

Comparative efficiency of microbial bioinputs in second-crop corn: integration of multivariate analyses and selection indices for field recommendations

Denner Moreira de Sousa¹, Diego Oliveira Ribeiro², Glicélia Pereira Silva³, José Tiago das Neves Neto⁴, Ivan Ricardo Carvalho⁵, Cristhian Milbradt Babeski⁶, Luiz Leonardo Ferreira⁷

¹ College of Agronomy, Mineiros University Center, Mineiros, Brazil. E-mail: souza@gmail.com, ORCID: <https://orcid.org/0009-0004-3905-5161>

² College of Agronomy, Mineiros University Center, Mineiros, Brazil. E-mail: ribeiro@gmail.com, ORCID: <https://orcid.org/0000-0003-2336-3042>

³ College of Agronomy and Medicine, University Center, Mineiros, Brazil. E-mail: silva@gmail.com, ORCID: <https://orcid.org/0000-0003-2440-8636>

⁴ College of Agronomy, Mineiros University Center, Mineiros, Brazil. E-mail: neto@gmail.com, ORCID: <https://orcid.org/0000-0003-4170-7670>

⁵ Regional University of Northwestern Rio Grande do Sul, Ijuí, Brazil. E-mail: ivan.carvalho@unijui.edu.br, ORCID: <https://orcid.org/0000-0001-7947-4900>

⁶ Federal University of Santa Maria, Santa Maria, Brazil. E-mail: babeski@gmail.com, ORCID: <https://orcid.org/0000-0003-2273-2512>

⁷ College of Agronomy, Mineiros University Center, Mineiros, Brazil. E-mail: luizleonardo@unifimes.edu.br, ORCID: <https://orcid.org/0000-0001-5444-8503>

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Abstract

This study was developed to evaluate the effects of inoculating beneficial microorganisms in the second-crop corn culture, with an emphasis on the correlations between morphological and productive traits. The experiment was carried out in the municipality of Mineiros, Goiás state, Brazil. The experimental design included seed and foliar treatments, involving *Trichoderma asperellum*, *Bacillus subtilis*, and a standard chemical treatment. Statistical analyses were performed using R software. It was demonstrated that inoculation promoted significant changes in root development, resulting in greater accumulation of aboveground biomass and gains in productive potential. Overall, the microorganisms evaluated, particularly *T. asperellum* and *B. subtilis*, showed potential for optimizing early growth, physiological efficiency, and productivity in second-crop corn, in addition to contributing to reducing dependence on mineral fertilizers. Therefore, the use of the TS3 treatment is technically recommended as a viable alternative for maximizing corn productivity, maintaining a balance between yield components and plant structural characteristics. However, it is emphasized that further research should be conducted in different harvests, soil and climate conditions, and management systems to validate and refine these recommendations, ensuring greater robustness and applicability of the results obtained.

Keywords: *Bacillus subtilis*. *Trichoderma asperellum*. *Zea mays*. Plant growth promotion. Sustainable agriculture.

Introduction

Agriculture is currently undergoing a process of intensification, driven by the adoption of modern management technologies aimed at increasing crop production and productivity. However, this intensification has also contributed to the increased occurrence of phytosanitary problems in commercial cropping systems, particularly those associated with soil-dwelling phytopathogens. These microorganisms persist in the soil for most of their life cycle, infect underground plant organs and stems, and often exhibit saprophytic survival capacity, which makes their management challenging (Ferreira, Bettiol, 2019).

Brazilian agricultural production is considered one of the largest in the world, due to the country's territorial size and its grain production potential. Corn (*Zea mays* L.) is produced for commercial grains, which are used for human and animal consumption, and also for the production of silage, which consists of a fermentation process used in animal feed during the dry season (Chagas Júnior *et al.*, 2022).

In recent years, technologies have been implemented in agricultural crops, particularly corn, aiming to improve seed quality and, consequently, increase production. Seed treatment with fungicides, insecticides, micronutrients, biostimulants, and microorganisms is one of the

fundamental technologies in this field, which can be applied to protect the crop in its early stages (Ferreira, Bettiol, 2019).

Given the current scenario, where Brazil's main economy is agriculture, alternatives are being sought to increase agricultural production of key crops, such as soybeans and corn, as well as the production of healthy foods (Chagas Júnior *et al.*, 2022). However, assessing the health of corn seeds through seed health testing has become important today, as healthy seeds produce strong, vigorous, well-developed seedlings that establish themselves in different soil and climate conditions, with faster emergence and plant development (Borsatti *et al.*, 2021).

Plant diseases result from continuous irritation of host cells and tissues by a pathogen or environmental factor, leading to their malfunction and the development of symptoms (Ferreira, Bettiol, 2019). In contrast, beneficial microorganisms can act as plant growth promoters and induce plant resistance to diseases (Chagas Júnior *et al.*, 2022). Therefore, the importance of bioprotective microorganisms as an alternative strategy for pathogen control is evident (Bezerra *et al.*, 2022).

One option for increasing agricultural productivity naturally and sustainably is the use of microorganisms, such as bacteria and fungi, that promote plant growth (Souto *et al.*, 2020). Microorganisms have a broad influence on plant development, including beneficial effects on seed germination, seedling emergence, plant development and growth, and phytosanitary control, resulting in increased productivity (Steffen *et al.*, 2021).

Although bioproducts (bioinsecticides, biofungicides, and plant biostimulants) are marketed under different names due to their compliance with Brazilian agricultural product legislation, they are being widely tested and disseminated, demonstrating their effectiveness in a wide variety of agricultural crops across all regions

of the country (Steffen *et al.*, 2021). According to Ferreira and Bettiol (2019), *Bacillus* isolates have promising characteristics for promoting plant growth and biocontrol of soil-borne phytopathogens in corn. The *Trichoderma* fungus inoculated via seed increased root volume and biomass of corn plants (Chagas Júnior *et al.*, 2022).

