Australian Journal of Crop Science AJCS

Aust J Crop Sci. 19(10):994-1006 (2025) | https://doi.org/10.21475/ajcs.25.19.10.p16 ISSN:1835-2707

Agronomic feasibility of bacterial consortium with *Bacillus* species and its effects on the productivity of some important crops

Willyan Júnior Adorian Bandeira[1], Ivan Ricardo Carvalho[1]*, Jaqueline Piesanti Sangiovo[2], Lays Garcia Meireles[3], Luis Fernando Maranho Watanabe[3], Lucas Moraes Santos[3]

[1]Department of Agricultural Studies, Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), Ijuí, Brazil
[2]Plant Science Department, Federal University of Santa Maria (UFSM), Santa Maria, Brazil
[3]Research and Development, Total Biotecnologia Indústria e Comércio SA, Santo Antônio de Posse, Brazil

***Corresponding author: carvalho.irc@gmail.com**

Submitted:* *16/04/2025

Revised:* *05/06/2025

Accepted:* *05/07/2025

Abstract: The objective of the study was to evaluate the efficiency and agronomic practicality of the bacterial consortium composed of *Bacillus aryabhattai*, *Bacillus circulans*, and *Bacillus haynesii* in lettuce, beans, maize, and sugarcane crops. Experiments were carried out from 2021 to 2023, in different soil and climate regions, to verify the efficiency and stability of the bacteria evaluated. The product is a pre-commercial liquid inoculant, tested to complement chemical fertilizers and compared with commercial products. Variance components and genetic parameters were estimated by the restricted maximum likelihood (REML) procedure. Best Linear Unbiased Predictor (BLUP) determined the effect of interaction with environments and treatment ranking. Linear correlation analyses were used to assess the relationships and magnitudes of the evaluated variables in each crop. REML analysis showed treatment effects for lettuce and beans, while maize and sugarcane showed treatment-environment interactions. Inoculation improved yields, especially in lettuce and beans. Correlation analyses revealed complex nutrient-productivity relationships. Sugarcane showed high environmental variation, limiting treatment selection, while maize responses varied by site, emphasizing tailored fertilization strategies. The bacterial consortium with *Bacillus* species can efficiently complement chemical fertilization in lettuce, beans, and maize crops, with the potential to reduce chemical fertilization by up to 50%. While chemical fertilizers provide quick yield benefits but involve high recurring costs and potential long-term soil degradations, bacteria consortia have lower input cost and improve nutrient efficiency, tough initial gains may be modest. Over time, bacterial consortia can reduce dependency on chemical inputs, making them a more sustainable and cost-effective option.

Keywords: Plant Growth Promoting Bacteria, Sustainability, *Lactuca sativa* L., *Phaseolus vulgaris* L., *Zea mays* L., *Saccharum* spp.

Introduction

The challenge of food security requires the ability to deal with the increased demand for food promoted by the constant expansion of the world population, expected to increase by 1.7 billion people by 2050 (McCarthy et al., 2018). Consequently, global demand for all agricultural products is expected to increase by 1.1% per year over this period (Noulas et al., 2023). Therefore, the challenge for future decades will be to guarantee food security for the growing population using sustainable agricultural practices that, at the same time, allow the maintenance of soil health, environmental quality and food productivity (Beltran-Peña et al., 2020; Li et al., 2020). In this scenario, soil fertility management is a key element for maintaining and increasing productivity, to meet the nutritional demand of crops in cultivation systems over time (Zhang et al., 2020).

Brazil is one of the largest food producers in the world, thanks to the large amount of arable land and climatic conditions that favor cultivation systems. From this perspective, the crops of lettuce (*Lactuca sativa* L.), beans (*Phaseolus vulgaris* L.), maize (*Zea mays* L.) and sugarcane (*Saccharum* spp.) play a high importance in the supply of food, bioactive beneficial to health and raw material for industrialized products (Kottwitz and Silva, 2024; Soratto et al., 2015; Silva et al., 2020; Nicchio et al., 2020). Given the importance of these species, we seek to understand their nutritional demands, which must be met so that they can express their productive potential (Paramesh et al., 2023). In this scenario, several studies show that nutrients including nitrogen (N), phosphorus (P) and potassium (K) significantly improve the yield performance and quality of lettuce (Hong et al., 2022), sugarcane (Castro et al., 2023), maize (Ichami et al., 2022) and beans (Noulas et al., 2023).

In this way, the increase in agricultural production to meet the demands of consumer markets and the growing world population depends on the use of a large number of chemical fertilizers (Kumar et al., 2017), being the most common way of supplying nutrients to the soil. However, the excessive use of these products increases the production cost and poses environmental problems. The correct recommendation of management that favors the absorption of nutrients by plants and the assertive positioning of new technologies are essential to increase crop productivity (Castro et al., 2023), which can help reduce the potential negative environmental impact of chemical fertilizers. The use of practices such as inoculation with plant growth-promoting bacteria (PGPBs) can represent a sustainable alternative to increase the efficiency of nutrient use and, consequently, the optimization of the use of chemical fertilizers (Galindo et al., 2018).

The mechanisms by which PGPBs promote plant growth can be direct or indirect (Vejan et al., 2016). Directly, improving the absorption and accumulation of essential plant nutrients, phosphate solubilization, hormone production and biological nitrogen fixation, directly affecting plant metabolism (Santos et al., 2020). Indirectly, they promote the biocontrol of pests and pathogens and the induction of plant resistance to biotic and abiotic stresses caused by heavy metals, water deficiency and salinity (Pietro Souza et al., 2020). Among several species of PGPBs, *Pseudomonas* and *Bacillus* spp. have been identified as the predominant communities (Kang et al., 2015), where some species have been isolated and commercialized due to their survival within a diverse range of environments (Radhakrishnan et al., 2017).

Inoculants produced with bacteria from the *Bacillus* genus are low cost, do not cause environmental damage and can even be used to supplement chemical fertilizers (Kalayu, 2019). B. haynesii is an endospore-forming and thermophilic bacterium (Rehman et al., 2019), capable of resisting high temperatures. Its growth-promoting properties have already been described by several authors (Sahu et al., 2021; Reang et al., 2022; Maheshwari et al., 2022). The growth-promoting capacity promoted by *B. aryabhattai* was demonstrated with *Xanthium italicum* as a model plant (Lee et al., 2012), in addition to reports by Park et al. (2017) who show that *B. aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes legume growth by modulating the production of phytohormones. Studies also show that *B. circulans* is capable of promoting growth and disease tolerance in cotton and tomato growth, mainly by solubilizing phosphorus (Mehta et al., 2015; Qin et al., 2021).

The combined use of multiple species of PGPBs has the advantage of the effect that each agent provides or even interaction processes, in addition to increasing the probability of establishing one or more agents in unfavourable environmental conditions (Prasad & Babu, 2017; Santiago et al., 2017). Thus, the objective of the study was to evaluate the efficiency and agronomic practicality of the bacterial consortium composed of *Bacillus aryabhattai*, *Bacillus circulans* and *Bacillus haynesii* in lettuce, beans, maize and sugarcane crops. Nitrogen and phosphate fertilization was used in a complementary way to identify the potential for reducing the use of chemical fertilizers promoted by plant growth-promoting bacteria, without the aim of identifying the best dose of fertilizer.

Results

Meteorological data stratified by environment

It was found that the air temperature varied between 15 and 25 ºC in most of the development cycle of the crops evaluated (Figure 1), in all environments, exceeding this limit only in Chapadão do Céu–GO and Chapadão do Sul-MS. The lowest volumes of precipitation occurred in Porto Amazonas-PR, in 2021, and in Chapadão do Céu-GO, in 2021 and 2023, with volumes of less than 20 mm. In other environments there is a good distribution of precipitation, with the highest volumes occurring in Santana da Vargem-MG, Lavras-MG and Minduri-MG.

Restricted maximum likelihood, variance components and genetic parameters

The REML (Table 1), supported by Deviance analysis at 5% probability level, indicates that only the treatment effect was significant for lettuce and beans. In contrast, for maize and sugarcane, the interaction between treatment and environment was significant. The lowest phenotypic productivity variances were attributed to bean and sugarcane crops (29377 and 17900, respectively).

