

RESPONSES TO THE USE OF GROWTH-PROMOTING BACTERIA IN THE GERMINATION AND EARLY DEVELOPMENT OF WHEAT AND SOYBEANS¹

RESPOSTAS DO USO DE BACTÉRIAS PROMOTORAS DE CRESCIMENTO NA GERMINAÇÃO E NO DESENVOLVIMENTO INICIAL DE TRIGO E SOJA

Ana Eloísa Furlan², Raquel Stefanello³, Ely Jhones Mello da Silva⁴,
Lusardo dos Santos da Silva⁵, Julio César Oliveira da Rosa⁶,
Tainá Mueller dos Santos⁷ e Anderson César Ramos Marques⁸

ABSTRACT

Soybean (*Glycine max (L.) Merrill*) and wheat (*Triticum aestivum L.*) are of great importance to the national agricultural sector. This study aimed to evaluate the effects of inoculating growth-promoting bacteria on germination, emergence, and initial development of soybean and wheat seedlings. Experiments were conducted under controlled conditions, using different bacterial treatments, isolated and in mixtures, in single and double doses. Vigor parameters, germination, emergence speed index, initial growth, and root system characteristics were evaluated. The results indicated no statistically significant differences between treatments for most variables analyzed in both soybean and wheat. The observed variations were only numerical, with no statistical impact compared to the control. These results suggest that, under the evaluated conditions, microorganisms do not directly influence the initial physiological performance of seedlings, possibly because internal seed reserves predominate and because more time is needed for the establishment of the plant-microorganism interaction. It can be concluded that the beneficial effects of plant growth-promoting rhizobacteria tend to manifest themselves in later stages of plant development, reinforcing the importance of evaluations in later stages of crops.

Keywords: Bioinputs; seeds; *Triticum aestivum L.*; *Glycine max (L.) Merrill*.

1 Trabalho de Mestrado Programa de Pós Graduação em Agronomia - UFSM.

2 Universidade Federal de Santa Maria - UFSM. E-mail: furlananaeloisa@gmail.com. ORCID: <https://orcid.org/0009-0000-9863-5167>

3 Universidade Federal de Santa Maria - UFSM. E-mail: raquelstefanello@yahoo.com.br. ORCID: <https://orcid.org/0000-0003-3079-2099>

4 Universidade Federal de Santa Maria - UFSM. E-mail: elyjhonesmelo@gmail.com. ORCID: <https://orcid.org/0000-0001-7106-3330>

5 Universidade Federal de Santa Maria - UFSM. E-mail: lusardo.ss2@gmail.com. ORCID: <https://orcid.org/0009-0002-4563-2696>

6 Universidade Federal de Santa Maria - UFSM. E-mail: julio.cezaroliveira134@gmail.com. ORCID: <https://orcid.org/0009-0003-1437-9904>

7 Universidade Federal de Santa Maria - UFSM. E-mail: taina.mueller@acad.ufsm.br. ORCID: <https://orcid.org/0009-0000-6898-5561>

8 Universidade Federal de Santa Maria - UFSM. E-mail: anderson.marques@ufsm.br. ORCID: <https://orcid.org/0000-0003-0866-8761>

RESUMO

A soja (*Glycine max* (L.) Merrill) e o trigo (*Triticum aestivum* L.) têm grande importância para o setor agrícola nacional. Este estudo teve como objetivo avaliar os efeitos da inoculação com bactérias promotoras de crescimento na germinação, emergência e desenvolvimento inicial de plântulas de soja e trigo. Foram conduzidos experimentos em condições controladas, utilizando diferentes tratamentos bacterianos, isolados e em mistura, em doses únicas e duplicadas. Avaliaram-se os parâmetros de vigor, germinação, índice de velocidade de emergência, crescimento inicial e características do sistema radicular. Os resultados indicaram ausência de diferenças estatisticamente significativas entre os tratamentos na maioria das variáveis analisadas, tanto em soja quanto em trigo. As variações observadas foram apenas numéricas, sem impacto estatístico quando comparadas à testemunha. Esses resultados sugerem que, nas condições avaliadas, os microrganismos não influenciam diretamente o desempenho fisiológico inicial das plântulas, possivelmente devido à predominância das reservas internas das sementes e à necessidade de maior tempo para o estabelecimento da interação planta-microrganismo. Conclui-se que os efeitos benéficos das rizobactérias promotoras do crescimento vegetal tendem a se manifestar em estágios mais avançados do desenvolvimento vegetal, reforçando a importância de avaliações em fases posteriores das culturas.