The use of *Trichoderma harzianum*, regardless of the concentration applied to the seeds, was effective in reducing incidence of *Aspergillus* sp., *Aspergillus niger*, *Penicillium* sp., and *Fusarium* sp. Furthermore, *Trichoderma harzianum* did not negatively interfere with the physiological quality of the seeds and provided an increase in the germination percentages (Bezerra *et al.*, 2022). Steffen *et al.* (2021) reported that the inoculation of *Trichoderma harzianum* via seed treatment provided a significant increase in the productivity of hybrid corn, making it possible to increase productivity by 40.56 bags of corn per hectare, starting from 13,800 kg ha⁻¹ and reaching a productivity of 16,232 kg ha⁻¹.

Medeiros *et al.* (2020) observed that the four *Trichoderma* species had antagonistic potential against *F. moniliforme*, and that soil treatment with application of *Trichoderma* strains promoted the greatest capacity to induce resistance and/or biocontrol in corn, with a rate of 90 %, followed by foliar application, with 85 % of plants considered resistant. Rosa *et al.* (2021), evaluating the efficiency of fungal and bacterial biological nematicides associated with poultry litter in the management of *Pratylenchus brachyurus* and *Heterodera glycines* in soybean and corn crops in succession, concluded that the use of *P. lilacinum* + *T. harzianum* and *B. subtilis* + *B. licheniform* is associated with the organic compost of poultry litter, which promotes an increase in fresh root weight and reduces the number of nematodes in soybean and second-season corn.

Araújo *et al.* (2023) concluded that *T. harzianum* influences vegetative growth in corn,

but that depending on the dose of the bioinput, it may result in different plant responses. However, Souto *et al.* (2020), studying the efficiency of microorganisms as growth promoters in the initial development of corn in a greenhouse, found that they did not provide any benefit to the crop.

Given the above, the present study was developed to evaluate the effects of seed treatments with beneficial microorganisms (*Trichoderma asperellum* and *Bacillus subtilis*) and chemical treatments, applied via seed and foliar, on the morphological and productive components of second-crop corn, using multivariate statistical analyses to understand the correlations between the variables and identify the most promising treatments in relation to the desired ideotype.

Material and methods

The experiment was carried out at Fazenda Invernadinha, located in the rural area of the municipality of Mineiros, state of Goiás, Brazil. Geographically, the site is located at coordinates

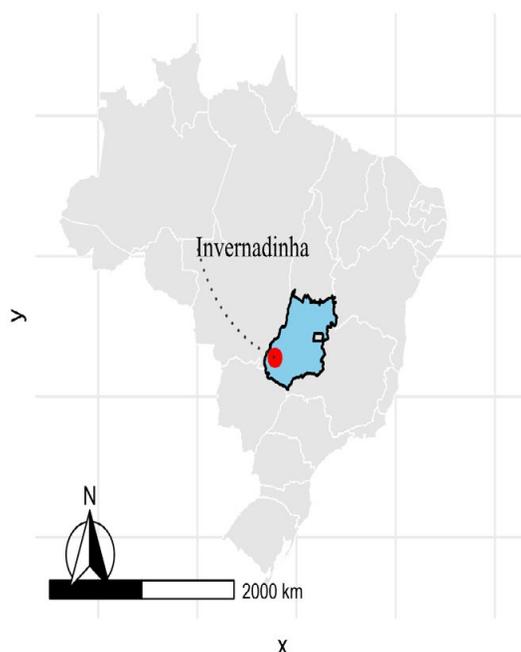
17°23'07.3"S latitude and 52°16'54.3"W longitude, at an approximate altitude of 800 meters (Figure 1).

The climatic variables related to the experimental period were obtained through the NASA POWER library of the R software (R CORE TEAM, 2025), and are presented in Figure 2. The experimental cycle began on February 28, 2022 and lasted until August 27, 2022. According to the Köppen and Geiger (1936) classification, the experimental area has an Aw climate (tropical with a well-defined dry season).

Soil chemical analyses were performed on samples collected from the 0–20 cm depth layer in the experimental area (Figure 3). The analytical procedures followed the methodology described by EMBRAPA (2009).

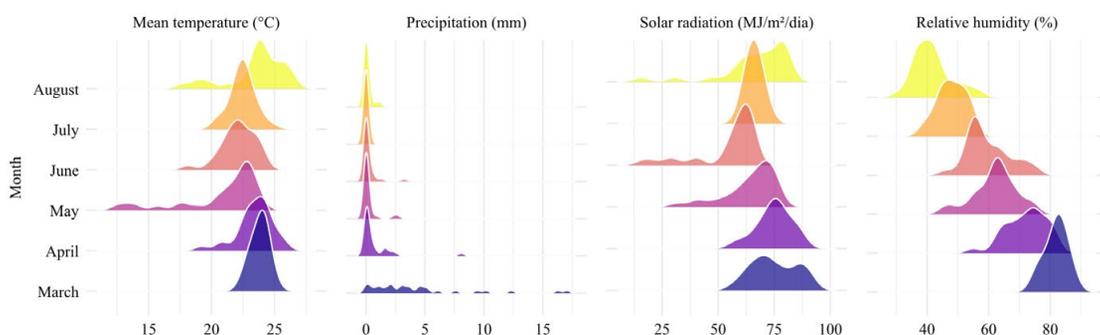
The experimental design adopted was a randomized complete block design, consisting of five treatments (Table 1). Each treatment corresponded to a specific method of seed treatment (ST) and foliar disease management

Figure 1. Geographical location of the Invernadinha experimental area in the municipality of Mineiros, State of Goiás, Brazil.



Source: authors (2022).

Figure 2. Monthly distribution of precipitation, global solar radiation, mean temperature and relative humidity during the experimental period at Fazenda Invernadinha, Mineiros, GO.



*Colors are used only to facilitate visual interpretation.

Source: authors (2022).