Estimated heritability values (H^2) suggest that environmental effects had less influence on the productivity of lettuce and maize, regardless of the application method, with H^2 values ranging between 0.405 (lettuce) and 0.544 (maize furrow). Conversely, environmental factors had a stronger impact on sugarcane and bean productivity. The null values estimated for sugarcane (h^2mg = 0) indicate no potential for selecting promising treatments for this crop.

Genetic coefficient of variation (CVg) values ranged from 4.92% to 15.5%, with the highest variation observed in the maize crop. Residual coefficients of variation (CVr) exceeded 10% for all crops, with the highest value recorded for lettuce (16.9%). The interactions observed were classified as complex, given the low estimated values for the coefficient of determination ($GEIr^2$) and correlation of the genotype-environment interaction (rge).

Lettuce and bean productivity

BLUP-based predictions for lettuce cultivation with nitrogen doses (Figure 2) show that inoculation as a complement to chemical fertilization, regardless of the dose used, was superior to the 0N and 50N treatments. The highest inoculation dose (50N+0.200BTP143-20) promoted higher yields (21161 kg ha^{-1}) than those obtained with 100% of the N dose (20304 kg ha^{-1}). For the bean crop (Figure 3), the treatments 50N+0.100BTP143-20 and 50N+0.200BTP143-20 promoted grain productivity of 1956 kg ha^{-1}, being similar to the result obtained with the dose of 100% N- fertilizer (1959 kg ha^{-1}). In contrast, the 50N and 0N treatments fell below the overall mean (1872 and 1734 kg ha^{-1}, respectively).

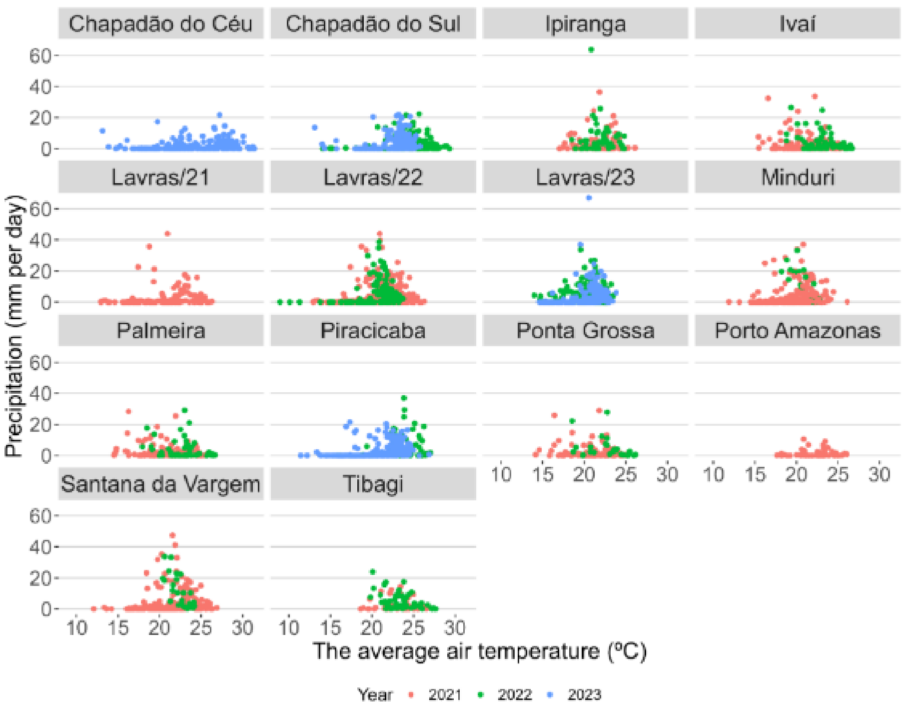

Fig 1. Satellite meteorological data on mean air temperature (ºC) and precipitation (mm) for the environments of Ponta Grossa-PR, Porto Amazonas-PR, Santana da Vargem-MG, Tibagi-PR, Lavras-MG, Minduri-MG, Palmeira -PR, Piracicaba-SP, Chapadão do Céu-GO, Chapadão do Sul-MS, Ipiranga-PR, Ivaí-PR for the years 2021, 2022 and 2023. Complete information regarding the experiments (location, sowing date and date of harvest) can be consulted in Supplementary Table 1.

Table 1. Restricted maximum likelihood test, variance components and genetic parameters for the variable productivity (PROD) in lettuce, beans, maize and sugarcane crops.

	Lettuce	Bean	Maize Foliar	Maize Seed Treatment	Maize Sowing Furrow	Sugarcane
Model	PROD (kg ha^{-1})	PROD (kg ha^{-1})	PROD (kg ha^{-1})	PROD (kg ha^{-1})	PROD (kg ha^{-1})	PROD (t ha^{-1})
GEN	0.00409*	0.00037*	0.00004*	0.00023*	0.00003*	1
GEN:ENV	0.21792	1	0.00000*	0.00097*	0.00019*	0.00002*
Parameters	-	-	-	-	-	-
PV	18915301	29377	3234209	32111128	3450764	17900
H^2	0.405	0.298	0.543	0.454	0.544	0
$GEIr^2$	0.0621	0	0.173	0.171	0.162	0.29
h^2mg	0.931	0.927	0.899	0.873	0.902	0
Ac	0.965	0.963	0.948	0.934	0.95	0
rge	0.104	0	0.379	0.314	0.355	0.29
CVg	14.8	4.92	15.5	12.6	16.0	0
CVr	16.9	7.54	11.2	11.5	11.8	0.118
CVratio	0.873	0.652	1.38	1.10	1.36	0

PV: phenotypic variance; H^2: heritability; $GEIr^2$: coefficient of determination of the genotype-environment interaction; h^2mg: genotype mean heritability; Ac: selection accuracy; rge: environmental genotype correlation coefficient; CVg: genetic coefficient of variation; CVr: residual variation coefficient; CVratio: relative coefficient of variation; *: significant at 5% using the $\chi 2$ test.

Maize and sugarcane productivity

The breakdown of the interaction for the application of inoculants via foliar in the maize crop (Figure 4) proves that all treatments were superior to the overall mean in the Lavras environment in 2021. The productivity obtained with the treatments 75P+100N+BTP143-20(0.1) and 100P+75N+BP143-20(0.1), with 13720 and 13629 kg ha^{-1}, were close to those obtained with 100% of the recommended P and N fertilization (13890 kg ha^{-1}). In the Minduri environment, all treatments were below the overall mean. The greatest variation in treatment performance was evaluated in the environments of Santana da Vargem and Lavras (in the year 2022). In this context, only the treatments 75P+100N+BTP143-20(0.1), 100P+100N+BP143-20(0.1) and 100P+75N+Biofree(0.1) were higher than the overall mean in Lavras (8797, 8607 and 8609 kg ha^{-1}), adding the treatment 100P+75N+BTP143-20(0.1) in the Santana da Vargem environment, with 9011 kg ha^{-1}.

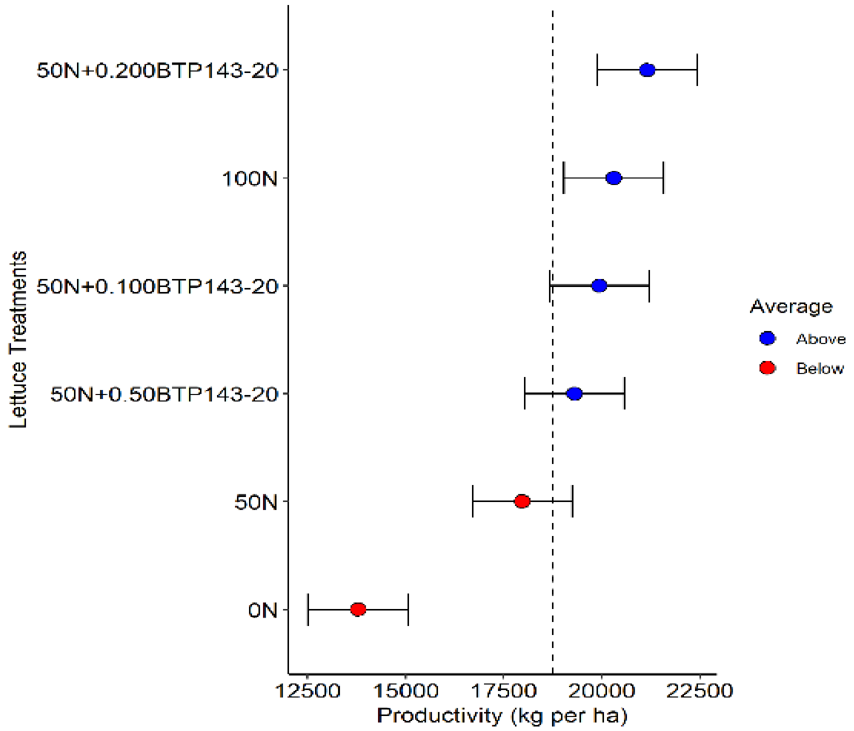

Fig 2. *Best Linear Unbiased Prediction* (BLUP) for the effect of treatments with different nitrogen doses and inoculation on lettuce crop productivity. Complete treatment information can be found in Supplementary Table 2.

