 million hectares, with a record production estimate of 136 million tons, representing an increase of 8.9% compared to the previous year [2]. In addition, it is estimated that Brazil will face another production record in the 2022/2023 season, with a 15% increase [3].

Soybean is a legume with great nutritional value, containing approximately 40% of the protein in the grains. Nevertheless, the crop demand is mainly for nitrogen (N), requiring approximately 80 kg ha^{-1} of the nutrient for every 1 ton of grain produced [4]. In this scenario, the use of the biological N fixation agents, using efficient strains associated with the selection of cultivars that are responsive to plant-microorganism interactions, has resulted in the dispensation or minimization of the requirement for nitrogen fertilization [5].

Furthermore, with the need to increase agricultural production and the emerging demand to reduce chemical fertilizers dependence throughout the crop cycle, continuous research has studied new strategies using the inoculation of plants with growth-promoting microorganisms. One of the advances is the association of two or more microorganisms that plays a role in different microbial processes, improving plant growth and its traits are so-called co-inoculation [6].

The use of microorganisms in agriculture can bring benefits to the farmer as it becomes possible to obtain a greater development of the crop and, consequently, save inputs, thus increasing the efficiency in the use of fertilizer [7]. When it comes to inoculants, some groups of soil bacteria that have already been studied have great potential for use in agriculture, such as *Pseudomonas, Bacillus*, *Agrobacterium*, *Herbaspirillum*, *Arthrobacter, Azospirillum, Azotobacter, Gluconacetobacter*, *Staphylococcus, Burkholderia* and *Flavobacterium* [8].

Species of the *Pseudomonas* generum stand out for their great versatility and ability to grow in a wide variety of environments and substrates. Although some species of *Pseudomonas* present phytopathogenicity, many members of this group have been related to having beneficial effects observed in plants [9], which characterizes them as promising plant growth-promoting rhizobacteria (root colonizing bacteria). Plant growth-promoting rhizobacteria use different mechanisms to suppress plant pathogens and promote plant growth, such as competition for nutrients through the production of siderophores (iron chelators) [10], antibiosis [11], synthesis of plant hormones such as auxins [12], cytokinins [13], gibberellins [14] and provide nutrients to plants, such as nitrogen, by biological nitrogen fixation [10], and as phosphorus, releasing phosphorus adsorbed to soil colloids [15].

In this context, increasing the efficiency in the use of agricultural resources through the inoculation of plants with growth-promoting microorganisms can result in the application of lower doses of pesticides and fertilizers in the agroecosystem and, consequently, reduce the cost of production. In addition, increasing crop productivity reduces pressure on new areas and increases the sustainability of current production systems. Nevertheless, the product containing *P. fluorescens* may lead to root improvements and vegetative development [16]. Thus, we aim to evaluate the soybean response to co-inoculation with *P. fluorescens* at two different doses and *P. fluorescens* + *Azospirillum brasilense*.

2. MATERIAL AND METHODS

A field experiment was conducted to determine the effects of seed inoculants in soybean crop in Ibiporã, Paraná State, Brazil (23°14' 3,54" S, 51°6'38,88" W) during the 2020/21 season. The climate region is classified as a humid mesothermal (subtropical) climate (Cfa) [17], with warm and rainy summer with an average temperature of 26°C. The experiments were conducted in Eutrophic Red Nitosol soil (USDA Red Alfisol) [18] [19]. Previously, the soil was collected from the upper layer (0-20 cm) and it was analyzed for its chemical and physical composition [20], resulting in pH (H_2O): 5,32; exchangeable Ca, Mg, and K, and CEC of 3.39, 1.18, 0.67, and 11.26 $\text{cmol}_c\ \text{dm}^{-3}$, respectively; C:12.49 g dm^{-3}; organic matter (OM): 21.49 g dm^{-3}; base saturation (V%): 46.51; and available P (Mehlich 1): 34.8 mg dm^{-3}. The concentrations of micronutrients were 39.66, 76.17, 5.36, and 7.17 mg dm^{-3} of Fe, Mn, Cu, and Zn, respectively. In addition, the respective contents of clay, silt, and sand were 745, 135, and 120 g kg^{-1}. Finally, the soil microbiological analysis resulted in 7.70×10^6 CFU of total bacterial g^{-1} soil, and the bacteria *Pseudomonas fluorescens* was not detected.