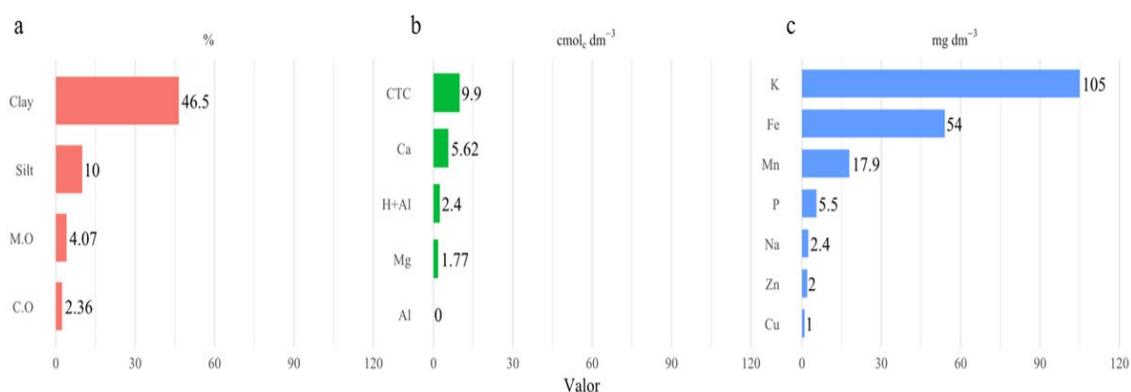
in corn, including combinations of standard chemical treatments and biological products based on growth-promoting microorganisms and pathogen control. Treatments were arranged in randomized blocks with four replicates. Each experimental plot consisted of eight 5-meter-long rows spaced 0.45 m apart, totaling 18 m² of usable area per plot.

Before sowing, pre-planting desiccation was performed, according to the methodology described by Cobucci, Portela and Silva (1999). Base fertilization consisted of applying 280 kg ha⁻¹ of the formulated fertilizer 14-14-10 in the sowing

furrow. Subsequently, topdressing was performed, with the application of 50 kg ha⁻¹ of potassium chloride (KCl) and 170 kg ha⁻¹ of urea, following the recommendations of Ferreira *et al.* (2020).

During the crop cycle, pest, disease, and weed management was carried out preventively and/or curatively, whenever necessary, following good agricultural practices and the principles of integrated management. At the end of the experiment, ten plants were randomly collected from the useful area of each experimental plot. Considering four replicates, a total of 40 plants were evaluated per treatment.

Figure 3. Spatial distribution of physical and chemical soil attributes in the 0–20 cm layer at Fazenda Invernadinha, Mineiros, GO.



(a) Physical attributes (e.g., texture – contents of clay, silt, and sand; or soil bulk density, as shown in the panel); (b) Chemical attributes related to fertility (e.g., pH, organic matter, available P and K); (c) Cationic chemical attributes (e.g., Ca, Mg, Al, and base saturation).

Source: authors (2022).

Table 1. Description of treatments in corn crops under seed treatment with rhizosphere capacitors

Treatments	Products	Dose (kg/seed)	Leaf disease management
TS1	Standard	2.5 mL	Standard fungicide application
TS2	Standard + Trichoplus	2.5 mL + 5 g	Standard fungicide application
TS3	Standard + BS10	2.5 mL + 5 mL	Standard fungicide application
TS4	Standard + Trichoplus + BS10	2.5 mL + 5 g + 5 mL	Standard fungicide application
TS5	Standard + Trichoplus + BS10 + (BS10 foliar)	2.5 mL + 5 g + 5 mL + (300 mL ha ⁻¹)	Standard fungicide application + BS10 Stages: V 4, V 8 and pre-tassel

Standard: Pyraclostrobin – 25 g/L (2.5 % m/v); Thiophanate methyl – 225 g/L (22.5 % m/v); Fipronil – 250 g/L (25 % m/v). Trichoplus: *Trichoderma asperellum* – Isolated BLF 1296, Concentration: 2×10^9 UFC g⁻¹
 BS10: *Bacillus subtilis* – Strain CBMAI 2479, Concentration: 1×10^8 UFC/mL.

Source: authors (2022).

The measured variables were: Plant height (PH) was determined by measuring the distance from soil level to the insertion of the flag leaf using a ruler graduated in centimeters. Stem diameter (SD) was obtained using a digital caliper, measuring the region of the first internode above ground. The number of rows per ear (NRE) was determined by directly counting the rows of grains in each ear. The number of grains per row (NGR) was obtained by counting the grains in a complete row, chosen randomly.

The number of grains per ear (NGE) was calculated by multiplying NRE by NGR. The thousand grain weight (TGW) was determined by weighing eight subsamples of 100 grains each, adjusting the value to thousand grain weight and correcting for 13 % moisture content. The mean number of ears per plant (NEP) was obtained by the ratio between the total number of ears harvested in the plot and the number of plants present in the useful area. Grain yield (GY) was calculated from harvesting and manual threshing of the ears in the useful area of the plot, with subsequent weighing and correction of the weight to 13 % moisture content, expressed in kg ha⁻¹.

The data obtained were initially subjected to verification of the assumptions of the statistical model, assessing the normality of residuals and

the homogeneity of variances. Subsequently, multivariate analysis of variance (MANOVA) was performed to assess the joint effect of treatments on the set of agronomic variables evaluated. Subsequently, Pearson's linear correlation analysis was applied, with significance established at 5 % probability using the t-test, to identify the magnitude and direction of the associations between variables. Path analysis was performed considering yield as the main variable to estimate the direct and indirect effects of the other variables on productivity.

Dissimilarity between treatments was determined by the generalized Mahalanobis distance, weighted by the residual matrix, and used to construct a hierarchical clustering dendrogram using the UPGMA method. Subsequently, canonical variate analysis, graphically represented by a biplot, was used to explore the overall variability and multivariate trends of the experiment. A bipartite network was also generated to graphically represent significant interactions between treatments and variables. Finally, the MGIDI index was applied to rank treatments based on the simultaneous performance of multiple traits. All analyses were performed using R software (R CORE TEAM, 2025).

Results

Pillai's multivariate test indicated a significant effect of treatments on all agronomic variables evaluated (Table 2), demonstrating that seed and foliar disease management simultaneously influenced characteristics such as plant height, stem diameter, number of rows per ear, number of grains per row, number of grains per ear, thousand grain weight, mean number of ears per plant, and yield.