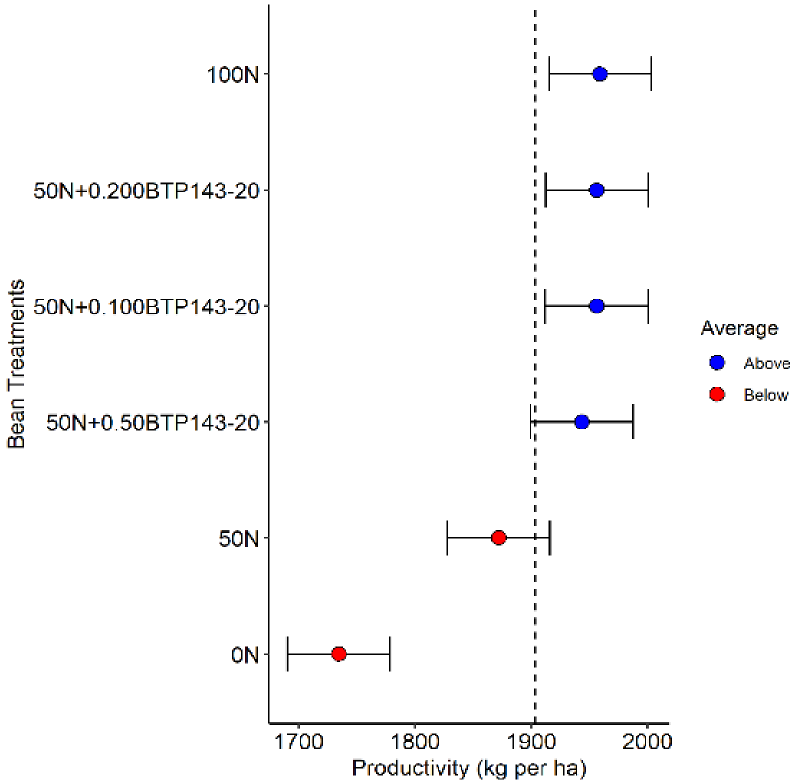

Fig 3. *Best Linear Unbiased Prediction* (BLUP) for the effect of treatments with different nitrogen doses and inoculation on bean crop productivity. Complete treatment information can be found in Supplementary Table 2.

In maize crops treated with inoculants via seed treatment (Figure 5), all treatments resulted in higher productivity than the overall mean in Lavras (in 2021) and Santana da Vargem. Conversely, in Minduri, all treatments were below the overall mean, with productivity ranging between CWF (4694 kg ha^{-1}) and 100P+100N+BTP143-20(0.1) (7319 kg ha^{-1}), leading to a 35.87% increase in productivity between treatments at this location.
In Lavras (in 2022), only the 100P+100N+BTP143-20(0.1) treatment surpassed the overall mean, reaching 10184 kg ha^{-1}. For maize inoculated in the furrow sowing system (Figure 6), all treatments outperformed the overall mean in Lavras. However, in Santana da Vargem, only the 100P+100N+BPT143-20(0.1) treatment exceeded the overall mean. In Lavras (in 2022), the highest predicted productivity levels were associated with the treatments 100P+100N+BPT143-20(0.1), 75P+100N+BPT143-20(0.1), 100P+75N+BPT143-20(0.1) and 100P+100N+Biofree(0.1).

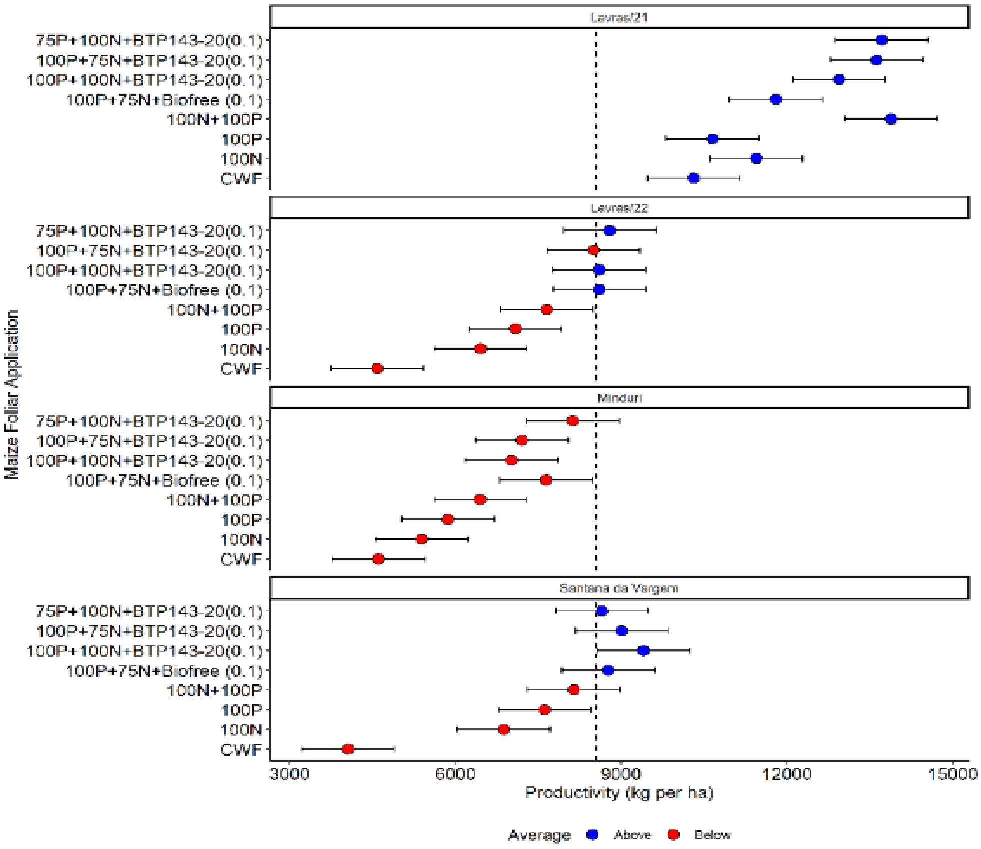

Fig 4. *Best Linear Unbiased Prediction* (BLUP) for breaking down the effect of the interaction between environments and treatments on maize productivity in a foliar application system. Complete treatment information can be found in Supplementary Table 2.

