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Agronomic efficiency of an inoculant based on *Bacillus amyloliquefaciens* FZB45 for corn and soybean crops

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ABSTRACT: The use of plant growth-promoting bacteria to replace or reduce the application of chemical fertilizers has been consolidated in recent decades. However, most of studies have been carried out under controlled conditions and tests under field conditions are necessary to validate this technology. The objective of this work was to evaluate the agronomic efficiency of the Phosbac-45 product based on *Bacillus amyloliquefaciens* FZB45 in promoting the growth and yield of corn and soybean crops. Tests were conducted in Brazilian territory under two different edaphoclimatic conditions for two consecutive harvests (2018-2019 and 2019-2020). The design used was randomized blocks in a 3 × 2 factorial scheme (inoculation treatments × levels of phosphate fertilization) and two additional treatments, with five replications of each treatment. It was verified that the use of Phosbac-45 product increased the variables shoot dry mass, root dry mass and grain yield, showing a positive correlation with P uptake by corn and soybean under the conditions evaluated. Thus, the Phosbac-45 product emerges as a promising technology for agricultural use.

Key words: *Glycine max*; plant growth promoting bacteria; Phosbac-45; phosphorus solubilization; *Zea mays*

Eficiência agronômica de inoculante a base de *Bacillus amyloliquefaciens* FZB45 para as culturas de milho e soja

RESUMO: Nas últimas décadas o uso de bactérias promotoras do crescimento de plantas tem se consolidado nos sistemas agrícolas com o intuito de substituir ou diminuir a aplicação de fertilizantes químicos. Contudo, a maioria dos trabalhos têm sido realizados em condições controladas tornando necessário ensaios em condições de campo para validação desta tecnologia. O objetivo deste trabalho foi avaliar a eficiência agronômica do produto Phosbac-45 a base de *Bacillus amyloliquefaciens* FZB45 na promoção do crescimento e rendimento das culturas de milho e soja. Para tanto os ensaios foram conduzidos em território brasileiro em duas condições edafoclimáticas distintas por duas safras consecutivas (2018-2019 e 2019-2020). O delineamento utilizado foi de blocos casualizados em esquema fatorial 3 × 2 (tratamentos de inoculação × níveis de adubação fosfatada) e dois tratamentos adicionais, com cinco repetições de cada tratamento. Foi verificado que a utilização do produto Phosbac-45 incrementou as variáveis massa seca de parte aérea, massa seca de raízes e rendimento de grãos, apresentando correlação positiva com a absorção de P pelas culturas milho e soja nas condições avaliadas. Desta forma, o produto Phosbac-45 mostra-se como uma tecnologia de bioinsumo promissora para uso agrícola.

Palavras-chave: *Glycine max*; bactérias promotoras do crescimento vegetal; Phosbac-45; solubilização de fosfato; *Zea mays*

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Introduction

In the coming years, the growth in world population brings to the agricultural sector the great challenge of meeting the demand for increased food production, with greater pressure on land use expected. In this scenario, Brazil is considered a strong candidate to lead this process, since it has 550 million hectares of potentially arable area, of which only 80 million are being cultivated (Saath & Fachinello, 2018). Currently, Brazil stands out worldwide in the production of grains, being considered the second largest world producer of corn (*Zea mays* L.) and soybeans (*Glycine max* (L.) Merrill), producing approximately 115 and 124 million tons in the 2021-2022 harvest, respectively (CONAB, 2022).

Phosphorus (P) is considered the second most limiting macronutrient for plant growth. However, the availability of P in the soil is low due to the complex dynamics of P in this environment. This is particularly relevant in Brazilian soils that have a high degree of weathering, with a predominance of iron and aluminum oxides (Tiecher et al., 2018). As a consequence, about 95% of the phosphate fertilizer applied to the soil is fixed by these metal ions, compromising P uptake by agricultural crops (Gong et al., 2022). Thus soil management alternatives and biological technologies that increase the availability and acquisition of P by plants are necessary to achieve satisfactory yields and provide a more sustainable agriculture.