Palavras-chave: Bioinsumos; sementes; *Triticum aestivum* L.; *Glycine max* (L.) Merrill.

1 INTRODUCTION

Soybeans (*Glycine max* (L.) Merrill) and wheat (*Triticum aestivum* L.) are important crops for Brazilian agribusiness. According to the National Supply Company (CONAB), Brazil is the world's leading soybean producer, with an estimated production of 154 million tons in the 2022/2023 agricultural year. Brazil's soybean production significantly impacts the national export sector, accounting for approximately 40.39% of total exports (CONAB, 2024). Wheat cultivation is most significant in southern Brazil, where Rio Grande do Sul is the main producer. It accounts for about 43.7% of the total area sown in the country, corresponding to approximately 1.34 million hectares (CONAB, 2024).

The productivity of these crops depends on proper initial establishment. High vigor values, seed germination, emergence, and emergence speed are essential. Seeds with high vigor can produce uniform, resilient seedlings, even under adverse environmental conditions. This directly affects the initial plant stand and productive potential (Rossi; Cavariani; França-Neto, 2017). Thus, new technologies based on microorganisms are employed in agricultural management to achieve adequate initial seedling stands. These stands contribute to the sustainability of production systems and help reduce stress while increasing productivity.

To achieve high yields of these agricultural crops, *Bradyrhizobium*, a genus of bacteria found in oilseeds, and *Azospirillum*, a genus of bacteria found in grasses, are applied as seed inoculants. These bacteria promote plant development and have been observed to be effective in soybeans and wheat. In addition to these bacterial genera, *Pseudomonas* and *Bacillus* have been documented to promote plant growth by producing metabolites or increasing nutrient absorption by roots.

Classified as rhizobacteria, these biological agents improve germination, emergence, and plant growth (Islam *et al.*, 2013; Araújo *et al.*, 2015; Santos *et al.*, 2017; Souza, 2017).

These genera play a key role in plant physiological responses. They promote growth by through phytohormones, such as auxins, gibberellins, and cytokinins. These phytohormones promote cell elongation and stimulate the formation of more lateral roots and root hairs, thereby expanding the plant's root system. These genera also solubilize essential nutrients, such as phosphorus, through the production of phosphatase enzymes.

The mechanisms of indirect action consist of environmental and resource changes, as well as changes in plant gene expression, which affect growth and development (Florentino *et al.*, 2022). Additionally, these microorganisms generate symbiotic or associative relationships, such as mycorrhizae and rhizobia, which aid in the absorption of water and nutrients.

These microorganisms also increase plant resistance to environmental adversity. With the aim of expanding knowledge of the use of rhizobacteria in soybean and wheat crops, this study analyzes the effects of applying microorganisms on the vigor, germination, emergence, and emergence speed index traits of these crops' seedlings.

2 MATERIALS AND METHODS

The test was carried out in the laboratories of the Department of Biology (Center for Natural and Exact Sciences - CCNE) at the Federal University of Santa Maria (UFSM). For the experiment, batches of seeds from the NEO 660 IPRO (soybean) and TBIO Audaz (wheat) cultivars recommended for the state of Rio Grande do Sul were used. Microorganisms were used to treat the seeds, with a total of seven treatments: treatment 1 - water only; treatment 2 - mixture of *Azospirillum brasilense*, *Pseudomonas fluorescens*, and *Bacillus megaterium*; treatment 3 - *Priestia aryabhattai*; treatment 4 - *Bacillus amyloliquefaciens*; treatment 5 - mixture of *Azospirillum brasilense*, *Pseudomonas fluorescens*, and *Bacillus megaterium* (double dose); treatment 6 - *Priestia aryabhattai* (double dose); treatment 7 - *Bacillus amyloliquefaciens* (double dose).

Prior to sowing, bacterial inoculation was performed using a solution containing 3 mL of distilled water and 1 mL of bacterial suspension at a dose of 4.0 mL per kg of seeds. The solutions were administered using sterile, disposable syringes. A total volume of 1 mL of solution was applied to 250 g of seeds, calculated based on the weight of 1,000 seeds (WTS). After applying the solution, the seeds were placed in plastic bags and shaken by hand for 3 minutes to ensure complete homogenization of the inoculant. These treatments were performed according to the methodology described by Souto (2025).