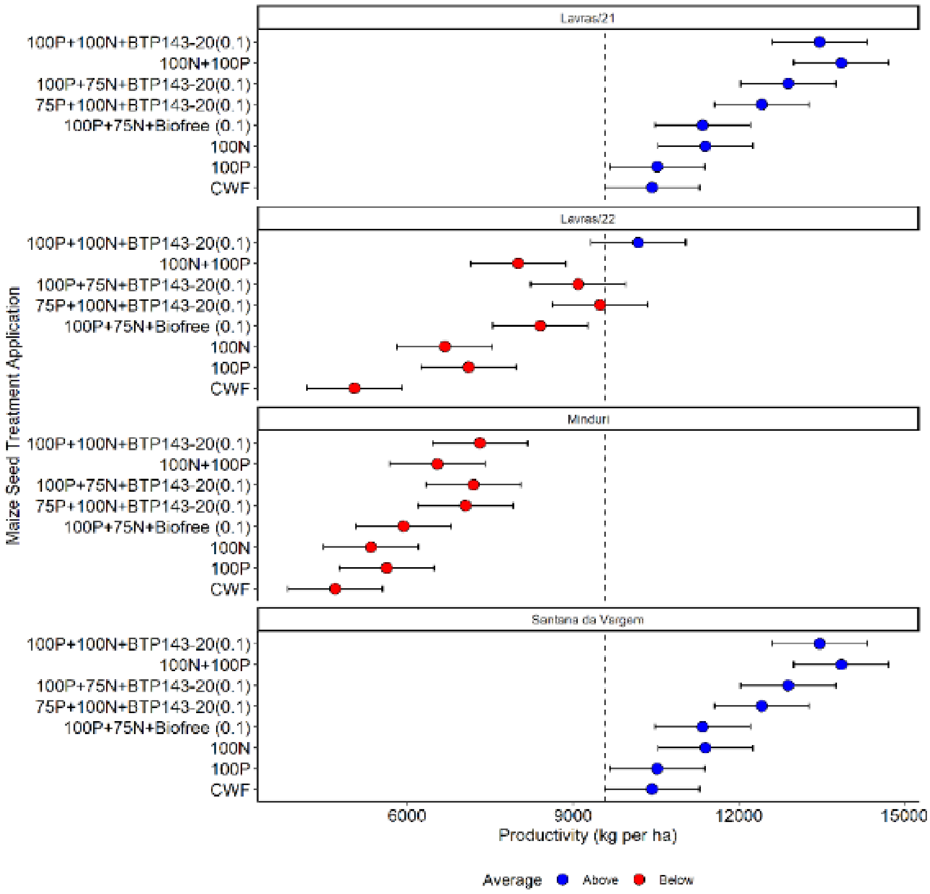

Fig 5. *Best Linear Unbiased Prediction* (BLUP) for breaking down the effect of the interaction between environments and treatments on maize productivity in an application system via seed treatment. Complete treatment information can be found in Supplementary Table 2.

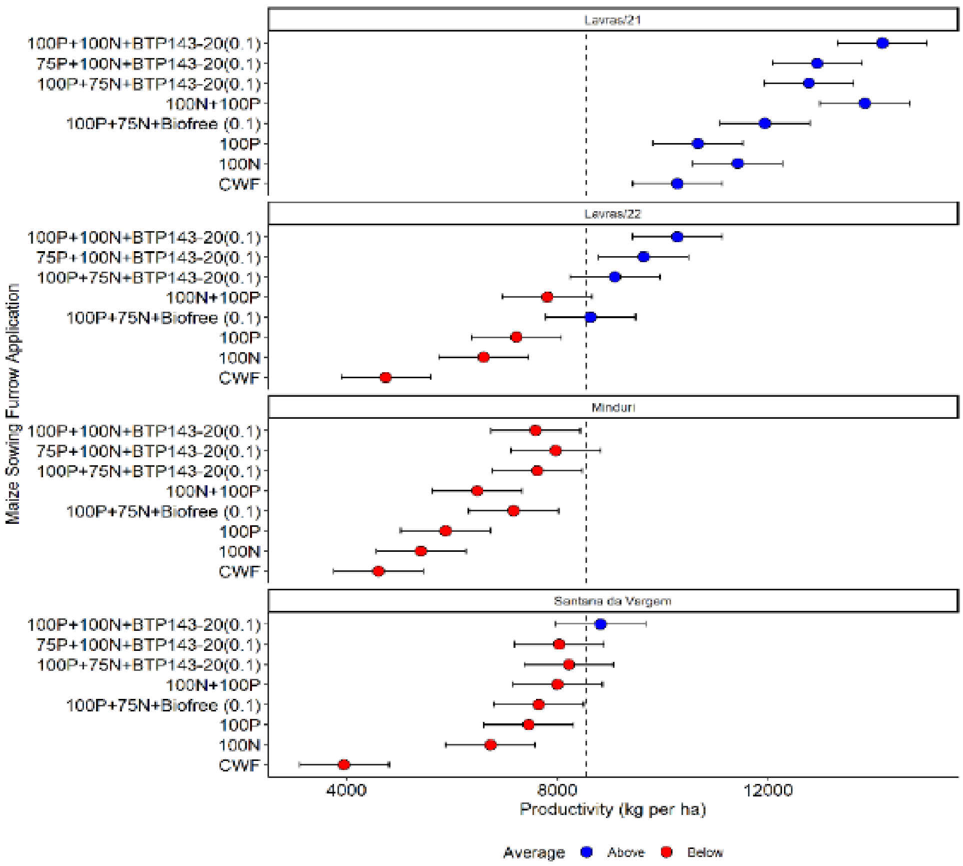

Fig 6. *Best Linear Unbiased Prediction* (BLUP) for breaking down the effect of the interaction between environments and treatments on maize productivity in an application system via furrow sowings. Complete treatment information can be found in Supplementary Table 2.

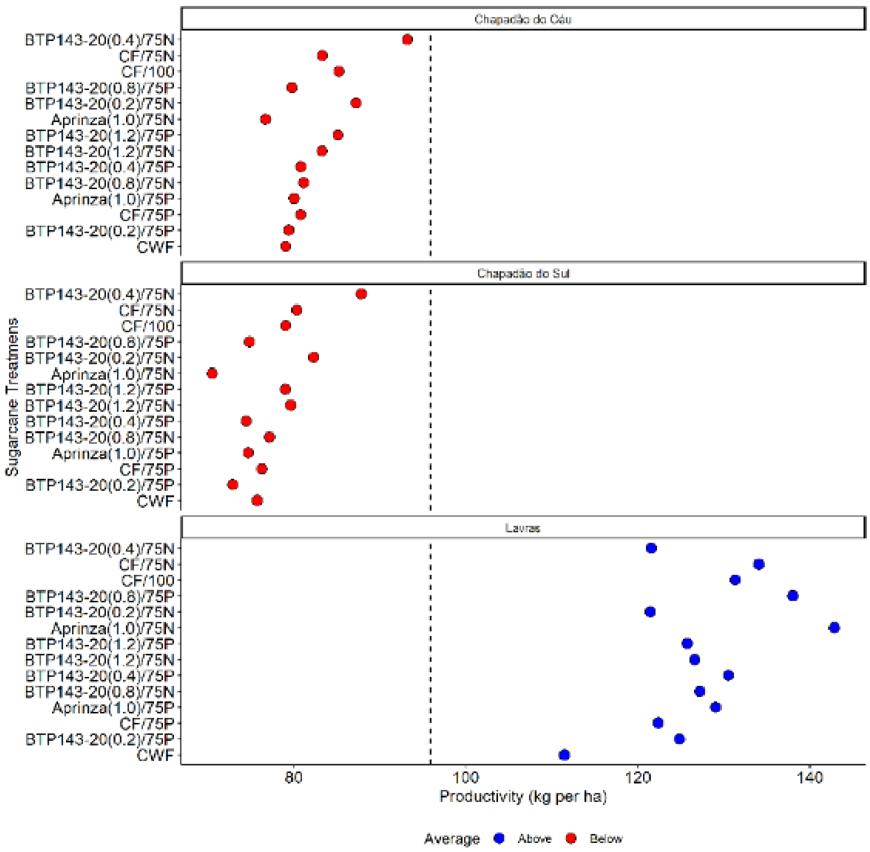

Fig 7. *Best Linear Unbiased Prediction* (BLUP) for breaking down the effect of the interaction between environments and treatments on sugarcane productivity. Complete treatment information can be found in Supplementary Table 2.

