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Growth promotion under inoculation with *Azospirillum brasilense* and *Trichoderma asperellum* in maize cultivation

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Resumo

A utilização de microrganismos promotores de crescimento vegetal constitui uma estratégia relevante para a intensificação sustentável da produção agrícola, especialmente em culturas de elevada importância econômica, como o milho. O estudo teve como objetivo avaliar os efeitos da inoculação isolada e da co-inoculação de *Azospirillum brasilense* e *Trichoderma asperellum* sobre o crescimento inicial de plantas de milho (*Zea mays* L.). O experimento foi conduzido em ambiente controlado, em delineamento inteiramente casualizado, com quatro tratamentos: controle (sem inoculação), *A. brasilense, T. asperellum* e a combinação *A. brasilense* + *T. asperellum*, com três repetições. Foram avaliados o crescimento da parte aérea ao longo do ciclo inicial e, ao final do período experimental, o comprimento e o volume do sistema radicular. Os resultados indicaram que a co-inoculação promoveu maior crescimento da parte aérea e incremento expressivo no desenvolvimento radicular, quando comparada aos tratamentos isolados e à testemunha. Os efeitos observados estão associados à complementaridade dos mecanismos de ação dos microrganismos, envolvendo fixação biológica de nitrogênio, produção de fitormônios, maior eficiência na absorção de nutrientes e estímulo à arquitetura radicular. Conclui-se que a co-inoculação de *A. brasilense* e *T. asperellum* apresenta potencial agronômico para promover maior vigor inicial do milho, configurando-se como uma prática promissora para sistemas agrícolas sustentáveis.

Palavras-chave: microrganismos promotores de crescimento; co-inoculação; *Zea mays*.

Abstract

Growth promotion under inoculation with *Azospirillum brasilense* and *Trichoderma asperellum* in maize cultivation

The use of plant growth-promoting microorganisms is an important strategy for the sustainable intensification of agricultural production, especially in crops of high economic importance, such as maize. The objective of this study was to evaluate the effects of isolated inoculation and co-inoculation of *Azospirillum brasilense* and *Trichoderma asperellum* on the initial growth of maize plants (*Zea mays* L.). The experiment was conducted in a controlled environment, in a completely randomized design, with four treatments: control (no inoculation), *A. brasilense, T. asperellum,* and the combination of *A. brasilense* + *T. asperellum*, with three replicates. Shoot growth was evaluated throughout the initial cycle and, at the end of the experimental period, the length and volume of the root system. The results indicated that co-inoculation promoted greater shoot growth and a significant increase in root development when compared to the isolated treatments and the control. The effects observed are associated with the complementary mechanisms of action of the microorganisms, involving biological nitrogen fixation, phytohormone production, greater efficiency in nutrient absorption, and stimulation of root architecture. It is concluded that the co-inoculation of *A. brasilense* and *T. asperellum* has agronomic potential to promote greater initial vigor in maize, making it a promising practice for sustainable agricultural systems.

Keywords: plant growth-promoting microorganisms; co-inoculation; *Zea mays*.

Resumen

Promoción del crecimiento mediante la inoculación de *Azospirillum brasilense* y *Trichoderma asperellum* en el cultivo de maíz

El uso de microorganismos promotores del crecimiento vegetal constituye una estrategia relevante para la intensificación sostenible de la producción agrícola, especialmente en cultivos de gran importancia económica, como el maíz. El objetivo del estudio fue evaluar los efectos de la inoculación aislada y la co-inoculación de *Azospirillum brasilense* y *Trichoderma asperellum* sobre el crecimiento inicial de plantas de maíz (*Zea mays* L.). El experimento fue conducido en un ambiente controlado, en un diseño completamente aleatorio, con cuatro tratamientos: control (sin inoculación), *A. brasilense*, *T. asperellum* y la combinación *A. brasilense* + *T. asperellum*, con tres repeticiones. Se evaluó el crecimiento de la parte aérea a lo largo del ciclo inicial y, al final del período experimental, la longitud y el volumen del sistema radicular. Los resultados indicaron que la co-inoculación promovió un mayor crecimiento de la parte aérea y un aumento significativo en el desarrollo radicular, en comparación con los tratamientos aislados y el testigo. Los efectos observados están asociados a la complementariedad de los mecanismos de acción de los microorganismos, que implican la fijación biológica de nitrógeno, la producción de fitohormonas, una mayor eficiencia en la absorción de nutrientes y el estímulo la arquitectura radicular. Se concluye que la co-inoculación de *A. brasilense* y *T. asperellum* presenta potencial agronómico para promover mayor vigor inicial del maíz, lo que la convierte en una práctica prometedora para los sistemas agrícolas sostenibles.