This result reinforces the need for complementary analyses to identify which variables and treatments contributed most to differentiation, which was addressed in the correlation, path, canonical variables, UPGMA, bipartite network, and MGIDI analyses.

Pearson's linear correlation analysis revealed significant associations between several agronomic variables. Yield was highly positively correlated with the number of grains per ear ($r = 0.81$; $p < 0.001$) and thousand grain weight ($r = 0.81$; $p < 0.001$), indicating that increases in these traits tend to directly reflect higher productivity. A moderate positive correlation was also observed with the mean number of ears per plant ($r = 0.58$; $p < 0.01$). On the other hand, the number of grains per row was negatively correlated with yield ($r = -0.54$; $p < 0.05$), suggesting a possible compensatory effect between ear length and width. Furthermore, stem diameter was positively correlated with the number of rows per ear ($r = 0.45$; $p < 0.05$), which may indicate a

relationship with grain-holding capacity and grain filling (Figure 4).

In the evaluated context, variables linked to grain filling and grain weight were more decisive for productivity than morphological variables such as plant height and stem diameter.

The path analysis, considering yield as the dependent variable, showed a high coefficient of determination ($R^2 = 0.92$) and a low residual effect (0.27), indicating that most of the variation in yield was explained by the set of variables evaluated. Among the direct effects, the thousand grain weight (TGW) stood out with a value of 0.60, followed by the mean number of ears per plant (NEP) with 0.26, evidencing its primary importance for productivity (Table 3).

Stem diameter (SD) had a low positive direct effect (0.11), but contributed indirectly via TGW and NEP. On the other hand, the number of grains per row (NGR) and the number of grains per ear (NGE) showed negative direct effects (-0.10 and -0.14, respectively), suggesting that increases in these variables, alone, did not translate into higher yield in the present study (Table 3). The most significant indirect effects were observed from TGW on yield via NEP (0.48) and from NEP via TGW (0.21), reinforcing the synergistic relationship between number of ears and grain weight (Table 3).

Canonical variate analysis (CVA) indicated that the first two axes, Can1 and Can2, jointly explained 76.85 % of the total variation

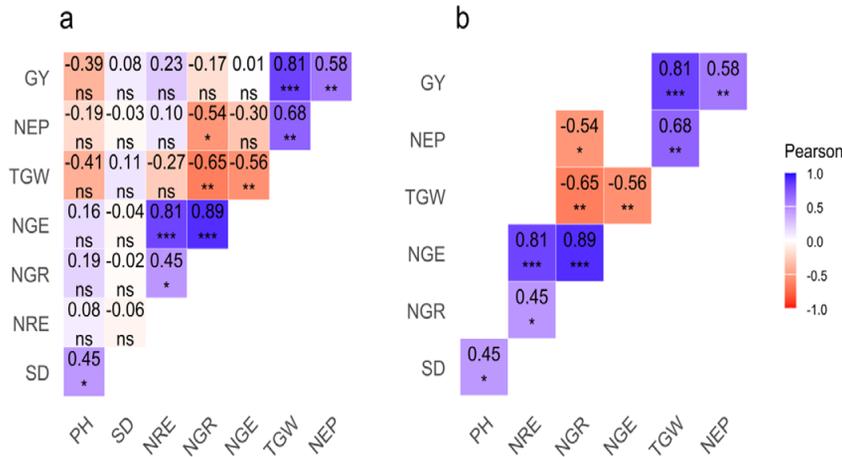
Table 2. Pillai test obtained in the multivariate analysis of variance (MANOVA) for the agronomic variables of the corn crop subjected to different seed treatments with rhizosphere capacitors.

	Df	Pillai approx F num	Df den	Df	Pr(>F)	
Replicates	3	1.7358	12.015	24	21	0.3376
TS	4	1.4684	0.58	32	32	0.0057**
Residuals	12					

Significance level: ** $p < 0.01$.

Source: authors (2022).

Figure 4. Pearson's linear correlation between agronomic variables of corn crops subjected to different seed treatments with rhizosphere capacitors.



Significance level: ns = not significant; * p < 0.05; ** p < 0.01; *** p < 0.001.

Source: authors (2022).

between treatments (Can1 = 52.30 % and Can2 = 24.55 %). In the Can1 × Can2 plane (Figure 5), it was observed that Can1 was the axis with the greatest discriminant capacity, mainly separating treatments TS3 and TS5 (negative values) from TS2 and TS4 (positive values). Treatment TS1 was positioned close

to the origin, indicating intermediate behavior (Figure 5).

The standardized coefficients indicated that Can1 was positively influenced by number of grains per row (NGR = 4.67), number of ears per plant (NEP = 2.02) and grain yield (GY = 1.74), and negatively by number of grains per ear (NGE = -7.58) and thousand

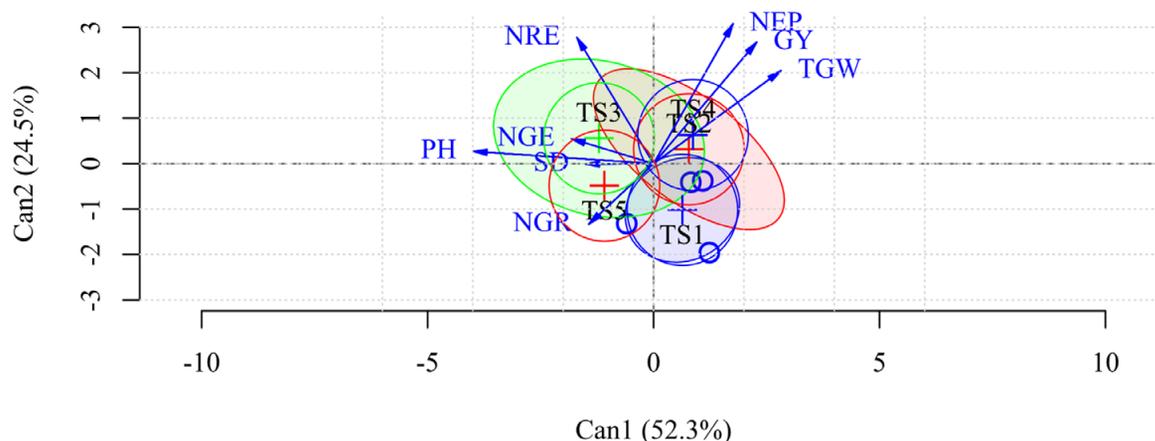
Table 3. Direct and indirect effects estimated by path analysis for agronomic variables of corn subjected to different seed treatments with rhizosphere capacitors.