The experiment was conducted as a randomized complete block design with five treatments and eight replications. Experimental units were 3-m wide by 8-m long (24 m^2) and plots were spaced 1-m from each other to avoid treatments contamination. The treatments consisted of an untreated control (Cont), one treatment with mineral fertilizer only representing the usual crop management (Min), and three treatments using mineral fertilizer in association with inoculants, consisting of inoculation of seeds with a mix of *Pseudomonas fluorescens* (strain CCTB03) + *Azospirillum brasilense* (strain AbV6) applied in furrow at 150 mL ha^{-1} (Min – Psf + Azb); isolated inoculation of seeds with *Pseudomonas fluorescens* (strain 1008) at 100 mL 100 kg^{-1} seeds (Min – Psf 100)$^{-1}$; isolated inoculation of seeds with *Pseudomonas fluorescens* (strain 1008) at 200 mL 100 kg^{-1} seeds (Min – Psf 200)$^{-1}$. Standard soybean seed inoculation was performed in all treatments, including treatment Cont, with peat-based inoculant with *Bradyrhizobium japonicum* (strains SEMIA 5079). Therefore, the experiment evaluated the effect of co-inoculation with *P. fluorescens* + *A. brasilense* (Min – Psf + Azb) and *P. fluorescens* (Min – Psf 100 and Min – Psf 200).

In all treatments with mineral fertilization, 440 kg ha^{-1} of single superphosphate and 190 kg ha^{-1} of potassium chloride were applied, equivalent to 80 kg ha^{-1} of P and 110 kg ha^{-1} of K. Phosphorus fertilization was carried out in planting furrow at the day of sowing. Potassium fertilizations were performed before sowing, 50 days after sowing (DAS), and 70 DAS, where the doses were divided in the proportion of 35%, 30%, and 30%, respectively. Nitrogen fertilization was not performed. In addition, a mixture containing the strains of *P. fluorescens* and *A. brasilense* was applied to the furrow before sowing in Min – Psf + Azb treatment. Inoculant at *P. fluorescens* was performed as seed treatments 10 days before sowing, in which Min – Psf 100 received 100 mL per 100 kg of seeds, whereas Min – Psf 200 received 200 mL per 100 kg of seeds.

The soybean cultivar NS 6700 IPRO was seeded on December 21st, 2020, with row spacing of 0.5-m and with 15 seed m^{-1}, aiming for 300,000 seeds per hectare^{-1}. The weather conditions during sowing were a temperature of 25.4°C and relative humidity of 60%. The plant emergence occurred five days after seeding. The experiment was conducted from 21 December 2020 to 20 April 2021. Weather conditions during the conduction of the experiment are shown in Fig. 1.