After treatment, germination, vigor by first count, and growth parameters were evaluated using paper roll tests. For the germination test, four replicates of 50 seeds each were used. After setting up the tests, the sample units were stored in a BOD (biochemical oxygen demand) growth chamber.

Constant temperatures of 25 and 20 °C were used for soybeans and wheat, respectively. Seedlings classified as normal were counted at predefined intervals according to the Rules for Seed Analysis (Brasil, 2026). After each count, ten plants per replicate were separated, and the length of the aerial part was measured with a millimeter ruler. According to Krzyzanowski *et al.* (2020), the seedlings were then placed in an oven at 65 ± 5 °C for 48 hours until a constant mass (g plant^{-1}) was obtained. The roots were frozen and scanned for root system analysis. To do so, the roots were suspended in 0.5 cm of water in a transparent acrylic tray measuring 30×40 cm² and scanned at 600 dpi using an Epson Expression 11000 scanner equipped with an additional TPU light. The effects of microorganisms on root length (cm), root volume ($\pi \times r^2 \times \text{length}$ (cm³)), and root diameter (mm) were determined.

For the emergence speed test, a sand experiment was set up in 7.5-liter plastic trays. The trays were filled with sand that had been previously washed, sieved, and sterilized. Twenty seeds were sown in each repetition. The trays were stored in a cultivation room at either 25 or 20 °C, depending on the species. The sand was kept at 70% field capacity; the trays were weighed daily, and evaporated water was replaced as needed. The evaluated parameters were emergence, emergence speed index, growth, and seedling dry mass. The emergence speed index (ESI) was calculated daily using Maguire's equation (1962) and evaluated by counting the number of emerged seedlings up to the eighth day. To analyze the root system, we scanned the roots of these plants using the same methodology described for the germination test.

The data were entered into SISVAR and analyzed using Tukey's test at the 5% significance level. The data were separated into evaluation categories: first count, germination, and root parameters.

3 RESULTS AND DISCUSSION

3.1 BACTERIA PROMOTING GROWTH IN SOYBEAN GERMINATION AND EARLY GROWTH

Data analysis revealed that inoculating soybean seeds with growth-promoting microorganisms did not produce statistically significant differences among treatments and had no impact on germination or initial development (Table 1). While some variables showed numerical variations between treatments, they remained in the same statistical group. In the control treatment, 52% of normal seedlings were obtained in the first count (FC), similar to the values obtained in the treatments with microorganisms, and 86% were obtained in germination (G). When inoculated with growth-promoting microorganisms, the percentage of normal seedlings ranged from 47 to 58% in the first count and from 83 to 90% at germination.

Similarly, there were no significant differences in total seedling length (TSL), surface area (SA), diameter (DR), or root volume (RV) between treatments, with mean values ranging from 161 to 194 mm, 34 to 41 cm², 0.50 to 0.68 mm, and 0.55 to 0.67 cm³, respectively.

Table 1 - Seed germination and initial growth of soybean seedlings inoculated with growth-promoting microorganisms.

Treatments	FC (%)	G (%)	TSL (mm)	SA (cm ²)	DR (mm)	RV (cm ³)
Witness	52 a	86 a	184 a	37 a	0.65 a	0.61 a
<i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus megaterium</i>	50 a	88 a	161 a	34 a	0.66 a	0.55 a
<i>Priestia aryabhatai</i>	58 a	85 a	175 a	38 a	0.68 a	0.64 a
<i>Bacillus amyloliquefaciens</i>	47 a	86 a	170 a	35 a	0.67 a	0.58 a
<i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus megaterium</i> (2x)	56 a	85 a	187 a	39 a	0.5 a	0.63 a
<i>Priestia aryabhatai</i> (2x)	54 a	83 a	194 a	41 a	0.66 a	0.67 a
<i>Bacillus amyloliquefaciens</i> (2x)	58 a	90 a	177 a	36 a	0.65 a	0.58 a
CV (%)	9.97	4.59	12.8	10.48	13.89	9.31

First count (FC), germination (G), total seedling length (TSL), surface area (SA), diameter (DR), and root volume (RV).

* Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p \leq 0.05$).