Palabras clave: microorganismos promotores del crecimiento vegetal; co-inoculación; *Zea mays*.

Introduction

Maize (*Zea mays* L.) is one of the main agricultural crops worldwide and in Brazil, playing a strategic role in food security, animal feed production, and as a raw material for several industrial sectors. In Brazil, maize shows broad edaphoclimatic adaptability and high economic relevance, being cultivated in diverse production systems ranging from smallholder farms to highly mechanized agricultural areas (CONAB, 2023; EMBRAPA, 2018).

However, the high yield potential of maize is strongly associated with the intensive use of mineral fertilizers, especially nitrogen fertilizers, which results in high production costs and significant environmental impacts, such as greenhouse gas emissions and water resource contamination (Hungria *et al*., 2022; Cassán *et al*., 2020).

The inoculation of plant growth-promoting microorganisms (PGPM) has emerged as a sustainable practice to increase crop productivity, reduce production costs, and mitigate environmental impacts. Among these microorganisms, the genus *Azospirillum* stands out due to its ability to fix atmospheric nitrogen, synthesize phytohormones, and improve root development (Bashan *et al*., 2004; Hungria *et al*., 2010). *Trichoderma*, in turn, is recognized as both a biocontrol agent and a plant growth promoter, capable of colonizing the rhizosphere, inducing systemic resistance, and releasing compounds that stimulate plant growth (Harman *et al*., 2004; Contreras-Cornejo *et al*., 2009).

The co-inoculation of compatible microorganisms such as *Azospirillum* and *Trichoderma* may enhance plant-soil benefits by promoting synergistic effects on nutrient uptake, stress tolerance, and crop productivity (Vinale *et al*., 2008; Yadav *et al*., 2022). Moreover, this practice contributes to reducing the dependence on synthetic nitrogen fertilizers, aligning with the principles of low-impact and sustainable agriculture (Souza *et al*., 2021).

Thus, the objective of this study was to evaluate the effects of single and combined inoculation of *Azospirillum brasilense* and *Trichoderma asperellum* on maize plant growth (*Zea mays* L.), aiming to identify potential positive interactions between these microorganisms and their impact on plant development.

Material and methods

The study was conducted in 2024 at the Teaching Laboratory of the State University of Rio Grande do Sul (UERGS), Cachoeira do Sul campus. The experimental design was completely randomized, with four treatments and three replicates: (a) control (without inoculation); (b) *Azospirillum brasilense*; (c) *Trichoderma asperellum*; and (d) *Azospirillum* + *Trichoderma*.

Maize seeds (variety SHS 5050) were sown in 500 mL plastic cups containing a sterilized substrate composed of two parts vermiculite and one part washed and autoclaved sand. Three seeds were sown per replicate, and after emergence, thinning was performed to maintain one plant per container.