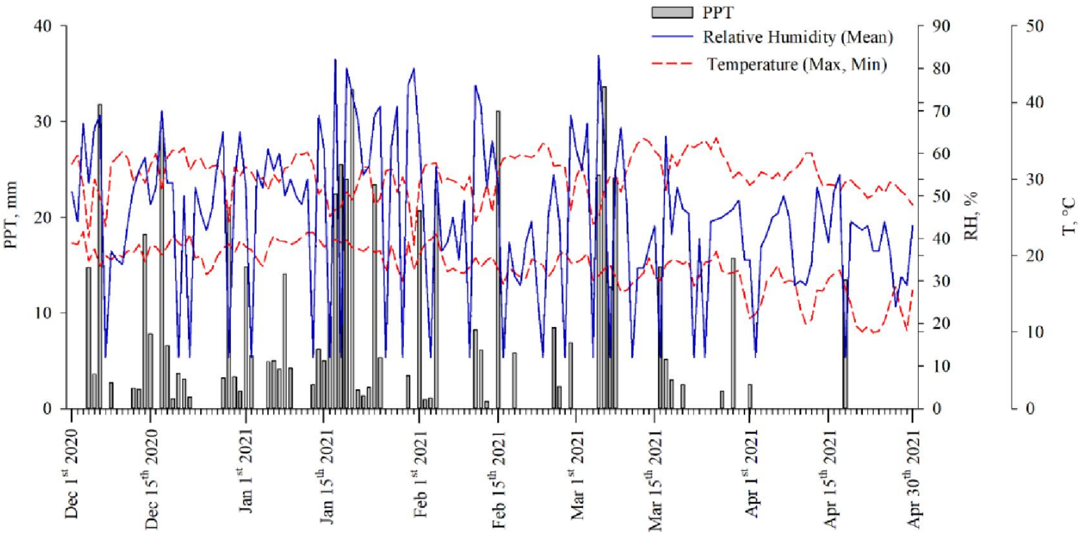

Fig. 1. Weather conditions during the cultivation of soy in Ibiporã, Parana, Brazil, 2020/21.

Precipitation (PPT, mm), maximum, mean, and minimum Temperatures (T, °C, Max, Mean, Min), and maximum, mean, and minimum Relative Humidity (RH, %, Max, Mean, Min)

The effect of the treatments in the soybean was assessed by determining the effect (a) on the seedling emergence speed; (b) on crop development; and (c) on crop yield. The evaluation of seedling emergence used the emergence speed index (ESI) by daily counting the number of seedlings that emerged from two planting lines [21]. The counting started from the day of sowing until the full establishment of emergencies.

In addition, soybean stand from each plot was evaluated once during vegetative stages V3 and V4. To evaluate crop development, during V4 and reproductive stage 1 (R1), visual ratings were recorded to assess plant vigor assigning scores from 1 to 10 in each plot. The ratings were assigned as 1 to 2 - plants were much worse than the untreated control (treatment 1); 3 to 4 - plants were a little worse than the untreated control; 5 - plants were equal to untreated control; 6 to 7 – plants were a little greater than the untreated control; 8 to 9 - plants were better than the untreated control; 10 – plants were much better than the untreated control. Therefore, the untreated control received a score of 5. Moreover, ten plants were harvested in each plot during the R1 stage to assess the effect of treatments on aboveground fresh biomass (AFB) and belowground fresh biomass (BFB). Each plant was weighed using a precision scale. In addition, soybean nodulation was evaluated from five plants per plot also at the R1 stage. Nodulation evaluation was assessed by carefully removing all nodules from the roots and weighing them.

Crop yield was assessed by hand harvesting 15 m^2 from each plot at crop physiological maturity, determining grain yield and the weight of 1000 grains on a 13% moisture base [22]. Furthermore, ten plants from each plot were randomly selected to assess the number of pods $plant^{-1}$ and grains pod^{-1}.

Finally, normality and homogeneity of variance were tested using the Shapiro-Wilks and Levene tests, respectively. Data were subject to ANOVA at $p < 0.05$. Treatments means were separated using the Duncan test at a 0.10 level of significance [23]. Statistical analyses were performed using the software R [24].

3. RESULTS AND DISCUSSION

Co-inoculation treatments with *P. fluorescens*, with or without *A. brasilense* did not affect ESI and Crop Stand. However, a significant effect on plant vigor was observed (Fig. 2). The co-inoculation treatments with *P. fluorescens* at two different doses (Min – Psf 100 and Min – Psf 200) resulted in the best vigor, with an increment of 15% and 18% in comparison to the untreated control, respectively. In addition, compared to the Min treatment, there was an increase in plant vigor of 7% and 14% for Min – Psf 100 and Min – Psf 200, respectively.