Plant growth promoting bacteria (PGPB) can act through different mechanisms (direct and indirect) in increasing agricultural productivity, being characterized as an innovative technology for increasing sustainability in agricultural systems (González et al., 2019; Nascimento et al., 2020a). Notoriously, the role played by PGPB in agriculture is consolidated in the provision of nutrients such as nitrogen (N), resulting in the wide use of commercial products used for this purpose (Quintas-Nunes et al., 2022). However, more recently, PGPB that act in the availability of other nutrients, such as P, have been receiving more attention, demonstrating themselves as a potential strategy for utilization of this nutrient (Ribeiro et al., 2018).

Several bacterial genera have already been described for their ability to increase the availability of P present in organic and inorganic forms in soil, for example, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia* (Novo et al., 2018). Among these, species of the genus *Bacillus* that have facultative anaerobic metabolism and are spore-formers capable of withstanding high temperatures and low pH values are worth mentioning. These characteristics allow bacteria belonging to this genus to survive under abiotic stress conditions (Grant et al., 2018; Nascimento et al., 2020b). Products based on different *Bacillus* species are commercially available in the European Union, primarily for use in biocontrol (Pertot et al., 2015), while application of this technology for increasing soil P availability is still not widespread (Mehnaz, 2016). Nevertheless, recently, the BiomaPhos® inoculant containing P-solubilizing PGPB was registered in Brazil, and its registration was granted for corn and soybean (Oliveira-Paiva et al., 2020).

The selection of PGPB strains that have the ability to increase P availability to plants begins in studies under controlled conditions, via P mineralization and/or solubilization tests. In this sense, Krebs et al. (1998) isolated *Bacillus amyloliquefaciens* strains from soils with high concentrations of pathogens, demonstrating that these strains had plant growth promoting characteristics. Further studies allowed selecting the FZB45 strain as the most promising for growth promotion of corn plants under P limitation, since it presented the highest activity of the extracellular phytase enzyme among the microorganisms evaluated (Idriss et al., 2002). However, these experiments were conducted in an *in vitro* system, and it is suggested by the authors that validation of these results under conditions more similar to the natural environment is pertinent. Subsequently, Ramirez & Kloepper (2010) pointed out that in the process of bioprospecting these strains, the responses may be less consistent and dependent on soil conditions. Thus, the objective of this study was to evaluate the agronomic efficiency of Phosbac-45, based on *B. amyloliquefaciens* FZB45, in promoting growth, yield, and acquisition of P in corn and soybean crops over two seasons in different soil and climatic conditions in Brazil.

Materials and Methods

Field experiments were conducted with corn and soybean to validate the efficiency and feasibility of a biological product called Phosbac-45 based on *B. amyloliquefaciens* FZB45, containing 2.5×10^{10} CFU mL^{-1}, in promoting the growth and productivity of these crops. The experimental conduction followed the protocols established by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) for product registration based on plant growth promoting microorganisms, contemplated in IN SDA 13, of 03/25/2011, and IN SDA 53, of 10/24/2013. To this end, two experiments were conducted for each crop in the municipalities of Assaí, PR, Brazil (23° 20’ 16.72” S; 50° 48’ 59.37” W) and Ponta Grossa, PR, Brazil (25° 5’ 50.96” S; 50° 3’ 13.29” W) in the 2018-2019 and 2019-2020 harvests (during the months of November and April considering the planting seasons of each location). The experiment with soybean planting in Assaí (PR) in 2018-2019 was conducted in an area with a history of soybean/fallow cultivation and in a no-till system, while in the experiment with corn the trial was conducted in an area with coffee stripping and in a conventional planting system. As for the tests conducted for soybeans and corn at this location in the year 2019-2020, corn was used as a predecessor crop, using the no-tillage system. The experimental area of Ponta Grossa (PR) in the 2018-2019 and 2019-2020 harvests the soybean trials were conducted in areas previously cultivated with soybean/wheat, while the corn trial was conducted in areas with a history of soybean/black oats cultivation, and the no-tillage system was adopted in both crops. No irrigation system was adopted in the experimental areas. The seed used for soybean in both harvests was the cultivar TMG 7062 and for corn the cultivar P30R50 and K9606 in the 2018-2019 and 2019-2020 harvests, respectively. During the cultivation, the management practices normally used in each location were adopted. Soil samples were collected in the 0-0.20 m depth layer for physical-chemical characterization as presented in Table 1.