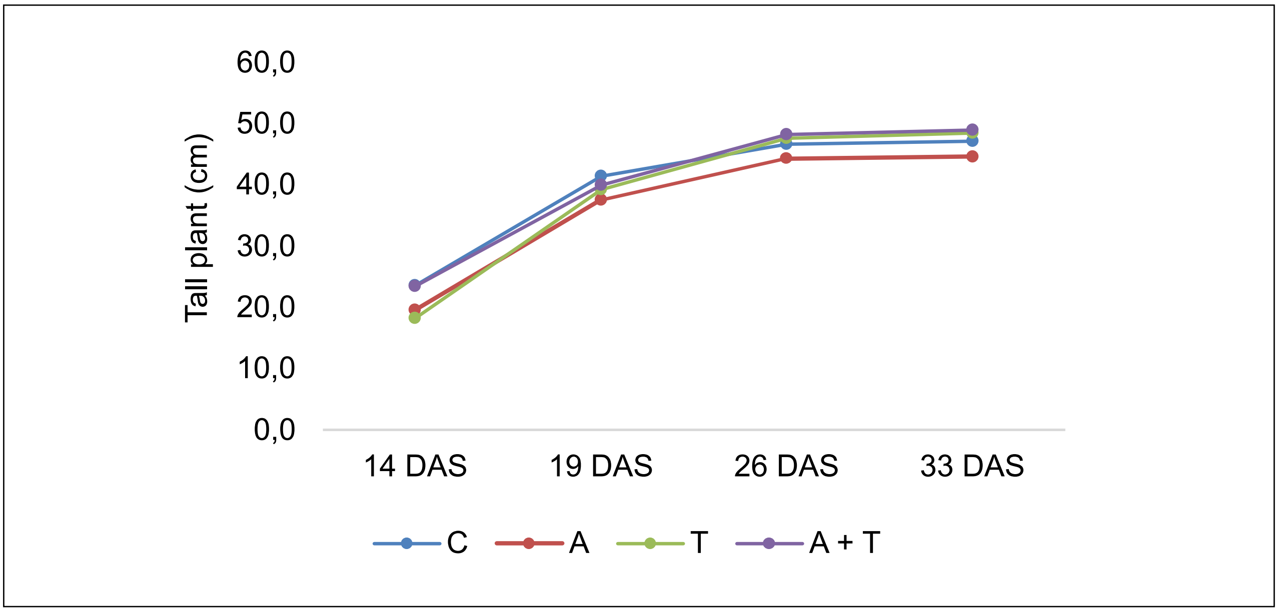
Inoculation was performed directly on the seeds, applying 0.5 mL of each microbial suspension per seed, totaling 1.5 mL per pot. The inoculant doses followed the manufacturer's recommendations. The commercial products Biomax Azum (*Azospirillum*, strain AbV5, concentration $3x10^8$ colony forming units per mL) and Tricho-turbo (Trichoderma asperellum, strain BV10, concentration $1x10^{10}$ viable conidia) were used, while distilled water was applied in the control treatment.

Shoot growth evaluations were conducted at 14, 19, 26, and 33 days after sowing (DAS). At the final evaluation, root length and root volume were measured using the water displacement method. Data were subjected to analysis of variance (ANOVA) and Tukey's test at a 5% significance level.

Results and discussion

Shoot growth of maize plants differed among treatments throughout the evaluation period, with the co-inoculation of *Azospirillum brasilense* and *Trichoderma asperellum* resulting in the highest growth increments at all evaluation times (Figure 1). This outcome reinforces the concept that microbial consortia often outperform single-strain inoculations due to synergistic interactions, providing multiple complementary plant growth-promoting mechanisms simultaneously (Hungria *et al*., 2022; Yadav *et al*., 2022).

Figure 1 – Shoot growth of maize



*C: control; A: *Azospirillum*; T: *Trichoderma*; A + T: *Azospirillum* and *Trichoderma;*
**DAS: days after sowing.