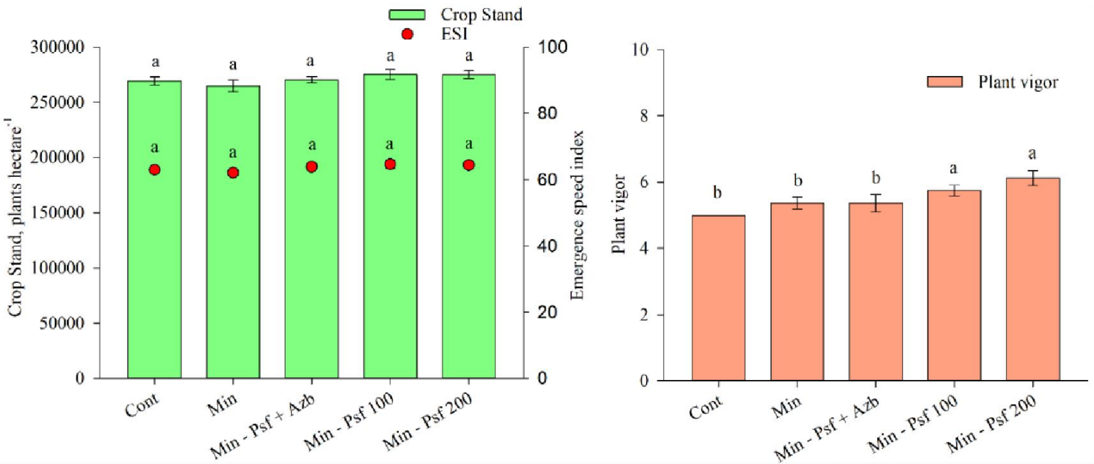

Fig. 2. Emergence speed index (ESI), crop stand, and plant vigor of soybean fertilized with and without co-inoculation with P. fluorescens and A. brasilense, in addition to the control without fertilization and co-inoculation at Ibiporã, Parana, Brazil, 2020/21.

* *P* = 0.10

The co-inoculation treatment with *P. fluorescens* using a dose of 200 mL 100 kg seeds^{-1} and with *A. brasilense* in soybean plants induced the greatest root production, resulting in 24% and 19%, respectively, presenting more BFB than the Min treatment (Fig. 3). Furthermore, there was no difference between Min and Min – Psf + Azb, and the Cont treatment showed the least BFB. Greater and more developed roots are essential to maintain higher yield, especially if considering their role in nutrient acquisition, and improving abiotic stresses adaptation [25], therefore, emphasizing the importance of a treatment that improves the root system. For aboveground biomass evaluation (ABF), the Min – Psf + Azb treatment showed the greatest result, leading to 30% more biomass than the Min treatment. Min – Psf 100 and Min – Psf 200 resulted in the second-best treatments, increasing AFB by approximately 17% in comparison to the Min treatment. Nevertheless, there was no difference between Cont and Min treatments.

Soybean plants co-inoculated with *P. fluorescens* at 100 mL 100 kg^{-1} seeds increased the number of nodules plant^{-1} compared to the Cont treatment (Fig. 3). The other treatments showed results similar to the Cont. Egamberdieva [24], testing the use of both *Bradyrhizobium japonicum* (USDA 110) and *Pseudomonas putida* (TSAU 1) on soybean, reported a significantly improvement in plant growth, nitrogen, and phosphorus content. Moreover, the treatments resulted in greater root volume and nodulation traits. Nevertheless, it has been reported a higher efficiency in using the combination of *B. japonicum* and *P. putida* in comparison to *B. japonicum* alone [26].