These results suggest that inoculating seeds with microorganisms did not affect germination or initial growth under the experimental conditions of this study. This finding is consistent with previous studies that reported microorganisms influence later stages of plant development when they interact with the rhizosphere and become fully established (Bashan; Bashan, 2005; Hungria *et al.*, 2010). Additionally, the benefits of inoculation with plant growth-promoting rhizobacteria (PGPR) tend to manifest after the initial emergence phase, primarily affecting vegetative growth and productivity (Marchão *et al.*, 2025). Thus, the absence of effect may be related to insufficient time for root colonization and the predominance of the internal reserves of the seeds, which sustain the initial metabolism independently of the interaction with rhizobacteria.

Kähkönen *et al.* (2025) found that although growth-promoting microorganisms show potential *in vitro*, they may not produce statistically significant effects in the early stages of plant development under experimental conditions. Other authors describe how growth-promoting bacteria act, including synthesizing phytohormones, increasing nutrient availability, modulating root signals, and stimulating water and nutrient absorption (Vessey, 2003; Marschner, 2012). However, some mechanisms depend on colonization of the root surface or rhizosphere, which does not occur during the interval between sowing and germination assessment.

The aim was to promote initial root growth and observe numerical variations in root length, surface area, diameter, and volume. It was evident that *Priestia aryabhatai* (2x) and *Azospirillum brasiliense* (2x) produced better results; however, none of the treatments differed statistically from the control. According to the literature, growth-promoting rhizobacteria have positive effects on the root system through auxin synthesis, nitrogen fixation, and phosphate solubilization (Spaepen; Vanderleyden; Remans, 2007; Lugtenberg; Kamilova, 2009). However, these effects depend on bacterial concentration, the compatibility of microorganisms with the plant species, the physical and chemical characteristics of the soil or substrate, and colonization. As the evaluations were carried out in the

initial phase, it is possible that physiological compatibility had not yet been established, preventing these mechanisms from manifesting in measurable ways.

The observed coefficients of variation (4-14%) are considered adequate for this type of test, indicating experimental precision. Thus, the absence of statistical significance is not due to excessive variability but rather the absence of an effect from the treatments under the evaluated conditions. This suggests that the germination phase may not be the optimal time to observe the physiological effects of microorganisms. Previous studies have demonstrated that more consistent responses occur after root establishment, when nutrient absorption and the exchange of chemical signals between the plant and microorganisms increase (Hungria, 2010; Marschner, 2012). It is hypothesized that sequential application or application at different phenological stages may intensify the beneficial effects, especially on vegetative growth and productivity.

From a physiological standpoint, the results indicate that inoculation with *B. megaterium*, *P. fluorescens*, *A. brasiliense*, *P. aryabhattai*, and *B. amyloliquefaciens* does not directly impact the initial performance of the seedlings, even at high doses (2x). This does not mean that microorganisms have no effect; rather, they can produce positive results during crop stages such as vegetative growth and grain filling. At these stages, microorganisms can more effectively influence nitrogen and phosphorus metabolism, phytohormone production, and stress tolerance (Bashan; Bashan; Hernández, 2014; Antonioli *et al.*, 2023). Therefore, to determine the agronomic potential of the tested microorganisms, it would be worthwhile to conduct evaluations at later stages.

No significant differences were observed between treatments in terms of the emergence speed index (ESI) or emergence percentage (E) in soybeans (Table 2). ESI values ranged from 2.6 to 3.2, and emergence ranged from 50 to 74%. Total seedling length (TSL), surface area (SA), diameter (DR), root volume (RV), leaf area (LA), and the dry mass of the roots (RDM) and shoot (SDM) did not differ statistically between treatments. Mean values ranged from 241.02 to 275.90 mm for length; 73.24 to 82.87 cm² for surface area; 0.95 to 1.03 mm for diameter; and 1.73 to 2.03 cm³ for root volume.

Table 2 - Emergence and initial development of soybean seedlings subjected to inoculation with growth-promoting microorganisms in sand.