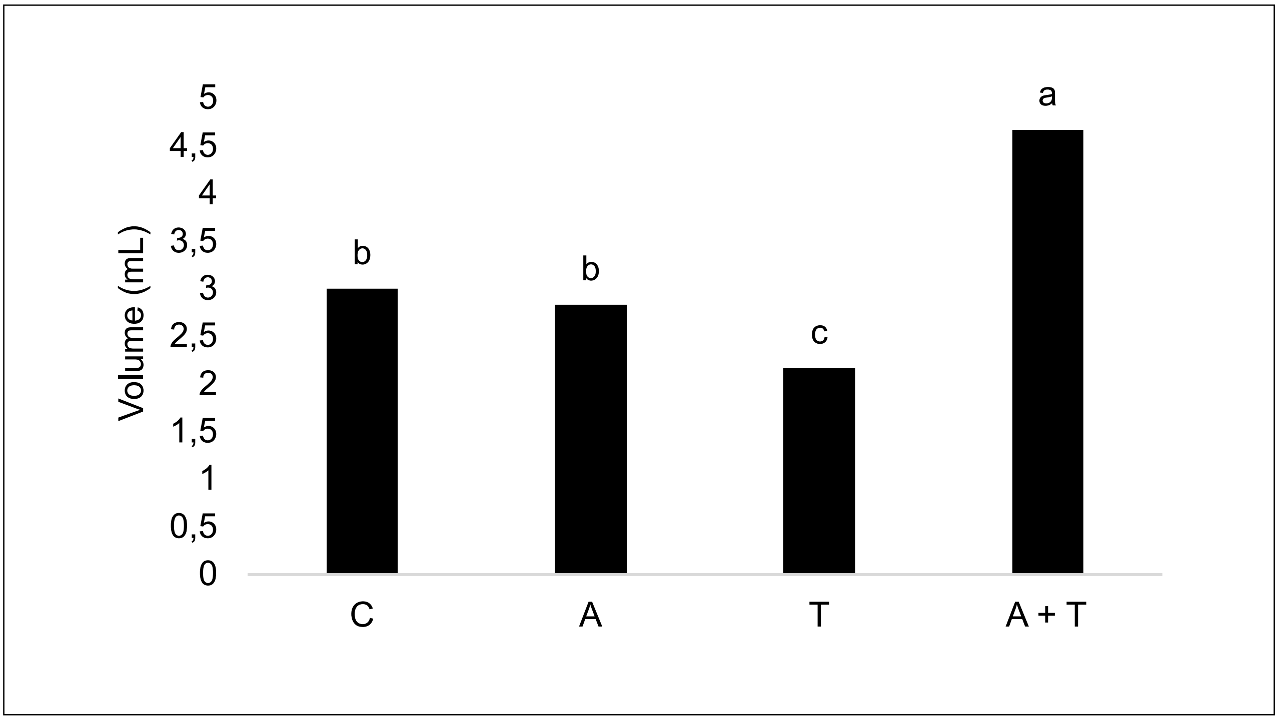
Microbial co-inoculation has gained increasing attention worldwide as an ecologically based strategy to enhance crop establishment while reducing dependence on synthetic fertilizers. Recent large-scale evidence suggests that inoculants can reshape the rhizosphere microbiome, improving nutrient cycling and plant vigor under field conditions (Francioli *et al.*, 2025).

Single inoculation with *A. brasilense* did not result in significant increases in shoot growth compared to the control. This response has also been reported in Brazilian meta-analyses, showing that maize responses to *Azospirillum* are variable and depend strongly on soil nitrogen availability, native microbial competition, and environmental conditions (Hungria *et al*., 2022). In early-stage or substrate-based experiments, the full benefits of biological nitrogen fixation may not be expressed, since plant–microbe associations often require time to stabilize (Cassán; Diaz-Zorita, 2016).

The superior performance of the combined treatment can be attributed to the complementary action of microbial mechanisms. *Azospirillum brasilense* is widely recognized for its capacity to stimulate plant growth mainly through phytohormone synthesis (auxins, cytokinins and gibberellins), which enhances cell expansion and root branching, rather than solely through nitrogen fixation (Cassán; Diaz-Zorita, 2016; Bashan; de-Bashan, 2010). These hormonal effects are particularly important during the early vegetative phase, when root architecture determines future nutrient uptake capacity.