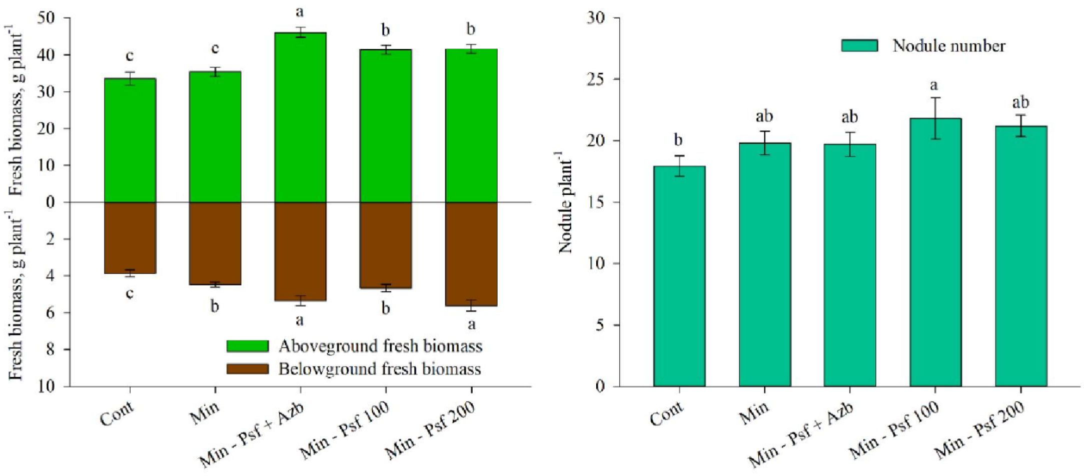

Fig. 3. Aboveground fresh biomass (AFB), belowground fresh biomass (BFB), and nodule number (NN) of soybean fertilized with and without co-inoculation with *P. fluorescens* and *A. brasilense*, in addition to the control without fertilization and co-inoculation at Ibiporã, Parana, Brazil, 2020/21.

* $P = 0.10$

For agronomic parameters, except for the weight of 1000 grains, the treatments resulted in significant effects on crop yield, number of pods plant^{-1}, and number of seeds pod^{-1} (Fig. 4 and Table 1). Considering crop yield, Min – Psf + Azb and Min – Psf 200 resulted in the greatest soybean yield, with about 4218 kg ha^{-1} and 4058 kg ha^{-1}, respectively (Fig. 4). Moreover, there was a yield increase of 12% in Min – Psf + Azb and 7% in Min – Psf 200, compared to Cont. On the other hand, treatments Min – Psf 100 and Min were statistically similar and was followed by Cont. Considering the number of pod plant^{-1}, inoculation with the *P. fluorescens* and *A. brasilense* mixture was the only treatment that showed a difference with the Cont treatment, increasing the number of pods (Table 1). Finally, the Min – Psf 200 treatment led to the greatest number of seeds pod^{-1}, with 8% more seeds than the Min treatment. Min – Psf + Azb presented a mean similar to Min – Psf 200, although it also did not differ from Min – Psf 100. All treatments with co-inoculation showed a higher number of seeds pod^{-1} results than the Min treatment.