Effect	Variable	PH	SD	NG	NGR	NGE	TGW	NEP
Direct effect	GY	0.02	0.11	-0.03	-0.10	-0.14	0.60	0.26
Indirect effect	SD	0.11	0.02	0.01	0.02	0.02	-0.01	-0.01
Indirect effect	NG	-0.01	-0.01	0.03	0.07	0.10	-0.06	-0.03
Indirect effect	NGR	-0.07	-0.06	0.04	0.01	-0.01	0.00	-0.02
Indirect effect	NGE	-0.12	-0.12	-0.05	-0.10	-0.07	0.09	0.09
Indirect effect	TGW	-0.37	-0.33	0.09	-0.53	-0.46	0.11	0.05
Indirect effect	NEP	-0.09	-0.07	0.18	-0.24	-0.10	0.21	0.48
Total		-0.52	-0.44	0.26	-0.88	-0.67	0.99	0.86

Coefficient of determination: 0.92; Residual variable: 0.27; K: 0.104. TGW: thousand grain weight; NEP: mean number of ears per plant; SD: stem diameter; PH: plant height; NG: number of grains; NGR: number of grains per row; NGE: number of grains per ear; GY: yield.

Source: authors (2022).

Figure 5. Canonical variate analysis (CVA) obtained by the Mahalanobis algorithm of agronomic traits of corn subjected to different seed treatments with rhizosphere capacitors. The arrows represent the contribution of the variables to each axis; the circles correspond to the clusters formed.



Source: authors (2022).

seed weight (TGW = -2.06). Can2 was strongly associated with TGW (3.59), NEP (3.15) and NGR (2.76), while GY (-2.23) and NGE (-2.86) contributed in the opposite direction. In the two-dimensional graph Figure 5, the 95 % confidence ellipses for each treatment are presented. Partial overlap between groups is observed, especially between TS1, TS2 and TS4, but also clear distancing from TS3 and TS5 along Can1. This suggests that, although there is intragroup variability, the variables NGR, NRE, GY, NGE and TGW play an important role in differentiating between treatments.

Hierarchical clustering using the UPGMA method, based on the generalized Mahalanobis distance, allowed the formation of three distinct groups among the evaluated treatments. The first group included TS4 and TS2, which were positioned close together and associated with high production characteristics. The second group consisted of TS1, located in an intermediate position in the multivariate space, while TS3 and TS5 comprised the third group, showing greater dissimilarity compared to the others (Figure 6).

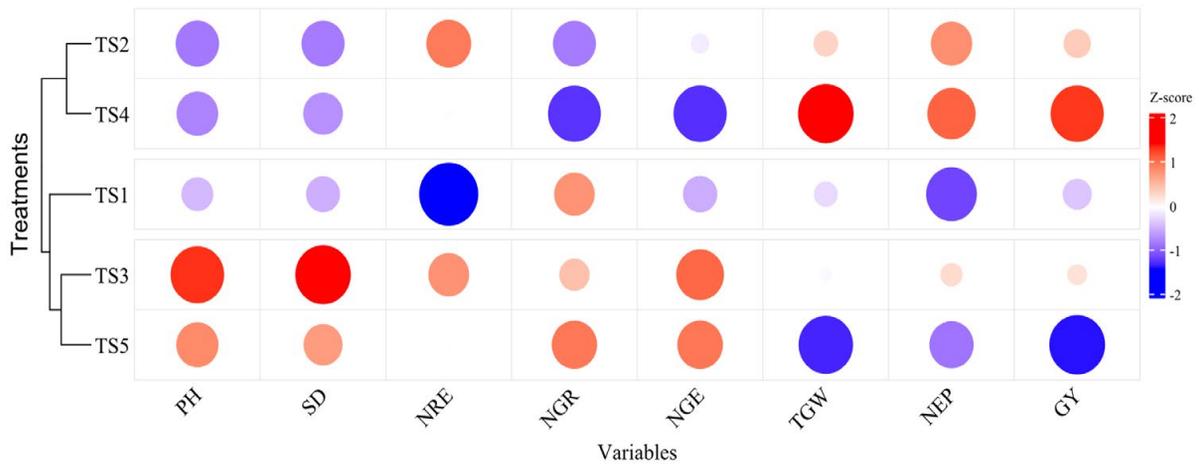
Differences between groups were most influenced by variables associated with thousand grain weight, mean number of ears and yield, as

observed in the canonical analyses. In Figure 7, a weighted bipartite network formed by two distinct sets of nodes: agronomic variables (green nodes) and treatments (yellow nodes). The connections (edges) represent the strength and direction of the associations between each treatment and the evaluated variables, with the thickness proportional to the magnitude of the association and the color indicating the direction of the relationship (blue for positive associations and red for negative ones). Variables such as thousand grain weight (TGW) and mean number of ears per plant (NEP) are strongly and positively associated with yield (GY) and certain treatments, especially TS3 and TS4. On the other hand, traits such as number of rows per ear (NRE) showed stronger negative correlations with some treatments, suggesting a possible limiting effect on productivity (Figure 7).

This graphical approach allows for the integrated identification of which treatments are most closely related to key performance variables, providing support for targeted technical recommendations.

Analysis using the MGIDI index, considering a selection intensity of 15 %, identified treatment TS3 as the closest to the crop's

Figure 6. Dendrogram constructed from the mean Euclidean distance between treatments of corn crops subjected to different seed treatments with rhizosphere capacitors, with UPGMA clustering and optimized groups.