Treatments	ESI	E (%)	TSL (mm)	SA (cm ²)	DR (mm)	RV (cm ³)	LA (cm ²)	RDM (mg)	SDM (mg)
Witness	3.2 a	70 a	259.86 a	80.46 a	0.98 a	1.98 a	14.41 a	82 a	99 a
<i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus megaterium</i>	2.9 a	74 a	245.69 a	73.24 a	0.95 a	1.73 a	12.94 a	71 a	85 a
<i>Priestia aryabhattai</i>	2.6 a	50 a	246.07 a	74.97 a	0.97 a	1.81 a	13.17 a	72 a	85 a
<i>Bacillus amyloliquefaciens</i>	3.0 a	65 a	241.02 a	75.05 a	0.99 a	1.88 a	11.96 a	67 a	120 a

<i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus megaterium</i> (2x)	2.8 a	74 a	275.90 a	82.87 a	0.95 a	1.98 a	11.65 a	69 a	91 a
<i>Priestia aryabhatai</i> (2x)	2.8 a	63 a	245.84 a	78.97 a	1.03 a	2.03 a	13.06 a	68 a	39 a
<i>Bacillus amyloliquefaciens</i> (2x)	3.1 a	70 a	273.96 a	81.18 a	0.96 a	1.93 a	14.16 a	65 a	91 a
CV (%)	15.69	15.47	14.29	12.41	6.27	14.46	18.84	17.87	49.65

Emergence speed index (ESI), emergence (E), total seedling length (TSL), surface area (SA), diameter (DR), root volume (RV), leaf area (LA), root dry mass (RDM) and shoot dry mass (SDM). *Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p < 0.05$).

In the absence of a significant response, one can see that the initial vigor of the seedling mainly comes from the seed's internal reserves and metabolism during germination. These are intrinsic processes controlled by the embryo's physiological conditions (Taiz *et al.*, 2017; Marcos-Filho, 2015). Therefore, the applied microorganisms have no influence at this stage, as colonization occurs after the establishment of the plant-microorganism interaction, which happens after emergence. Field studies indicate that the benefits of rhizobacteria are enhanced in higher stress environments, suggesting that the results obtained under controlled conditions may differ from those observed in real agricultural systems.

Seedling emergence ranged from 50 to 74%, with no significant difference between treatments. Microorganisms such as *Azospirillum brasilense*, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Priestia aryabhatai*, and *Bacillus amyloliquefaciens* are known to promote plant growth, phytohormone production, nutrient solubilization, and root system stimulation (Vessey, 2003; Lugtjenberg; Kamilova, 2009; Hungria *et al.*, 2010).

3.2 BACTERIA PROMOTING GROWTH IN WHEAT GERMINATION AND EARLY GROWTH

The results indicate no statistically significant differences between treatments for wheat germination and initial development (Table 3). The values ranged from 41 to 43% in the first count and from 82 to 89% in germination. These results suggest that inoculation, regardless of the double dose, was ineffective in influencing the germination rate or seed germination. According to Silva *et al.* (2019), inoculation with plant growth-promoting microorganisms does not always result in a significant increase in germination or emergence speed index, as effects depend on environmental conditions and the stage of plant development.

Table 3 - Seed germination and initial growth of wheat seedlings subjected to inoculation with growth-promoting microorganisms.

Treatments	FC (%)	G (%)	TSL (mm)	SA (cm ²)	DR (mm)	RV (cm ³)
Witness	42 a	86 a	450 a	71 a	0.49 a	0.89 a
<i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus megaterium</i>	41 a	83 a	516 a	83 a	0.51 a	1.06 a
<i>Priestia aryabhatai</i>	43 a	89 a	443 a	70 a	0.51 a	0.90 a
<i>Bacillus amyloliquefaciens</i>	43 a	87 a	483 a	75 a	0.50 a	0.95 a
<i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus megaterium</i> (2x)	43 a	88 a	455 a	67 a	0.46 a	0.8 a
<i>Priestia aryabhatai</i> (2x)	42 a	87 a	455 a	69 a	0.48 a	0.84 a
<i>Bacillus amyloliquefaciens</i> (2x)	41 a	82 a	388 a	68 a	0.56 a	0.95 a
CV (%)	7.28	7.04	14.55	14.98	9.94	19.58

First count (FC), germination (G), total seedling length (TSL), surface area (SA), diameter (DR), and root volume (RV).

* Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($p \leq 0.05$).

Analysis of germination and early development parameters shows that none of the microbial treatments produced statistically significant differences compared to the control. These results align with germination physiology, wherein the process is primarily regulated by the cotyledon's internal reserves and the embryo's metabolism. These factors are not heavily influenced by external interactions during the initial hours and days after sowing (Taiz *et al.*, 2017; Marcos-Filho, 2015).