According to Köppen classification, the predominant climate in Assaí (PR) is subtropical, with hot summers and rare frosts, rainfall generally concentrated in the summer months and no well-defined dry season (Cfa). On the other hand, the predominant climate in the municipality of Ponta Grossa (PR) is temperate oceanic with an average temperature below 22 °C and at least four months had an average temperature above 10 °C, with no significant difference in the level of precipitation between the seasons (Cfb). The average temperature and rainfall during the experimental conduction in the 2018-2019 and 2019-2020 harvests are presented in Figure 1, whose data were obtained from the Weather Station of BASF S.A being this representative of the Eastern region of the State of Paraná.

The corn and soybean experiments were conducted in a randomized block design in a 3 × 2 factorial scheme (3 inoculation treatments × 2 phosphate fertilization levels) and two additional treatments, with five replicates per treatment. The inoculation treatments were: non-inoculated (NI); Phosbac-45-1 (dose 1.0 mL kg^{-1} of seed) and Phosbac-45-2 (dose 2.0 mL kg^{-1} of seed). Phosphate fertilization treatments for corn consisted of no P fertilization (P_0) and the application of 50% of the recommended phosphate fertilization (P_{50}), corresponding to 56.25 kg ha^{-1} of P_2O_5. In addition to these, two additional treatments were tested for the corn crop: T-P_{100} - non-inoculated seeds and with application of 100% of the recommended phosphate fertilization, corresponding to 112.50 kg ha^{-1} of P_2O_5; T-Azo - treatment without phosphate fertilization (P_0) and seeds inoculated only with *Azospirillum brasilense* by applying the commercial product Grap NOD A (2 × 10^8 CFU mL^{-1}) at a dosage of 100 mL for 50 kg of seeds. The experimental plots had a total area of 40.0 m^2 (4.0 × 10.0 m), and were sown at 0.80 m spacing between rows with six seeds per meter.

In soybean, in addition to the Phosbac-45 treatments described above (Phosbac-45-1 and Phosbac-45-2), the treatments P_0 (no fertilization) and with 25 kg ha^{-1} of P_2O_5 (P_{50}) were tested, while the additional treatment T-P_{100}

Table 1. Physical-chemical characterization of the soils in the experimental areas for validation of a biological product based on *Bacillus amyloliquefaciens* FZB45 for corn and soybean.

Location/	pH	Ca/[1]	Mg/[1]	K/[2]	Al/[1]	P/[2]	C_{org}/[3]	Sand/[4]	Silt/[4]	Clay/[4]	Soil
Crop	$CaCl_2$	($cmol_c$ dm^{-3})				(mg dm^{-3})	(g dm^{-3})	(g kg^{-1})			classification
Assaí-PR (2018-2019)	4.5	6.0	1.8	0.74	0.3	7.8	32	360	120	520	Typic Dystrophic Red Latosol
Assaí-PR (2019-2020)	5.6	8.5	2.6	0.79	0.0	8.1	24	365	125	510	
Ponta Grossa (2018-2019)	4.2	1.8	1.0	0.51	1.3	51.1	28	483	128	389	Dystrophic Humic Cambisol
Ponta Grossa-PR (2019-2020)	4.4	2.3	0.8	0.61	0.8	18.8	38	494	133	373	

/[1] Extracted with 1 mol L^{-1} of KCl; /[2] Extracted by Mehlich-1; /[3] Walkley-Black method; /[4] Pipette method.