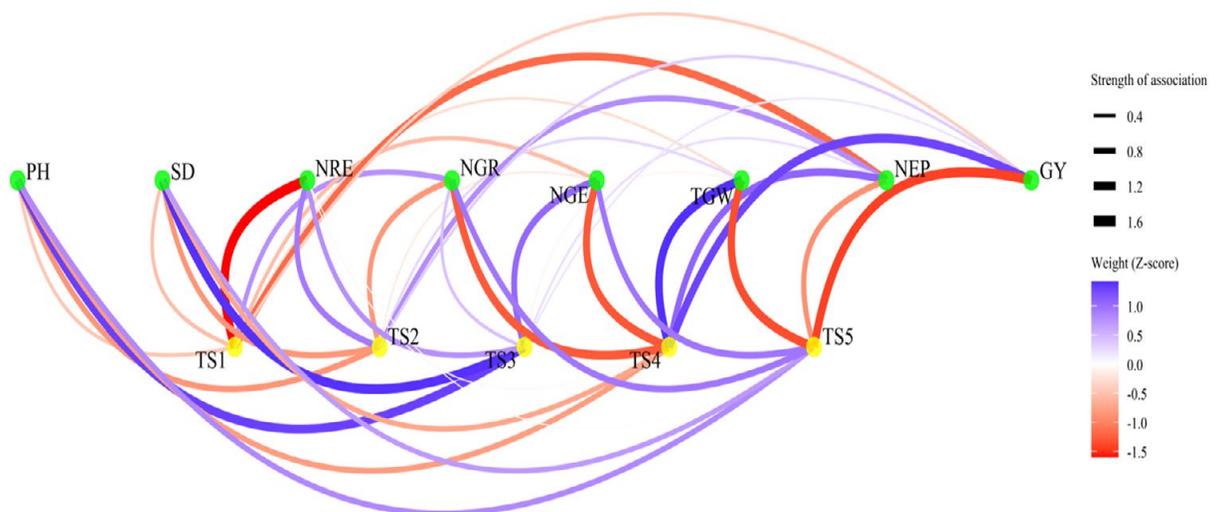
Source: authors (2022).

ideotype, presenting a lower index value and, therefore, superior performance across all the traits evaluated. Subsequently, treatments TS5, TS4, and TS2 presented intermediate values, indicating moderate and balanced performance in some variables, but far from the ideal standard. Treatment TS1 presented the highest MGIDI index value, being the furthest from the ideotype, reflecting inferior performance considering all variables simultaneously (Figure 8).

This result complements previous multivariate analyses, indicating that TS3, in addition to presenting favorable characteristics in isolation, maintains a general balance between morphological and productive attributes.

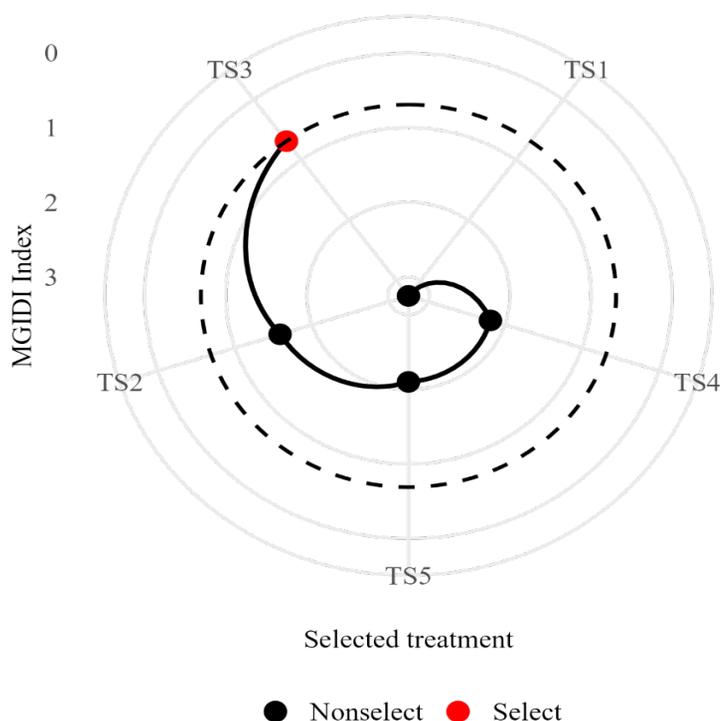
Multivariate analysis of variance indicated a significant effect of treatments on all agronomic variables evaluated, demonstrating that combinations of seed treatments and foliar disease management simultaneously influenced

Figure 7. Weighted bipartite network between treatments and agronomic variables of corn crop.



Source: authors (2022).

Figure 8. Classification of corn crop treatments subjected to different seed treatments with rhizosphere capacitors by the MGIDI index, considering a selection intensity of 15 % (Z). Lower values indicate greater proximity to the ideotype.



Source: authors (2022).

the productive and morphological characteristics of the corn crop.

Complementary analyses identified the most important factors for yield. Pearson's correlation and path analysis highlighted thousand grain weight (TGW) and mean number of ears per plant (NEP) as the variables most closely associated with yield. Canonical variate analysis showed that treatments closest to these variables performed better in the multivariate space. UPGMA clustering revealed the formation of three groups, reinforcing the dissimilarity between treatments.

The MGIDI index, which integrates multiple characteristics into a single measure of proximity to the ideotype, indicated the TS3 treatment as the closest to the ideal standard, reconciling good performance in the variables determining performance with general balance in the set of characteristics.

On this occasion, TS3 is technically recommended for the soil and climate conditions and management of Fazenda Invernadinha, Mineiros-GO, as it presents superior multivariate performance and potential to maximize the productive performance of the corn crop.