Initial root development showed no statistical difference (Table 3). However, root length varied from 388 to 516 mm, with the highest value observed in the treatment with *B. megaterium*, *P. fluorescens*, and *A. brasilense*. This trend suggests a possible initial stimulus for root elongation, but it is not statistically significant. Similarly, the surface area (67 to 83 cm²) and root diameter (0.46 to 0.56 mm) showed little variation between treatments, with no consistent differences. Root volume, ranging from 0.80 to 1.06 cm³, also showed no significant variation; however, numerically higher values were observed in some microbial treatments, such as *B. amyloliquefaciens*.

The lack of statistical difference between treatments is consistent with the evaluated phenological stage. Microbial action mechanisms depend on root colonization and establishing plant-microorganism interactions, which occur after germination (Vessey, 2003; Lugtvenberg; Kamilova, 2009). According to the coefficients of variation, the experimental precision is adequate, suggesting that the lack of response is due to the biological process rather than methodological limitations.

Thus, the results reinforce the idea that the effects of inoculation tend to manifest in the later stages of plant development, when nutritional demand and rhizosphere physiological interactions are greater. This does not invalidate the agronomic potential of these microorganisms (Hungria *et al.*, 2010; Marschner, 2012).

No significant differences were observed between treatments regarding the emergence speed index (ESI), emergence (E), total shoot length (TSL), diameter (DR), root volume (RV), leaf area (LA), dry root mass (RDM), and dry shoot mass (SDM) of wheat seedlings (Table 4).

Table 4. Emergence and initial development of wheat seedlings subjected to inoculation with growth-promoting microorganisms in sand.

Treatment	ESI	E (%)	TSL (mm)	SA (cm ²)	DR (mm)	RV (cm ³)	LA (cm ²)	RDM (mg)	SDM (mg)
Witness	4.01 a	86 a	450.71 a	73.13 a	0.51 a	0.95 a	8.41 a	51 a	25 a
<i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus megaterium</i>	5.24 a	98 a	496.60 a	78.37 a	0.50 a	1.0 a	6.14 a	52 a	22 a
<i>Priestia aryabhatai</i>	4.56 a	88 a	479.01 a	77.37 a	0.52 a	1.0 a	7.50 a	47 a	26 a
<i>Bacillus amyloliquefaciens</i>	4.74 a	95 a	460.65 a	69.33 a	0.48 a	0.83 a	6.86 a	45 a	27 a
<i>Azospirillum brasilense</i> + <i>Pseudomonas fluorescens</i> + <i>Bacillus megaterium</i> (2x)	4.46 a	83 a	459.32 a	71.37 a	0.49 a	0.89 a	6.15 a	53 a	27 a
<i>Priestia aryabhatai</i> (2x)	4.86 a	88 a	421.05 a	67.21 a	0.51 a	0.87 a	6.18 a	40 a	27 a
<i>Bacillus amyloliquefaciens</i> (2x)	4.83 a	88 a	459.76 a	70.96 a	0.49 a	0.87 a	6.77 a	44 a	22 a
CV (%)	11.65	10.65	16.02	17.01	10.73	21.89	34.08	20.48	23.43

Emergence speed index (ESI), emergence (E), total seedling length (TSL), surface area (SA), diameter (DR), root volume (RV), leaf area (LA), root dry mass (RDM) and shoot dry mass (SDM). *Means followed by the same letter in the column do not differ statistically from each other by Tukey’s test (p<0.05).

The emergence percentage (83-98%) and ESI (4.01-5.24) results indicate that inoculation with plant growth-promoting rhizobacteria, either alone or in combination, did not negatively affect germination. Initial growth variables (total length, surface area, diameter, and root volume) showed no significant responses, with mean values similar to those of the control across all treatments. This shows that there were no gains in the initial development of the plants.

There were no statistically significant differences between treatments, and the coefficient of variation (CV) was less than 15%, indicating high experimental precision. Compared with the control, the combination of *B. megaterium*, *P. fluorescens*, and *A. brasilense* showed improvements in emergence and ESI, whereas *Bacillus amyloliquefaciens* showed greater improvements in emergence alone. Overall, ESI improvements were observed with all treatments, but not with the doubled doses. No improvement in emergence was observed when the dose was doubled (2x).

Marchão *et al.* (2025) demonstrated that inoculation twice with an interval period with *Azospirillum*, *Pseudomonas*, *Priestia*, and *Bacillus* in soybean cultivation promoted significant increases in plant growth and grain yield but had little effect in the early stages of development. Therefore, beneficial microbial effects are more effective after the plant is established. According to Bhattacharyya and Jha (2012), this development is natural because the mechanisms of growth promotion mediated by bacteria—including the production of phytohormones, nutrient solubilization, and hormonal modulation—intensify as the plant matures.