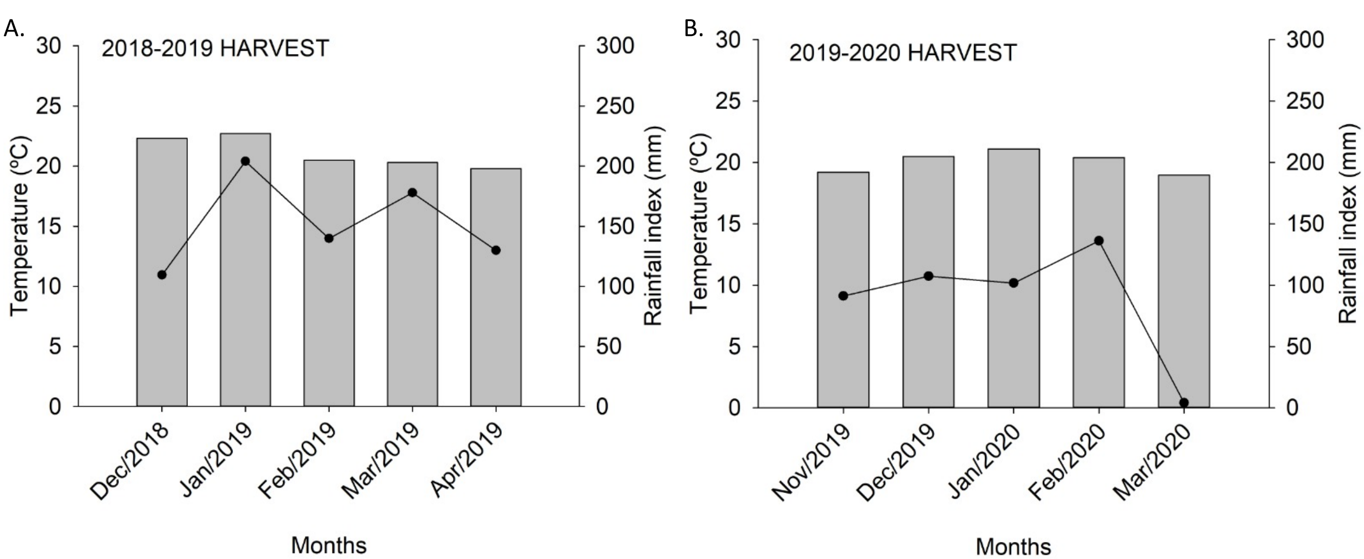


Figure 1. Climatological data of temperature (°C) and rainfall index (mm) in the 2018-2019 and 2019-2020 harvests during the period of conducting the trials for corn and soybean crops.

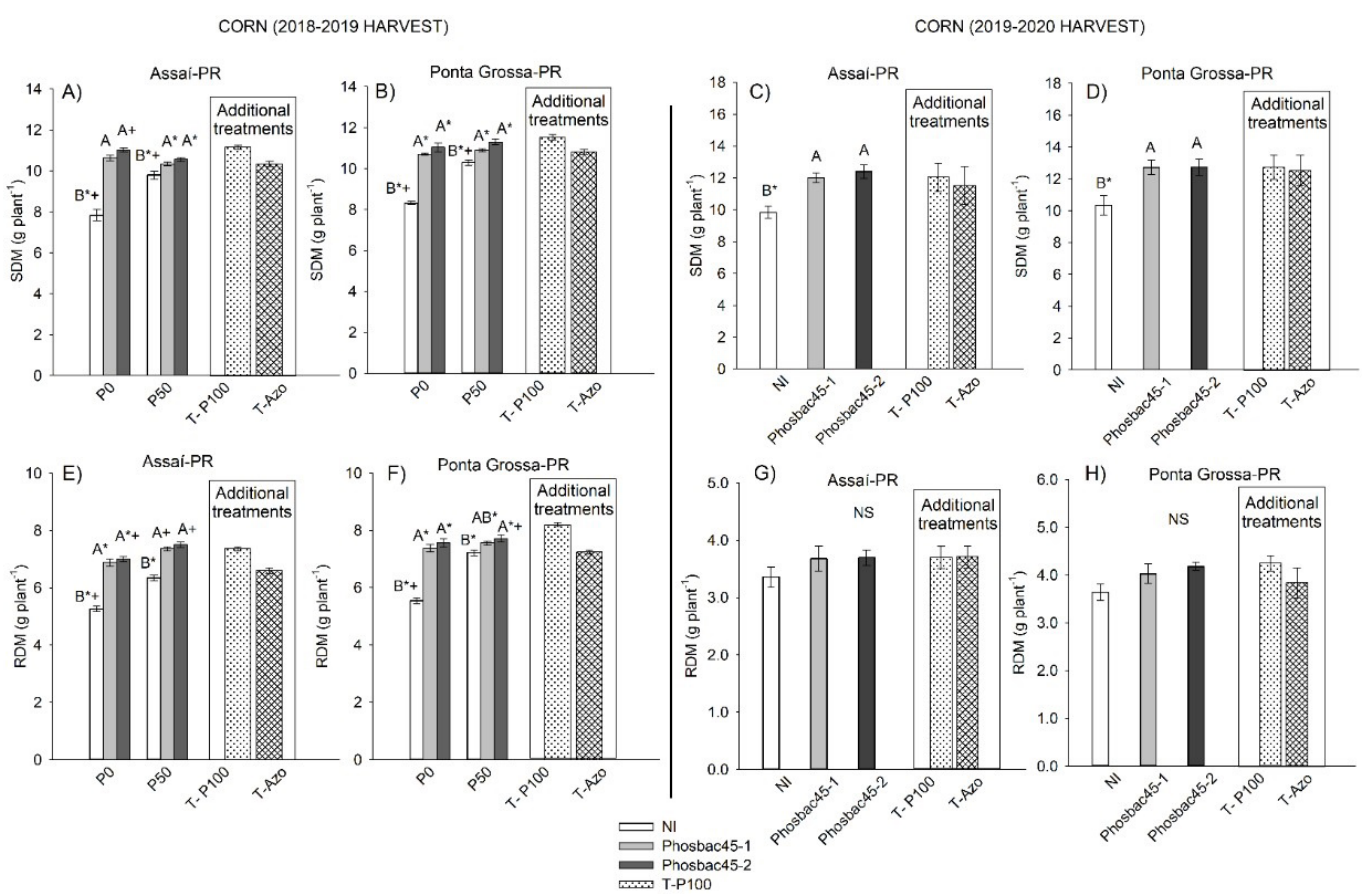
corresponded to seed without inoculation and with the application of 50 kg ha^{-1} of P_2O_5. Additionally, the additional T-Brady treatment was tested, which corresponded to the treatment without phosphate fertilization (P_0) and seeds inoculated only with *Bradyrhizobium japonicum* by applying the commercial product Grap NOD L (Agrocete Indústria de Fertilizantes Ltda) based on *B. japonicum* (strains SEMIA 5079 and SEMIA 5080 containing 5×10^9 CFU mL^{-1}) at a dosage of 100 mL for 50 kg of seeds. The experimental plots used for soybean cultivation had a total area of 15.0 m^2 (3.0 × 5.0 m) and were sown at a spacing of 0.45 m between rows, with 13 seeds per meter.