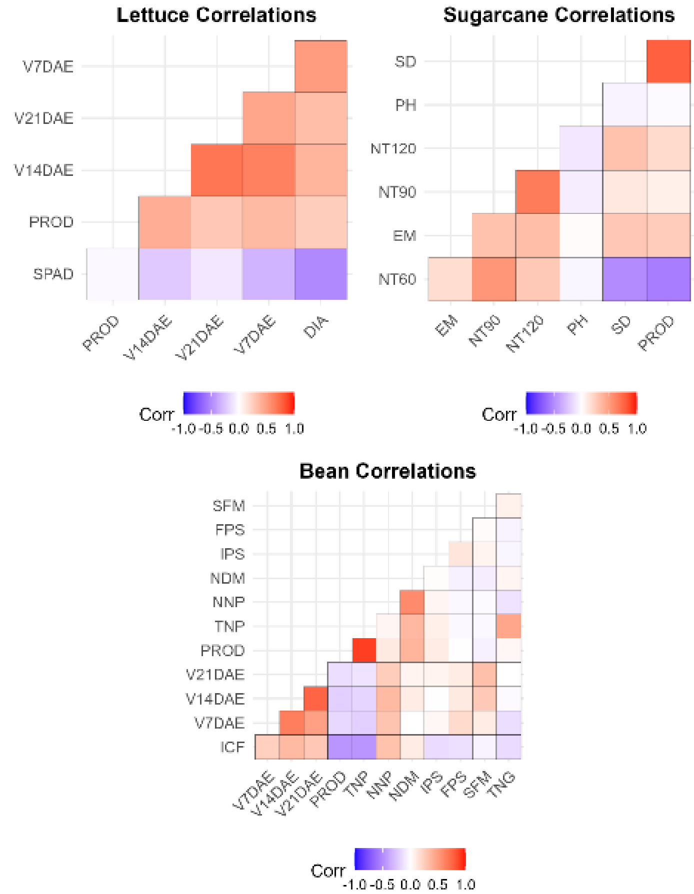

Fig 8. Pearson's linear correlations for the variables measured in the lettuce, bean and sugarcane crops. V7DAE: vigor seven days after transplanting; V14DAE: vigor at fourteen days after transplanting; V21DAE: vigor at twenty-one days after transplanting; ICF: chlorophyll content; NNP: number of nodules per plant; NDW: nodule dry weight; SFW: shoot fresh weight; IPS: initial plant stand; FPS: final plant stand; NCG: nitrogen content in grains; TNP: total nitrogen in plants; NT60: number of tillers at 60 days; NT90: number of tillers at 90 days; NT120: number of tillers at 120 days; PH: plant height; EM: emergency; SD: stem diameter; DIA: head diameter; SPAD: chlorophyll content PROD: productivity. Corr: correlation coefficient.

The BLUP for sugarcane productivity (Figure 7) demonstrates wide variation in the performance of treatments, explained by the high environmental effect. All treatments were below the overall mean in Chapadão do Céu and Chapadão do Sul, with the highest predicted average for BTP143-20(0.4)/75N, where the lowest predicted average in both environments was attributed to the Aprinza(1.0)/75N treatment. However, the use of Aprinza(1.0)/75N in Lavras resulted in greater productivity, in addition to all treatments having a performance greater than 110 t ha^{-1}. Consequently, it was not possible to confidently identify the most promising treatments for sugarcane productivity.

Linear correlation diagrams for lettuce, bean and sugarcane crops

For lettuce (Figure 8), there was a medium magnitude correlation between vigor measurements, while the correlation between head diameter and SPAD index exhibited a moderate negative correlation. This indicates that the increase in chlorophyll index was indirectly proportional to the increase in diameter. Furthermore, grain productivity showed weak correlations with other variables.

Regarding the bean crop (Figure 8), a strong negative correlation was found between total nitrogen content in grains and productivity, which suggests that higher productivity reduces nitrogen accumulation in grains. On the other hand, a positive correlation of strong magnitude was seen between total nitrogen in the plant and productivity. Weak correlations were found between the number of nodules per plant and dry weight of nodules, as well as between vigor assessments.

For sugarcane (Figure 8), strong positive correlations were detected between the number of tillers at 60, 90, and 120 days, indicating the maintenance of these structures over time. The stem diameter correlated positively and strongly with sugarcane productivity. A weak positive correlation was observed between emergence and both productivity and number of tillers, regardless of the evaluation period. In contrast, a strong negative correlation was evaluated between the number of tillers at 60 days and stem diameter and productivity.

Linear correlation diagrams for maize crop

In maize under foliar inoculant application (Figure 9), phosphorus content exhibited a moderate negative correlation with productivity. The macronutrients calcium and potassium showed a positive correlation of medium magnitude with

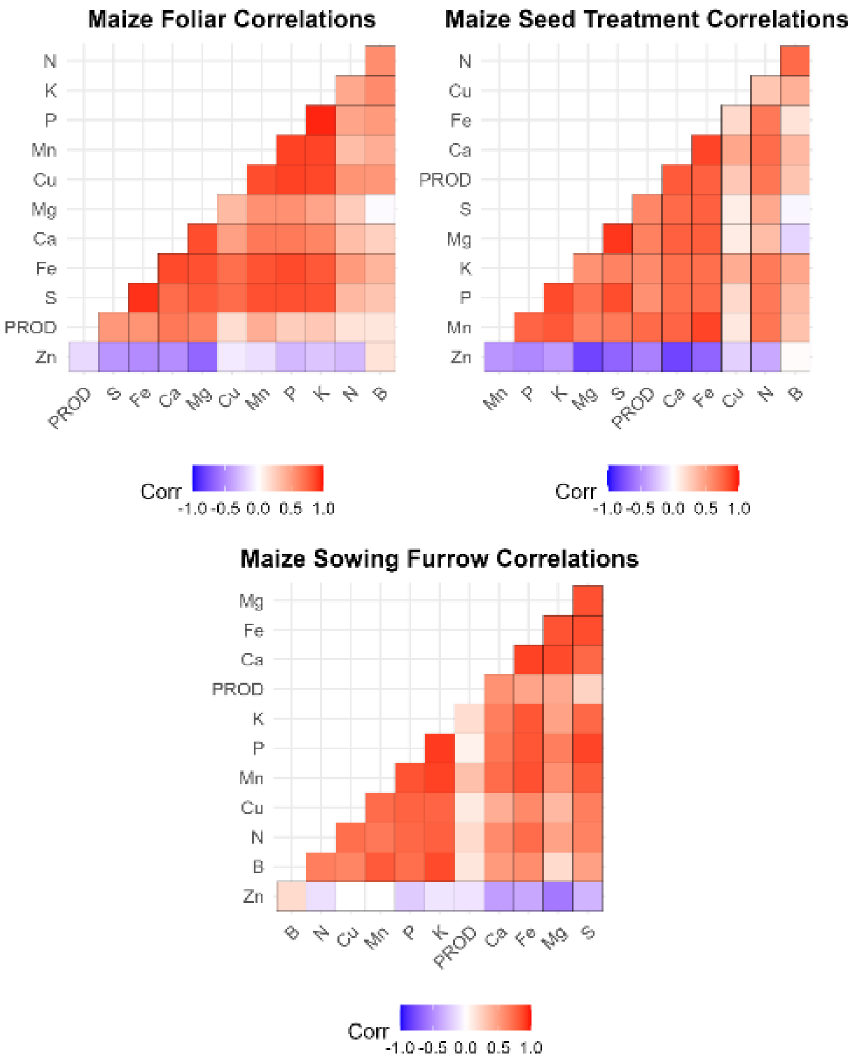

Fig 9. Pearson's linear correlations between the levels of macronutrients, micronutrients and productivity of the maize crop in the foliar application, seed treatment and furrow sowing systems. N: nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; S: sulfur; B: boron; Zn: zinc; Fe: iron; Mn: manganese; Cu: copper; PROD: productivity; Corr: correlation coefficient.

productivity, as did the micronutrient zinc. Strong correlations were identified between phosphorus and potassium, while medium-magnitude associations were observed between copper and nitrogen, phosphorus and magnesium, zinc and manganese, nitrogen and boron, sulfur and copper, and phosphorus and copper. These associations also revealed certain antagonisms, with a strong negative correlation between sulfur and calcium, as well as between potassium and magnesium. Furthermore, a weak negative correlation can be observed between zinc and nitrogen, magnesium and potassium, as well as between phosphorus and sulfur.

For maize in the seed treatment inoculation system (Figure 9), nutrient-productivity correlations were generally weak. However, nitrogen and calcium were positively and strongly associated with boron, phosphorus and sulfur in addition to nitrogen and iron. The correlation of copper with potassium, calcium and zinc, phosphorus and potassium and manganese with iron and potassium was positive of medium magnitude. Strong antagonistic relationships were found between boron and both iron and magnesium, as well as between calcium and zinc.

The correlations obtained for the maize crop in the furrow sowing inoculation system (Figure 9) show a strong negative correlation of phosphorus with grain productivity. Antagonistic relationships were observed between potassium and both zinc and calcium, manganese with both phosphorus and nitrogen, sulfur with calcium, and boron with both iron and manganese. Strong positive correlations were found between phosphorus and potassium, boron and calcium, and sulfur and magnesium. The nutrients zinc, copper, potassium and calcium were positively correlated with productivity, but with a low magnitude.