Some factors that may exemplify the response during the early stages include the short evaluation period, limited root system development, and insufficient microbial colonization. Examples of these factors include auxin production and phosphorus solubilization. Additionally, controlled

conditions tend to reduce abiotic and biotic stresses, in which growth-promoting bacteria demonstrate better outcomes (Backer *et al.*, 2018; Compant *et al.*, 2019). According to Compant *et al.* (2019), the benefits of beneficial microorganisms depend on the environment and are generally amplified under stressful conditions, such as water deficiency, low nutrient availability, or pathogen pressure.

These effects also occur in wheat cultivation. Responses to microorganism inoculation are used in later stages, and the plants are subjected to stressful environments (Lugtjenberg; Kamilova, 2009; Backer *et al.*, 2018). The effects of plant growth-promoting rhizobacteria are evidenced under abiotic stress conditions, such as water deficit and low fertility, proving to be efficient against environmental adversities. Given this, results obtained under controlled conditions generally differ from those worked in the field, due to the complexities of agricultural systems with environmental interactions (Vessey, 2003; Glick, 2012; Bashan *et al.*, 2014; Timmusk *et al.*, 2017).

4 CONCLUSIONS

This study shows that applying microorganisms (*Azospirillum brasilense*, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Priestia aryabhatai*, and *Bacillus amyloliquefaciens*) did not produce significant results during the early stages of soybean and wheat development. During this period, the seedlings directly use the energy reserves of the cotyledons, with the need for integration with soil microorganisms. The colonization by rhizobacteria depends on a broader, more developed root system, that is, with a larger contact area and exudation to support the associated microbiota.

Moreover, in the initial stages of development, the root system still has low structure, which can limit establishment. Thus, some effects tend to manifest in more advanced phenological stages, where there is greater root metabolic activity and intensification of exchanges with the soil and the environment.

To achieve greater results, it is necessary to conduct analyzes in later stages of plant development, especially in phases that involve higher physiological demands. Also, exposing plants to environmental stresses, such as water deficit or low nutrient availability, can enhance the effects of plant growth-promoting bacteria, boosting their performance in the face of adversities. Future studies under field conditions are essential, allowing for the consideration of the complexity of the soil-plant-microorganism system, aiming for results that are more representative of agricultural systems.

REFERENCES

ANTONIOLLI, H. R. M. *et al.* *Horizontal transfer and the widespread presence of Galileo transposons in Drosophilidae (Insecta: Diptera)*. **Mobile DNA**, London, v. 14, p. 1-13, 2023.

- ARAÚJO, E. O. *et al.* *Effects of nitrogen fertilization associated with diazotrophic bacteria inoculation on nitrogen use efficiency and its biological fixation by corn determined using N*. **African Journal of Microbiology Research**, v. 9, n. 9, 2015.
- BACKER, R. *et al.* *Plant growth-promoting rhizobacteria: mechanisms and applications*. **Frontiers in Plant Science**, v. 9, p. 1473, 2018.
- BASHAN, Y.; BASHAN, L. E. *How the plant growth-promoting bacterium Azospirillum promotes plant growth: a critical assessment*. In: SPARKS, D. L. (ed.). **Advances in Agronomy**. San Diego: Academic Press, 2005. v. 86, p. 77-136.
- BASHAN, Y.; BASHAN, L. E.; HERNÁNDEZ, J.-P. *The multiple effects of inoculants of Azospirillum spp. on the growth of crops*. In: MARCO, G. (ed.). **Azospirillum-Plant Interactions**. Boca Raton: CRC Press, 2014. p. 195-212.
- BHATTACHARYYA, P. N.; JHA, D. K. *Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture*. **Trends in Biotechnology**, v. 30, n. 9, p. 483-490, 2012.
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Brasília, DF: MAPA, 2026. Disponível em: https://wikisda.agricultura.gov.br/pt-br/Laborat%C3%B3rios/Metodologia/Sementes/RAS_2025/cap_4_Germinacao_rev_1.
- COMPANT, S. *et al.* *The plant endosphere world - bacterial life within plants*. **Nature Reviews Microbiology**, v. 17, p. 747-760, 2019.
- CONAB. *Safra 2023/24: Rio Grande do Sul é o terceiro maior produtor de grãos do país*. Agência Gov, 14 nov. 2024. Disponível em: <https://agenciagov.ebc.com.br>. Acesso em: 02 dez. 2025.
- FLORENTINO, A. L. *et al.* *Integrated Ca, Mg, Cu, and Zn supply upregulates leaf anatomy and metabolic adjustments in Eucalyptus seedlings*. *Plant Physiology and Biochemistry*, v. 208, p. 108446, 2024.
- GLICK, B. R. *Plant growth-promoting bacteria: mechanisms and applications*. *Scientifica*, v. 2012, Article ID 963401, 2012.
- HUNGRIA, M. *et al.* *O. Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil*. **Plant and Soil**, v. 331, n. 1-2, p. 413-425, 2010.
- ISLAM, M. R. *et al.* *Nitrogen-fixing bacteria with multiple plant growth-promoting activities enhance growth of tomato and red pepper*. **Journal of Basic Microbiology**, v. 53, n. 12, 2013.
- KÄHKÖNEN, M. A. *et al.* *Inoculation with in vitro promising plant growth-promoting bacteria isolated from nitrogen-limited boreal forest did not translate to in vivo growth promotion of agricultural plants*. **Biology and Fertility of Soils**, v. 61, 2025.