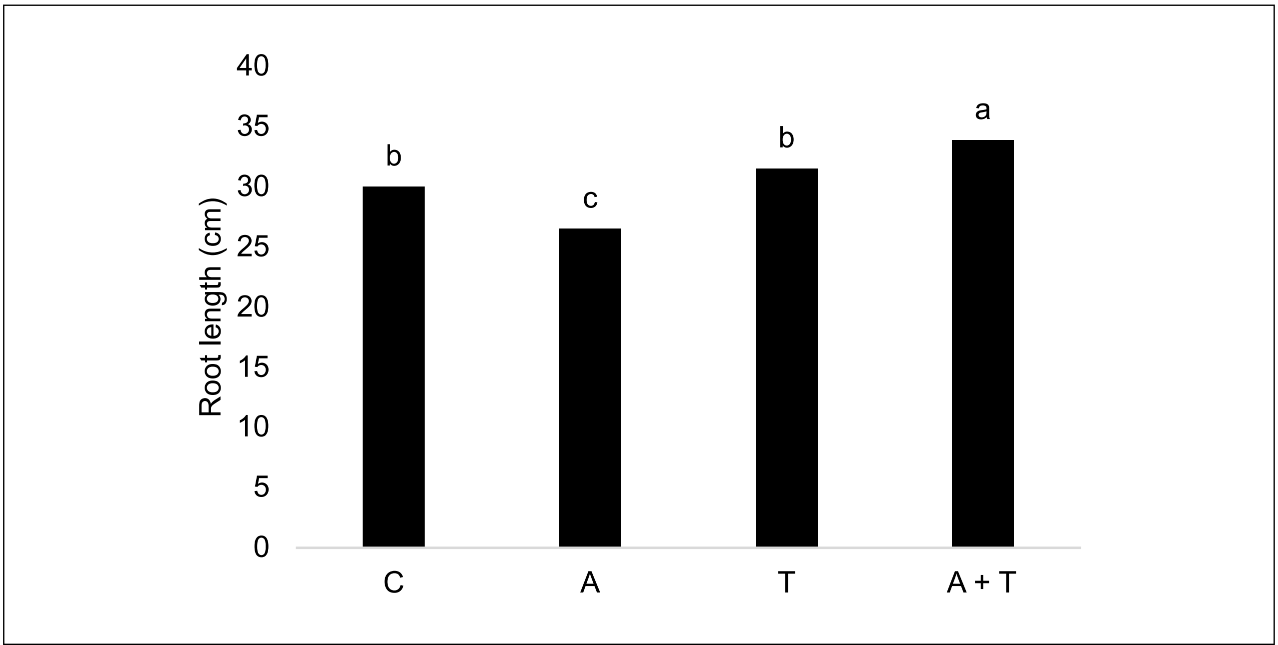
In contrast, *Trichoderma asperellum* acts as a multifunctional biostimulant fungus, improving nutrient solubilization, producing secondary metabolites, modulating plant metabolism, and enhancing stress tolerance (Lorito *et al*., 2010; Medeiros *et al*., 2022). Additionally, *Trichoderma spp.* are known to colonize the rhizosphere efficiently and induce systemic resistance, contributing indirectly to better plant growth by reducing biotic stress (Harman *et al*., 2004).

Figure 2 – Root volume of maize



*C: control; A: *Azospirillum*; T: *Trichoderma*; A + T: *Azospirillum* and *Trichoderma*.

Figure 3 – Maize root length



*C: control; A: *Azospirillum*; T: *Trichoderma*; A + T: *Azospirillum* and *Trichoderma*.

Table 1 – Summary of analysis of variance tables for the evaluated parameters

Evaluated parameter	DF	F	p	VC
Plant heigtht	3	2,81	0,047	18,4%
Root volume	3	4,18	0,045	18,4%
Root length	3	4,40	0,042	17,1%

Table 1 presents a summary of the analysis of variance (ANOVA) for the evaluated parameters, showing a significant effect of the treatments on the analyzed variables. These results demonstrate confirm that the plants responded significantly to the treatments, reinforcing the sensitivity of these parameters as performance indicators.