Discussion

Understanding the meteorological conditions during the cultivation period of the evaluated crops is essential for interpreting the results. The observed temperature range of 15 to 25 ºC supports the optimal growth of all evaluated crops. When assessing the efficiency of PGPBs, water availability throughout the crop cycle is a key factor. A well-distributed precipitation pattern is not only important for plant development but also creates favorable conditions for bacteria to thrive in the soil. According to Askari-Khorasgani et al. (2019), the optimal temperature for rhizobacteria development ranges from 25 to 30 ºC, provided there are adequate moisture levels in the soil.

Our study demonstrates that supplementing nitrogen fertilization with the bacterial consortium effectively supports lettuce cultivation at a dose of 0.200 L per hectare, allowing for up to a 50% reduction in N-fertilization. These findings align with those of Hong et al. (2022), who reported that increased nutrient input significantly enhanced stem diameter and plant height in lettuce, even with just half of the recommended fertilizer dose.

For the bean crop, yield with the bacterial consortium matched the yield achieved with the full dose of N-fertilization at both 0.200 and 0.100 L per hectare. This outcome is particularly promising, considering the traditionally low efficiency of biological nitrogen fixation in microorganism associated with bean roots. According to Pastor-Bueis et al. (2019), the effectiveness of new bacterial strains in beans is attributed to the combined effects of different modes of action, which collectively support high productivity.

The results indicate less variation in treatment performance in the inoculation system via seed treatment in maize crops. However, the highest predicted averages were observed with furrow sowing application of the bacterial consortium, likely due to reduced bacterial exposure to chemical and salts (common in seed treatments) and a longer period for root colonization compared to foliar application. In this case, the natural soil fertility must also be considered. Ichami et al. (2022) reported that spatial variability in the distribution of nitrogen, phosphorus, and potassium in soil previously classified as fertile ultimately limited maize productivity. Although phosphate is abundant in many environments, it is mostly insoluble and unavailable to plants, making bioavailable forms essential for growth, especially in infertile soils (Lee et al., 2012), being one of the key benefits of using *Bacillus* species.

Variance components and genetic parameters indicated that environmental factors played a significant role in sugarcane productivity (Table 1), leading to variations in treatment performance. This variation can be attributed to differences in the chemical properties and morphological characteristics of the soil, as well as the dynamics of tillering, nutrient use efficiency, and the expression of stem diameter across different environments evaluated. Similarly, Castro et al. (2023), reported high variability in sugarcane productivity related to the timing of fertilizer application, the type of fertilizer (solid or liquid), and the interaction between factors.

Correlation is the measure of a monotonic association between two variables, where the change in the magnitude of one variable is associated with the change in the magnitude of the other, in the same or opposite direction, mathematically described in terms of the covariance between these variables (Wackerly et al., 2008; Schober et al., 2018). Correlation coefficients consist of a dimensionless measure of covariance, which is scaled such that it ranges from -1 to +1 (Wackerly et al., 2008).

Greater bean productivity occurs at the expense of an increase in nitrogen content in the grains. Pastor-Bueis et al. (2019) found a difference only in the total biomass of bean nodules when inoculated with *Rhizobium leguminosarum*, with no difference in the number of nodules. The same study also demonstrated that total nitrogen absorption was higher by up to 37% when comparing treatments without inoculation and inoculation with bacteria. This bioavailability of nitrogen may be mainly related to the input of ammonia and organic acids, with *B. haynesii* standing out in this process (Maheshwari et al., 2022).

The stem of grasses, such as sugarcane and maize, is characterized as a structure that accumulates photoassimilates throughout the plant's development, making them available at the end of the cycle. Therefore, a larger stem diameter typically correlates with higher final productivity. This linear association occurred in our study, and is the main cause of the variability in sugarcane productivity. The bacterial consortium's effectiveness in promoting growth in Lavras appeared greater for sugarcane than in other environments, likely due to increased phosphorus solubilization and availability, along with the synthesis of growth-promoting hormones, particularly auxins (IAA) and cytokinins (Santos et al., 2020).

The application of linear correlations is also one of the ways to indicate associations and interactions between nutrient absorption by plants. These linear associations do not precisely indicate the causal relationships that occur in the soil, they only show indications of the possible magnitude of the associations at the time of carrying out the experiments. Crop responses to inoculation are complex and depend on plant-microorganism interactions, many of which remain unknown, but several IAA-producing bacteria have shown positive effects on maize shoot and root weight, as well as nutrient uptake (Santos et al., 2020; Galindo et al., 2018).

Materials and Methods

Location of experiments

The experiments were carried out during the years 2021, 2022 and 2023, in different soil and climate regions (Supplementary Figure 1). The trials with lettuce and bean cultivation were conducted in the following municipalities in the state of Paraná: Palmeira, Ivaí, Ipiranga and Ponta Grossa, in addition to the municipality of Porto Amazonas for lettuce and Tibagi for bean cultivation, totaling five environments by culture. The maize crop trials were conducted in three municipalities in the state of Minas Gerais, namely: Santana da Vargem, Lavras and Minduri. For sugarcane cultivation, experiments were carried out in Chapadão do Sul-MS, Chapadão do Céu-GO and Lavras-MG. All information regarding location, soil type and climate is described in Supplementary Table 1.

Description of inoculants, cultivars and management used

The product BTP 143-20 is a pre-commercial liquid inoculant composed of the bacteria *Bacillus aryabhattai* (isolate CMFMA529), *Bacillus circulans* (isolate CCTB15) and *Bacillus haynesii* (isolate CMFMA162), formulated with a guarantee of 9.9×10^{10} UFC L^{-1} and absence of contaminants. They all have the same number of bacteria in the formulation. The commercial product Biofree® (*Pseudomonas fluorescens* [CCTB03] + *Azospirillum brasilense* [AbV6], concentration of 1×10^{11} viable cells) was used as a standard inoculation treatment for maize crops. As standard treatment in sugarcane cultivation, the commercial product Aprinza® was used, composed of the bacterium *Nitrospirillum amazonense* [BR11145], with a guaranteed concentration of 1.0×10^{8} UFC mL^{-1}.

The Vanda lettuce cultivar was used, which is characterized as a curly type, large in size, long leaves, bright light green in color and moderately crispy. The seedlings were transplanted in the second half of September of 2021. The sowing of the IPR Urutau bean cultivar was carried out in the second half of November 2021, with a density of 12 seeds per linear meter. The corn hybrid NS 90 PRO was sown in the second half of August, with a density of 5 viable plants per linear meter. The planting of sugarcane, variety IACSP95-3028, was carried out in the second half of August 2022.

In lettuce cultivation, the inoculant was applied to the substrate in which the seedlings were sown, as well as to the beds where the seedlings were later transplanted. In beans and maize, the inoculant was applied via the furrow sowing using a flow rate controller adapted to the seeder, with a volume of 100 L ha^{-1}, with a Solid Jet nozzle with 3 KGF Nozzle Valve and a pressure of 3 bar. Foliar application was carried out with a pressurized CO_2 sprayer, using a spray volume of 100 L ha^{-1} and constant pressure of 35 lb in^{-2}, when the corn plants were at the V4 stage. In maize cultivation, the inoculant was also applied via seed treatment, with a volume of 100 mL for 20 kg of seeds. In sugarcane cultivation, inoculants were applied via the planting furrow with a CO_2 pressurized knapsack sprayer with the bar adjusted to operate 30 cm from the surface of the planting furrow, with a pressure of 40 psi and a spray volume of 150 L ha^{-1}.

Experimental design

The experimental design used in all experiments was randomized blocks. For lettuce cultivation, six treatments and four replications were used, in a system with different doses of nitrogen. Each experimental unit was composed of 30 plants, which were distributed in 3.0 m^2 beds, taking only the 10 central plants as the useful area. In the bean crop, six treatments were evaluated with six replications. The experimental units were composed of six lines spaced 0.45 m apart, totaling 24 m^2, with a useful area consisting of six lines three meters long (9 m^2). The treatments for lettuce and bean were: 0N – control without N fertilizer; 50N – control with 50% N; 100N – control with 100% N; 50N+0.50BTP143-20 – 0.50 mL of BTP 143-20 + 50%N; 50N+0.100BTP143-20 – 0.100 mL of BTP 143-20 + 50% N; 50N+0.200BTP143-20 – 200 mL of BTP 143-20 + 50%N, varying only in the dosage of N-fertilizer applied to each crop.