- KRZYŻANOWSKI, F. C. *et al.* *J. Testes de vigor baseados em desempenho de plântulas*. In: **Vigor de sementes: conceitos e testes**. Londrina: ABRATES, 2020. p. 601.
- LUGTENBERG, B.; KAMILOVA, F. *Plant-growth-promoting rhizobacteria*. **Annual Review of Microbiology**, v. 63, p. 541-556, 2009.
- MARCOS-FILHO, J. **Fisiologia de sementes de plantas cultivadas**. Londrina: ABRATES, 2015. 650p.
- MAGUIRE, J. D. Speed of germination aid in selection and evaluation for seedling emergence and vigor. **Crop Science**, v. 2, n. 2, p. 176-77, 1962.
- MARCHÃO, R. L. *et al.* *Improving soybean development and grain yield by complementary inoculation with growth-promoting bacteria*. **Plants**, v. 14, n. 3, p. 402, 2025.
- MARSCHNER, H. **Marschner's Mineral Nutrition of Higher Plants**. 3. ed. London: Academic Press, 2012.
- ROSSI, R. F.; CAVARIANI, C.; FRANÇA-NETO, J. B. *Vigor de sementes, população de plantas e desempenho agrônomo de soja*. **Revista de Ciências Agrárias**, v. 60, n. 3, p. 215-222, 2017.
- SANTOS, K. F. D. N. *et al.* *Wheat colonization by Azospirillum brasilense ammonium-excreting strain reveals upregulation of nitrogenase and superior plant growth promotion*. **Plant and Soil**, v. 415, n. 1, 2017.
- SILVA, J. D. *et al.* *Effect of plant growth-promoting rhizobacteria on Salicornia ramosissima seed germination under salinity, CO₂ and temperature stress*. **Agronomy**, v. 9, n. 10, p. 655, 2019.
- SOUTO, M. S. **Alterações fisiológicas e produtivas em soja e trigo pelo uso de bioinsumos**. 2025. Dissertação (Mestrado em Agrobiologia) - Universidade Federal de Santa Maria, Santa Maria, RS, 2025.
- SOUZA, A. A. **Resposta do milho e do tomateiro à inoculação com bactérias diazotróficas isoladas da superfície de folhas**. 2017. Dissertação (Mestrado em Solos e Nutrição de Plantas) - Universidade de São Paulo, Piracicaba, 2017.
- SPAEPEN, S.; VANDERLEYDEN, J.; REMANS, R. *Indole-3-acetic acid in microbial and microorganism-plant signaling*. **FEMS Microbiology Reviews**, v. 31, n. 4, p. 425-448, 2007.
- TAIZ, L. *et al.* **Plant Physiology and Development**. 6. ed. Sunderland: Sinauer Associates, 2017.
- TIMMUSK, S. *et al.* Perspectives and challenges of microbial application for crop improvement. *Frontiers in Plant Science*, v. 8, 2017.
- VESSEY, J. K. *Plant growth promoting rhizobacteria (PGPR)*. **Biological Reviews**, v. 78, n. 4, p. 641-653, 2003.