Root volume showed a marked increase under co-inoculation (Figure 2), indicating that microbial association strongly favored root system development. Root stimulation is a central mechanism explaining yield gains from plant growth-promoting microorganisms, as increased root volume improves soil exploration and nutrient acquisition efficiency (Bashan; de-Bashan, 2010).

The synergistic effect observed may result from the combined influence of bacterial auxin production and fungal enhancement of nutrient availability, leading to accelerated formation of lateral roots and root hairs (Vinale *et al*., 2008). Similar findings were reported by Souza *et al*. (2021), who demonstrated that co-inoculation of *Azospirillum* and *Trichoderma* improved maize nutritional status and biomass accumulation.

Root length was also greater in the combined treatment (Figure 3), suggesting enhanced substrate exploration. Alterations in root architecture induced by microbial inoculants are critical because maize is highly responsive to early root establishment, which determines competitive ability and drought resilience (Cassán *et al*., 2020).

Recent studies indicate that microbial consortia promote positive changes in root morphology and physiology, improving water and nutrient uptake and increasing tolerance to abiotic stresses such as salinity and drought (Yadav *et al*., 2022; Medeiros *et al*., 2022). Silva *et al*. (2024) further reported that *Azospirillum* inoculation in maize-based systems not only enhances productivity but also promotes beneficial chemical and biological shifts in soil quality.

Overall, the results corroborate national and international literature by demonstrating that co-inoculation of *A. brasilense* and *T. asperellum* is more efficient than single inoculation, particularly during early maize growth stages. This phase is critical for crop establishment, and improvements in root vigor may translate into higher yield stability under field conditions (Hungria *et al*., 2022; Cassán *et al*., 2020).

Laboratory experiments that evaluate plant growth promotion present important limitations, mainly due to the simplification of biological and environmental interactions. Under controlled conditions, inoculated microorganisms interact with the plant in a sterile or semi-sterile environment, without the presence of the native soil microbiota, which may overestimate their effects. "Plant growth-promoting rhizobacteria can enhance plant growth under controlled conditions, but their performance in the field is often inconsistent due to environmental variability" (Kloepper; Ryu; Zhang, 2004).

In addition, the absence of microbial competition and natural limiting factors favors root colonization by the inoculated microorganisms. Another relevant point is that, at early stages, seedling growth may still be partially supported by seed reserves, which can mask the real contribution of microorganisms during this period.

In contrast, experiments conducted under field conditions expose microorganisms to a range of factors that directly influence their survival, colonization, and efficiency. The native soil microbiota, for example, exerts strong competition for the environment, affecting the establishment of inoculated microorganisms. According to Compant *et al*. (2010), "the success of introduced beneficial microorganisms depends largely on their ability to compete with indigenous microflora and to adapt to environmental conditions". Furthermore, variables such as temperature, moisture, soil pH, and nutrient availability modulate microbial activity and plant–microorganism interactions.

Thus, results obtained in laboratory conditions often do not replicate in the field, highlighting the limited external validity of such studies. Therefore, although laboratory assays are essential for initial screening and understanding the mechanisms of action of plant growth-promoting microorganisms (PGPMs), validation under field conditions is indispensable to confirm their agronomic efficiency.

Finally, it is important to highlight that this experiment was conducted under controlled and substrate-based conditions, which may limit the extrapolation of results to field environments. Future studies should validate these responses under diverse edaphoclimatic conditions and evaluate impacts on grain yield and fertilizer savings, as recommended for sustainable intensification strategies (Yadav *et al*., 2022; Francioli *et al.*, 2025).

Conclusions

Co-inoculation with *Azospirillum brasilense* and *Trichoderma asperellum* significantly increased shoot and root growth of maize plants compared to single inoculations and the control treatment. The results demonstrate a synergistic interaction between these microorganisms, leading to greater plant vigor and development.

Therefore, co-inoculation represents a promising strategy to improve nutrient use efficiency and reducing reliancen on chemical inputs, contributing to more sustainable agricultural systems.

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