For the maize crop, eight treatments were tested with four replications. The experimental units were composed of lines spaced 0.50 m apart, totaling 24.0 m^2 of useful area. The treatments were: CWF (absolute control); 100N (100% of N-fertilizer dosage); 100P (100% of P-fertilizer dosage); 100N+100P (100% of N-fertilizer and P-fertilizer dosage); 100P+75N+Biofree(0.1) (100% of P-fertilizer dosage + 75% of N fertilizer dosage + 100 mL of Biofree); 100P+75N+BTP143-20(0.1) (100% of P-fertilizer dosage + 75% of N-fertilizer dosage + 100 mL of BTP 143-20); 75P+100N+BTP143-20(0.1) (75% of P-fertilizer dosage + 100% of N-fertilizer dosage + 100 mL of BTP 143-20); 100P+100N+BTP143-20(0.1) (100% of P-fertilizer dosage + 100% of N-fertilizer dosage + 100 mL of BTP 143-20).

In sugarcane, fourteen treatments were evaluated, with four replications, where the experimental units totaled a useful area of 40.0 m^2. Nitrogen was applied as a complementary treatment, corresponding to 50% and 100% of the total nitrogen requirement for beans and lettuce, and 75% and 100% of the total nitrogen and phosphorus requirement for corn and sugarcane. The treatments were: CWF (control without fertilizer); CF/100 (absolute control with 100% of fertilization); CF/75N (control with 75% of N-fertilizer); CF/75P (control with 75% of P-fertilizer); Aprinza(1.0)/75P (1000 mL of Aprinza + 75% of P-fertilizer); Aprinza(1.)/75N (1000 mL of Aprinza + 75% of N-fertilizer); BTP143-20(0.2)/75N (200 mL of BTP 143-20 + 75% of N-fertilizer); BTP143-20(0.4)/75N (400 mL of BTP 143-20 + 75% of N-fertilizer); BTP143-20(0.8)/75N (800 mL of BTP 143-20 + 75% of N-fertilizer); BTP143-20(1.2)/75N (1200 mL of BTP 143-20 + 75% of N-fertilizer); BTP143-20(0.2)/75P (200 mL of BTP 143-20 + 75% of P-fertilizer); BTP143-20(0.4)/75P (400 mL of BTP 143-20 + 75% of P-fertilizer); BTP143-20(0.8)/75P (800 mL of BTP 143-20 + 75% of P-fertilizer); BTP143-20(1.2)/75P (1200 mL of BTP 143-20 + 75% of P-fertilizer).

This approach was based on methodologies described by Sandini et al. (2024) and Tinna et al. (2020) to assess the potential for reducing mineral fertilizer use in maize and onion cultivation. A detailed description of the treatments used per culture can be found in Supplementary Table 2.

Measured variables

In lettuce cultivation, head diameter (DIA, cm) and chlorophyll content (SPAD) were evaluated in ten plants per experimental unit at the time of harvest. Productivity (PROD, kg ha^{-1}) was determined by harvesting ten heads per plot, which were weighed and the data extrapolated to one hectare. For the bean crop, the plant stand was measured at 7 (IPS, units) and 30 (FPS, units) days after emergence. At 40 days after emergence, five plants were collected per experimental unit to evaluate the shoot fresh weight (SFW, g), as well as the number of nodules on the plant (NNP, units) and nodule dry weight (NDW, mg). Grain productivity (PROD, kg ha^{-1}) was determined by harvesting the central rows of the experimental unit, with values corrected for 13% moisture. The nitrogen content in the grains (NCG, g kg^{-1}) was determined from a sample of grains collected using the calorimetry method. In both cultures, the vigor of the plants was evaluated visually at 7 (V7DAE), 14 (V14DAE) and 21 (V21DAE) days after transplanting, with grades assigned from 1 (low vigor) to 5 (high vigor).

Chlorophyll contents (SPAD and ICF) were measured with the ClorofiLOG CFL 1030 electronic chlorophyllometer, in order to estimate the total plant nitrogen content.

In maize cultivation, the accumulation of macro (N, P, K, Ca, Mg and S) and micronutrients (B, Zn, Fe, Mn and Cu) in shoot dry weight was carried out according to the methodology proposed by Teixeira *et al*. (2017). All ears in the useful area of the plot were harvested, threshed and the grain weight was measured on a precision scale. With grain moisture corrected to 13%, grain productivity (PROD, kg ha^{-1}) was estimated. In sugarcane cultivation, emergence (EM) was obtained by counting the plants that emerged in the three central rows of the experimental unit after emergence. Plant height (PH, cm) was determined by measuring ten random plants per experimental unit, using a graduated ruler. The stem diameter (SD, mm) was evaluated in ten random plants, measured at the height of the third internode above the soil surface. The number of tillers was counted in the three central rows of the useful area of the plot at 60 (NT60), 90 (NT90) and 180 (NT180) days after planting. The number of stalks was counted in two meters from the selection of 20 industrializable stalks, which were harvested to determine fresh weight and estimate productivity (t ha^{-1}).

As a way of helping to understand the results, satellite climate data on mean air temperature (in ºC) and precipitation (in mm) were obtained for the cultivation environments with the help of the NASA Power tool (Nasa Power, 2023).

Statistical analysis

The productivity data obtained were subjected to analysis and removal of discrepant values and, subsequently, to the assumptions of the statistical model, based on tests of normality of errors, homogeneity of variances and independence of errors using the Shapiro-Wilk, Bartlett and Durbin Watson tests, respectively. With the assumptions met, the variance components and genetic parameters were estimated using the restricted maximum likelihood (REML) procedure, with the results supported by Deviance at 5% probability using the $\chi 2$ test. The model used to determine the effect of the interaction between treatment and environment was:

$$y_{ijk=\mu+\alpha_i+\tau_j+(\alpha\tau)_{ij}+\gamma_{jk}+\epsilon_{ijk}},$$

where y_{ijk} is the response variable observed in the kth block of the ith genotype of the jth environment; μ is the overall mean; α_i is the effect of the ith genotype; τ_j is the effect of the jth environment; $(\alpha\tau)_{ij}$ is the effect of the interaction between the ith genotype and the ith environment; γ_{jk} is the effect of the kth block within the jth environment; and ε_{ijk} is the random error. In a complementary way, linear correlation was obtained between productivity and the other variables measured in each crop, with the results supported by the Student's t test, at 5% probability. Statistical analyzes were performed with the R software (R Core Team, 2023), using the metan package version 1.18.0 (Olivoto and Lúcio, 2020) and correlation version 0.8.5 (Makowski *et al*., 2019).

Conclusion

In lettuce cultivation, the bacterial consortium proved to be efficient in maximizing the use of nutrients contributed to the soil. The inoculation of beans with *Bacillus* species allowed an increase in nitrogen accumulation in the plant and grain yields similar to the total dose of chemical fertilizers. The application of the bacterial consortium via furrow sowing resulted in higher grain yields in maize. The bacterial consortium with *Bacillus* species can be efficiently used as a complement to chemical fertilization in lettuce, beans and maize crops, with the potential to reduce chemical fertilization by up to 50%. While chemical fertilizers provide quick yield benefits but involve high recurring costs and potential long-term soil degradations, bacteria consortia have lower input cost and improve nutrient efficiency, tough initial gains may be modest. Over time, bacterial consortia can reduce dependency on chemical inputs, making them a more sustainable and cost-effective option.

References

Beltran-Peña A, Rosa L, D'Odorico P (2020) Global food self-sufficiency in the 21st century under sustainable intensification of agriculture. Environ Res Lett 15:095004.

Castro SGQ, Coelho AP, Castro SAQ, Souza Chiachia TR, Castro RA, Lemos LB (2023) Fertilizer source and application method influence sugarcane production and nutritional status. Front Plant Sci 14:1099589.