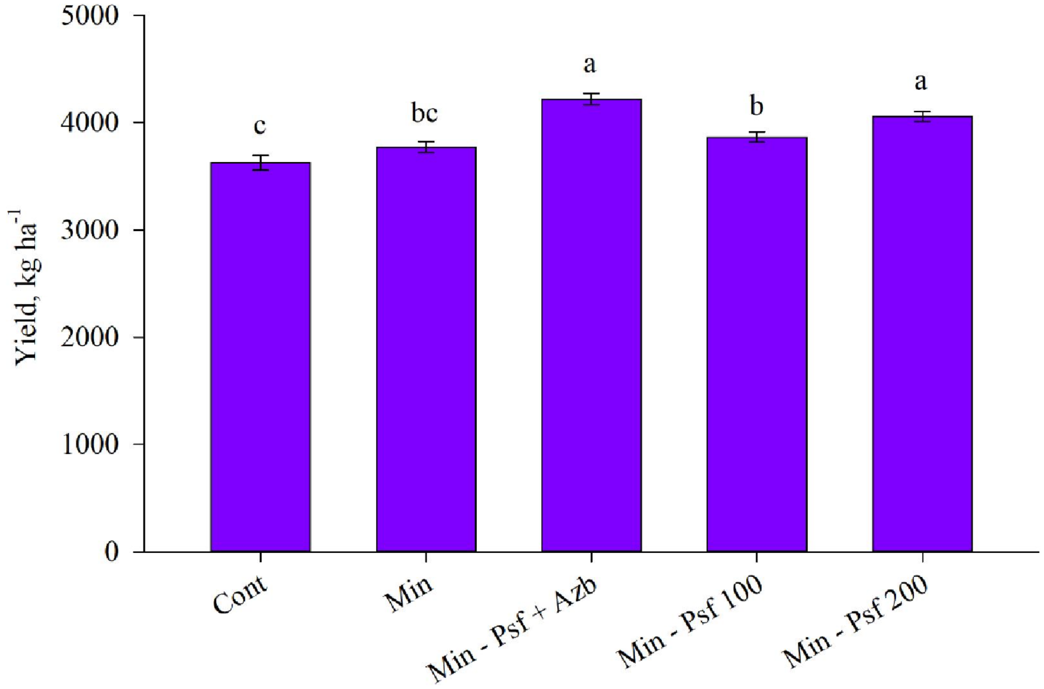

Fig. 4. Crop Yield (kg ha^{-1}) of soybean fertilized with and without co-inoculation with *P. fluorescens* and *A. brasilense*, in addition to the control without fertilization and co-inoculation at Ibiporã, Parana, Brazil, 2020/21.

* *P* = 0.10

Table 1. Weight of 1000 grains, number of pod plant^{-1}, and number of seeds pod^{-1} of soybean fertilized with and without co-inoculation with *P. fluorescens* and *A. brasilense*, in addition to the control without fertilization and co-inoculation at Ibiporã, Parana, Brazil, 2020/21

Treatments	**Weight of 1000 grains (g)**	**Number of pod plant^{-1}**	**Number of seeds pod^{-1}**
Cont	141.49 a	52.96 b	2.18 cd
Min	142.58 a	58.09 ab	2.13 d
Min – Psf + Azb	143.87 a	61.45 a	2.26 ab
Min – Psf 100	143.67 a	58.36 ab	2.23 bc
Min – Psf 200	143.66 a	59.00 ab	2.31 a
Coefficient of variance, %	2.10	12.80	3.20

Means within a column followed by the same letter are not significantly different according to Duncan`s test at the 10% level of significance.

The increase in crop yield on Min – Psf + Azb and Min – Psf 200 treatments should be closely related to the increase in pod plant^{-1} and seeds pod^{-1}, leading to an increase in crop production. That is, although there was no effect on the grain weight, the inoculants may have induced greater production of pods and the number of seeds pod^{-1} [6]. Inoculants applied before sowing are the great responsible for most of the nodule formation in legumes, such as soybean [27]. In addition, initial nodule formation is critical, and achieving numerous functional nodules at the beginning is desired [28]. In this sense, co-inoculation with rhizobia and root colonizing beneficial bacterial promotes greater plant growth and nodulation [29], as well as allowing the disease management [30].

Plant growth-promoting rhizobacteria, such as *P. fluorescens*, have shown the ability to increase plant growth by N_2-fixation and promote plant growth through different mechanisms [10]. In addition to N fixation, these bacteria increase nutrient availability in the soil, such as P and Fe solubilization, and promote root and shoot growth through the influence of phytohormones, such as indole-3-acetic acid (IAA), and volatile organic compounds [31] [32]. The association of *Azospirillum brasilense* and *Bradyrhizobium* spp can favor root development and nodulation, leading to greater grain yield and soybean quality. Furthermore, co-inoculation of soybean with these two traits has been shown to increase root mass and nodule number by 11% and 10.6%, respectively, resulting in a 3.6% yield increment [6].

4. CONCLUSION

This study carried out in field conditions revealed that the co-inoculation of soybean with *P. fluorescens* (strain 1008) at both doses and with *P. fluorescens* (strain CCTB03) + *A. brasilense* (strain AbV6) presented the greatest results. In addition, the study highlights the treatment with *P. fluorescens* (strain 1008) with 200 mL 100 kg seeds^{-1} showing the greatest plant vigor, belowground biomass, and crop yield. Therefore, the use of co-inoculation resulted in great crop improvements, increasing plant nodulation and better agronomic parameters. Co-inoculation of soybean cultivation is a sustainable agronomic strategy that improves its development.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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