Discussion

The application of MANOVA allowed evaluating the effect of beneficial microorganisms on multiple morphological components of corn. Integrated responses were observed, with simultaneous increases in plant height, aerial biomass, and root biomass. Similar results were reported by Chagas Júnior *et al.* (2022), who observed significant morphophysiological changes in corn and rice inoculated with *T. asperellum*, demonstrating the systemic action of

the fungus. Bezerra *et al.* (2022) also highlighted that the use of *T. harzianum* in seeds reduced the incidence of fungi and maintained physiological quality, confirming that the effects of inoculation are not limited to an isolated variable but extend to different aspects of plant performance.

When analyzed from the perspective of other microorganisms, MANOVA also revealed integrated response patterns. Buchelt *et al.* (2019) observed that, although the application of *B. subtilis* did not alter seed germination, it did impact initial shoot growth. Machado *et al.* (2020) showed that foliar inoculation with *B. subtilis* promoted a significant increase in plant height and grain yield, in addition to a higher economic return compared to the use of nitrogen fertilization alone. These results reinforce the importance of multivariate analysis for understanding the systemic effects of bioinputs across different application routes.

The linear correlations obtained in this study indicated positive associations between root traits and total biomass, suggesting that root system development is a determining factor for gains in aerial growth. This result is in agreement with Araújo *et al.* (2023), who observed increased root growth and, consequently, greater accumulation of aerial dry matter in plants inoculated with *Trichoderma*.

Additionally, recent studies with rhizobacteria also support these relationships. Moreno *et al.* (2021) observed that coinoculation with *Azospirillum brasilense* and *Bacillus subtilis* promoted greater root growth, resulting in positive correlations with plant height and yield. Similarly, Buchelt *et al.* (2019) reported that the use of biostimulants and *B. subtilis* in seeds favored increases in dry weight, although it did not directly influence germination. These findings corroborate that the associations observed between traits in the present study reflect physiological interactions promoted by microorganisms in the rhizosphere.

Path analysis highlighted the central role of the root system as a variable with a direct effect on potential corn yield. In this multivariate context, thousand grain weight (TGW) and number of ears per plant (NEP) were identified as the traits with the highest direct effect on yield, indicating that root-mediated responses are ultimately expressed through improvements in key yield components. Chagas Júnior *et al.* (2022) demonstrated that increased root volume in inoculated plants resulted in a higher photosynthetic rate, establishing a causal relationship between root development and physiological performance.

In the same vein, Machado *et al.* (2020) showed that foliar application of *B. subtilis* generated gains in height and productivity, indicating a direct effect of the microorganism on traits of greater agronomic importance. This interpretation is consistent with the MGIDI results, which identified the treatment based on *B. subtilis* as the most balanced and superior, linking multivariate selection with enhanced grain filling (TGW) and plant reproductive efficiency (NEP). Path analysis is an essential tool for identifying which traits should be prioritized in microorganism management, with the root system being the main link between bioinputs and productivity.

In the present study, clear clusters were observed between variables related to photosynthesis and biomass accumulation, explaining much of the total variation. Chagas Júnior *et al.* (2022) reported a similar pattern, in which intermediate doses of *T. asperellum* were identified by CVA as more efficient in promoting growth. Araújo *et al.* (2023) also used CVA to differentiate corn genotypes under inoculation, reinforcing the technique's potential to summarize complex response patterns.

Studies with rhizobacteria have also used CVA to characterize multivariate responses. Moreno *et al.* (2021) showed that treatments with *A. brasilense* and *B. subtilis*, alone or in combination, were discriminated based on

multiple productive traits. This evidence supports the effectiveness of CVA in identifying treatments most responsive to microbial inoculation.

Cluster analysis revealed distinct groups among treatments, reflecting the variability in microbial activity. Lima *et al.* (2021) found significant differences between *Trichoderma* species in terms of growth promotion, demonstrating that the effects are not homogeneous. Bezerra *et al.* (2022) also observed variation between *T. harzianum* concentrations in fungal control, reinforcing the importance of dose.

Among rhizobacteria, Moreno *et al.* (2021) highlighted that the combination of *A. brasilense* and *B. subtilis* clustered separately from treatments with only one of the microorganisms, indicating a differentiated interaction. Machado *et al.* (2020) found that the application of *B. subtilis* alone promoted better performance than its association with *A. brasilense*, which can be explained by competition between microorganisms, also reflected in the cluster analysis.

The bipartite network constructed in this study illustrated the multiplicity of connections between microorganisms and morphological traits. Similarly, Bezerra *et al.* (2022) showed that *T. harzianum* was able to reduce different fungal genera simultaneously, acting on multiple fronts. Araújo *et al.* (2023) reinforced that seed inoculation affects root, physiological, and growth variables concomitantly.

Using rhizobacteria, Machado *et al.* (2020) showed that foliar-applied *B. subtilis* simultaneously impacted plant height, productivity, and economic return. Buchelt *et al.* (2019), in turn, found that biostimulants affected both root and aerial biomass. This evidence supports the network representation as an appropriate model for interpreting complex interactions of multiple factors in agricultural systems.

The MGIDI index allowed an integrated interpretation of treatment performance. Treatment TS3 showed the shortest distance to the ideotype, indicating superior overall performance. This response reflects the combined influence of traits related to yield and vegetative development, suggesting that *B. subtilis* improves plant performance through coordinated effects on plant architecture and resource allocation, as previously reported for rhizobacteria-based bioinputs (Moreno *et al.*, 2021; Machado *et al.*, 2020).

Conclusions

Under the soil and climate conditions of Fazenda Invernadinha, Mineiros, Goiás, the TS3 seed treatment, comprising the use of BS10 combined with the farm's standard fungicide management, outperformed across all productive and morphological characteristics, particularly in terms of thousand grain weight and mean number of ears per plant. These factors proved decisive in increasing yield and positioned TS3 as the treatment closest to the ideal production standard.

Therefore, the use of TS3 treatment is technically recommended as a viable alternative to maximize corn productivity, maintaining a balance between yield components and structural characteristics of the plant.

However, it is emphasized that new research must be conducted in different harvests, soil and climate conditions and management systems, in order to validate and refine these recommendations, ensuring greater robustness and applicability of the results obtained.