Galindo FS, Teixeira Filho MCM, Buzetti S, Rodrigues WL, Fernandes GC, Boleta EHM, Barco Neto M, Biagini AC, Baratella EB, Souza JS (2018) Nitrogen doses associated with *Azospirillum brasilense* inoculation and Si application: effects on micronutrients and silicone concentration in irrigated corn. Open Agric 3:510–523.

Hong J, Xu F, Chen G, Huang X, Wang S, Du L, Ding G (2022) Evaluation of the effects of nitrogen, phosphorus, and potassium applications on the growth, yield, and quality of lettuce (Lactuca sativa L.). Agronomy 12:2477.

Ichami SM, Karuku GN, Sila AM, Ayuke FO, Shepherd KD (2022) Spatial approach for diagnosis of yield-limiting nutrients in smallholder agroecosystem landscape using population-based farm survey data. Plos One 17(2):e0262754.

Kalayu G (2019) Phosphate solubilizing microorganisms: promising approach as biofertilizers. Int J Agron 2019:4917256.

Kottwitz D, Silva VN (2024) Growing crisp lettuce with different nitrogen sources. Agric Sci 22. Available at: https://www.seer.ufal.br/index.php/revistacienciaagricola/article/view/15815/11552 (Accessed 12 July 2024)

Kumar A, Maurya BR, Raghuwanshi R, Meena VS, Tofazzal Islam M (2017) Co-inoculation with Enterobacter and rhizobacteria on nutrient production and uptake by wheat (*Triticum aestivum* L.) in alluvial soil beneath the Indo-Gangetic Plain of India. J Plant Growth Regul 36:608–617.

Lee S, Ka JO, Song HG (2012) Growth promotion of *Xanthium italicum* growth by application of *Bacillus aryabhattai* rhizobacteria isolates in microcosm soil. J Microbiol 50:45–49.

Li C, Hoffland E, Kuyper TW, Yu Y, Zhang C, Li H, Zhang F, Van der Werf W (2020) Syndromes of production in intercropping impact yield gains. Nat Plants 6:1

Maheshwari R, Kumar P, Butão N, Suneja P (2022) Exploration of plant growth-promoting endophytic bacteria from *Pisum sativum* and *Cicer arietinum* from South–West Haryana. J Basic Microbiol 62(7):857–874.

Makowski D, Ben-Shachar MS, Patil I, Lüdecke D (2019) Methods and algorithms for correlation analysis in R. J Open Source Softw 5(51):2306.

Mehta P, Walia A, Kulshrestha S, Chauhan A, Shirkot CK (2015) Efficiency of plant growth-promoting P-solubilizing *Bacillus circulans* CB7 for enhancement of tomato growth under net house conditions. J Basic Microbiol 55:33–44

NASA POWER. Prediction of worldwide energy resource applied science program. Available at: https://power.larc.nasa.gov/docs/ (Accessed 4 July 2023)

Nicchio B, Santos GA, Lino ACM, Ramos LA, Pereira HS, Korndorfer GH (2020) Effect of foliar fertilization on sugarcane. Acta Iguazu 9(2):10–24.

Noulas C, Torabian S, Qin R (2023) Crop nutrient requirements and advanced fertilizer management strategies. Agronomy 13(8):1–10.

Olivoto T, Lúcio AD (2020) metan: An R package for multi-environment trial analysis. Methods Ecol Evol 11(6):783–789.

Paramesh V, Kumar RM, Rajanna GA, Gowda S, Nath AJ, Madival Y, Jinger D, Bhat S, Toraskar S (2023) Integrated nutrient management for improving crop yields, soil properties, and reducing greenhouse gas emissions. Front Sustain Food Syst 7:1173258.

Park YG, Mun BG, Kang SM, Hussain A, Shahzad R, Seo CW, Kim AY, Lee SU, Oh KY, Lee DY (2017) *Bacillus aryabhattai* SRB02 tolerates oxidative and nitrosative stress and promotes soybean growth by modulating the production of phytohormones. Plos One 12:1–28.

Pastor-Bueis R, Sánchez-Cánizares C, James EK, González-Andrés F (2019) Formulation of a highly effective inoculant for common bean based on an autochthonous elite strain of *Rhizobium leguminosarum* bv. *phaseoli*, and genomic-based insights into its agronomic performance. Front Microbiol 10:2724.

Pietro Souza W, Campos Pereira F, Mello IS, Stachack FFF, Terezo AJ, Cunha CN, White JF, Li H, Soares MA (2020) Mercury resistance and bioremediation mediated by endophytic fungi. Chemosphere 240:124874.

Qin L, Tian P, Cui Q, Hu S, Jian W, Xie C, Yang X, Shen H (2021) *Bacillus circulans* GN03 alters the microbiota, promotes cotton seedling growth and disease resistance, and increases the expression of phytohormone synthesis and disease resistance-related genes. Front Plant Sci 12:15.

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Radhakrishnan R, Hashem A, Abdallah EF (2017) *Bacillus*: a biological tool for crop improvement through bio-molecular changes in adverse environments. Front Physiol 8:667.

Reang L, Bhatt S, Tomar RS, Joshi K, Padhiyar S, Vyas UM, Kheno JK (2022) Plant growth promoting characteristics of halophilic and halotolerant bacteria isolated from coastal regions of Saurashtra Gujarat. Sci Rep 12:4699.

Rehman S, Jermy BR, Akhtar S, Borgio JF, Azeez A, Ravinayagam V, Jindan RAL, Alsalem ZH, Buhameid A, Gani A (2019) Isolation and characterization of a novel thermophile, *Bacillus haynesii*, applied for the green synthesis of ZnO nanoparticles. Artif Cells Nanomed B 47:2072–2082.

Sahu PK, Singh S, Singh UB, Chakdar H, Sharma PK, Sarma BK, Teli B, Bajai R, Singh AHV, Saxena AK (2021) Inter-genera colonization of *Ocimum tenuiflorum* endophytes in tomato and their complementary effects on Na+/K+ balance, oxidative stress regulation, and root architecture under elevated soil salinity. Front Microbiol 12:744733.

Sandini IE, Pacentchuk F, Franco DAS, Sandini AH (2024) Bacterial consortium of *Azospirillum brasilense* and *Pseudomonas fluorescens* on the stimulation of growth of corn culture. Cienc Rural 54:09.

Santos D, Diaz RM, Lobo PAE, Rigobelo EC (2020) Use of plant growth-promoting rhizobacteria in maize and sugarcane: characteristics and applications. Front Sustain Food Syst 4:136.

Schober PMD, MMedStat B (2018) Correlation coefficients: appropriate use and interpretation. Anesth Analg 126(5):1763-1768.

Silva LEB, Silva JCS, Souza WCL, Lima LLC, Santos RLV (2020) Development of maize (*Zea mays* L.) culture: literature review. Diversitas J 5(3):1636–1657.

Soratto RP, Schlick GDS, Fernandes AM, Oliveira LFF (2015) Growth and yield of two bean cultivars as a function of doses of 2,3,5-triiodobenzoic acid. Cienc Rural 45(12):2181–2186.

Teixeira PC, Donagemma GK, Fontana A, Teixeira WG (2017) Soil analysis methods manual. 3rd ed. rev. e ampl. Embrapa, Brasília, DF.

Tinna D, Garg N, Sharma S, Pandove G, Chawla N (2020) Utilization of plant growth promoting rhizobacteria as root dipping of seedlings for improving bulb yield and curtailing mineral fertilizer use in onion under field conditions. Sci Hortic 270:25.

McCarthy U, Uysal I, Badia-Melis R, Mercier S, O'Donnell C, Ktenoudaki A (2018) Global food security – issues, challenges and technological solutions. Trends Food Sci Technol 77:11–20.

Vejan P, Abdullah R, Khadiran T, Ismail S, Boyce AN (2016) Role of plant growth promoting rhizobacteria in agricultural sustainability — a review. Molecules 21(5):673.

Wackerly DD, Mendenhall III W, Scheaffer RL (2008) Multivariate probability distributions. In: Mathematical statistics with applications. 7th ed. Brooks/Cole, Belmont, CA, 223–295.
Zhang X, Davidson EA, Zou T, Lassaletta L, Quan Z, Li T, Zhang W (2020) Quantifying nutrient budgets for sustainable nutrient management. Global Biogeochem Cy 34:e2018GB006060.