References

ARAÚJO, T. B. de; SCHUELTER, A. R.; SOUZA, I. R. P. de; COELHO, S. R. M.; CHRIST, D. Promoção de crescimento em milho inoculado

com *Trichoderma harzianum*. **Revista Brasileira de Milho e Sorgo**, v. 22, p. 1–11, 2023.

BEZERRA, M. C. L.; GOMES, R. S. S.; CARVALHO, T. K. N.; RODRIGUES, R. M.; SILVA, T. B. M.; MEDEIROS, J. G. F. Redução de fungos e qualidade fisiológica de sementes de milho inoculadas com *Trichoderma harzianum*. **Nativa**, v. 10, n. 1, p. 69–73, 2022.

BORSATTI, J.; GUEDES, S. F.; BARCELOS, A. I. H.; DIPPLE, F. L.; OLIVEIRA, K. C. L. de. Sanidade de diferentes cultivares de sementes de milho. **Revista Ibero-Americana de Ciências Ambientais**, v. 12, n. 12, p. 196–203, 2021.

BUCHELT, A. C.; CUSTÓDIO, T. N.; ANTONIOLLI, Z. I.; ARAÚJO, E. S.; WAGNER JÚNIOR, A. Aplicação de bioestimulantes e *Bacillus subtilis* na germinação e desenvolvimento inicial da cultura do milho. **Revista de Agricultura Neotropical**, v. 6, n. 4, p. 69–74, 2019.

CHAGAS JÚNIOR, A. F.; LUZ, L. L.; MARTINS, A. L. L.; QUEIROZ, A. S. B.; CHAGAS, L. F. B.; GIONGO, M.; PORTELLA, A. C. F.; SCHEIDT, G. Alterações morfofisiológicas em plantas de arroz e milho ocasionadas por *Trichoderma asperellum*. **Journal of Biotechnology and Biodiversity**, v. 10, n. 4, p. 287–296, 2022.

COBUCCI, T.; PORTELA, C. M. O.; SILVA, J. G. **Manejo de plantas daninhas na cultura do milho**. Santo Antônio de Goiás: Embrapa Arroz e Feijão, 1999. 60 p.

EMBRAPA. **Manual de análises químicas de solos, plantas e fertilizantes**. 2. ed. rev. e ampl. Brasília, DF: Embrapa Informação Tecnológica; Rio de Janeiro: Embrapa Solos, 2009. 627 p.

FERREIRA, T. C.; BETTIOL, W. Biocontrole de patógenos de solo e promoção de crescimento vegetal promovidos por *Bacillus* spp. em milho.

BIOFARM – Journal of Biology & Pharmacy and Agricultural Management, v. 15, n. 4, p. 337–356, 2019.

FERREIRA, L. L.; MOREIRA, N. V.; CARVALHO, I. R.; SANTOS, N. S. C.; FERNANDES, M. S.; PEREIRA, A. I. A.; CURVÊLO, C. R. S.; SILVA, R. V.; AMARAL, U.; SILVA, R. A. Spacing between rows of soybean genotypes: averages, regressions and correlations in yield levels. **Research, Society and Development**, v. 9, n. 9, e952997793, 2020.

KÖPPEN, W.; GEIGER, R. **Das geographische System der Klimate**. Berlin: Gebrüder Borntraeger, 1936. 44 p. Disponível em: [https://upload.wikimedia.org/wikipedia/commons/3/3c/Das_geographische_System_der_Klimate_\(1936\).pdf](https://upload.wikimedia.org/wikipedia/commons/3/3c/Das_geographische_System_der_Klimate_(1936).pdf). Acesso em: 24 dez. 2025.

LIMA, R. S.; SILVA, C. F.; ALVES, F. R.; OLIVEIRA, J. A.; DIAS, D. C. S. Diversidade de espécies de *Trichoderma* e seu potencial de uso agrícola. **Revista de Ciências Agrárias**, v. 44, n. 2, p. 223–234, 2021.

MACHADO, R. W. B.; CALVI, V. O.; PACCOLA, E. A. S.; SCHMIDT FILHO, E.; GASPAROTTO, F. Inoculação foliar de plantas de milho com *Bacillus subtilis* e *Azospirillum brasilense*. **Enciclopédia Biosfera**, v. 17, n. 34, p. 289–295, 2020.

MEDEIROS, J. C. D.; MARTINS, W. S.; MIRANDA, F. F. R. de. Antagonismo de *Trichoderma* spp. no biocontrole de *Fusarium moniliforme* na cultura do milho. **Revista Sítio Novo**, v. 4, n. 4, p. 169–178, 2020.

MORENO, A. L.; PEREIRA, M. A. R.; SANTOS, F. C.; FERREIRA, A. A.; OLIVEIRA, A. R. Coinoculação de milho com *Azospirillum brasilense* e *Bacillus subtilis* e suplementação

com nitrogênio e zinco. **Revista Brasileira de Ciências Agrárias**, v. 16, e8902, 2021.

R CORE TEAM. **R: a language and environment for statistical computing**. Vienna: R Foundation for Statistical Computing, 2025. Disponível em: <https://www.r-project.org/>. Acesso em: 24 dez. 2025.

ROSA, T. E. A.; SOUZA, J. C. de; PEREIRA, W. J.; MOREIRA, J. A. A.; ARAÚJO, F. G. de. Nematicidas biológicos associados à cama de frango no controle de nematoides na soja e milho. **Revista Caatinga**, v. 34, n. 3, p. 580–589, 2021.

SOUTO, A. C.; BELONI, G.; AZEVEDO, V. H.; MORAES, C. A.; CAMPOS, D. Desenvolvimento inicial do milho com uso de microrganismos. **Enciclopédia Biosfera**, v. 17, n. 32, p. 1–8, 2020.

STEFFEN, G. P. K.; TOMAZZI, D. J.; STEFFEN, R. B.; GABE, N. L.; SILVA, R. F. da; MORTARI, J. L. M.; MALDANER, J. Incremento da produtividade de milho pela inoculação de *Trichoderma harzianum*. **Brazilian Journal of Development**, v. 7, n. 1, p. 4455–4468, 2021.