After 40 days after planting (40 DAP), 10 plants were collected from the central rows of the experimental plots to determine the variables shoot dry mass (g $plant^{-1}$), root dry mass (g $plant^{-1}$), and leaf P content (g kg^{-1}), determined by the nitropercloric digestion method according to Malavolta et al. (1989). From the shoot dry biomass and P leaf content, the P uptake was determined (mg $plant^{-1}$ of P). For soybean, the dry mass of nodules (mg $plant^{-1}$) was also measured at 40 DAP. At the end of each crop cycle, the plants of the useful plot were harvested and the grain yield was estimated (kg ha^{-1}) at 13% humidity.

The data were subjected to normality (Shapiro-Wilk) and homogeneity of variance (Bartlett) analysis. Given the assumptions, we proceeded to the analysis of variance by Tukey test at 5% probability level. All statistical analyses were performed using R Software (R Development Core Team, 2009), using the package Treatments.ad with the command factorial2.ad.dbc in order to compare the treatments inoculated with Phosbac-45 (its isolated effect or its unfolding in each P dose) and the additional treatments T-P_{100} (corn and soybean), T-Azo (corn), and T-Brady (soybean). Error bar graphs and regressions were generated with Sigma-Plot Software v. 12.5 (Systat Corp., San Jose, USA).

Results and Discussion

In the present study, we evaluated the agronomic efficiency of a product based on the *B. amyloliquefaciens* FZB45 strain and its contribution to increasing the acquisition of P by corn and soybean, in consecutive years of production and different soil and climate conditions. It was found for corn in the 2018-2019 harvest, there was a significant interaction between inoculation treatments and phosphate fertilization ($p < 0.05$) for shoot dry mass (SDM) and root dry mass (RDM) yield at 40 DAP (Figure 2A-B and 2E-F). Overall, inoculation



T-P_{100} = additional treatment corresponding to 100% of P; T-Azo = additional treatment corresponding to the standard inoculation with *Azospirillum brasilense*; * = significant difference between inoculation and T-P_{100} treatments; + = significant difference between inoculation and T-Azo treatments; NS = no significant effect between treatments. Means followed by the same letter do not differ by the Tukey test at 5% probability.

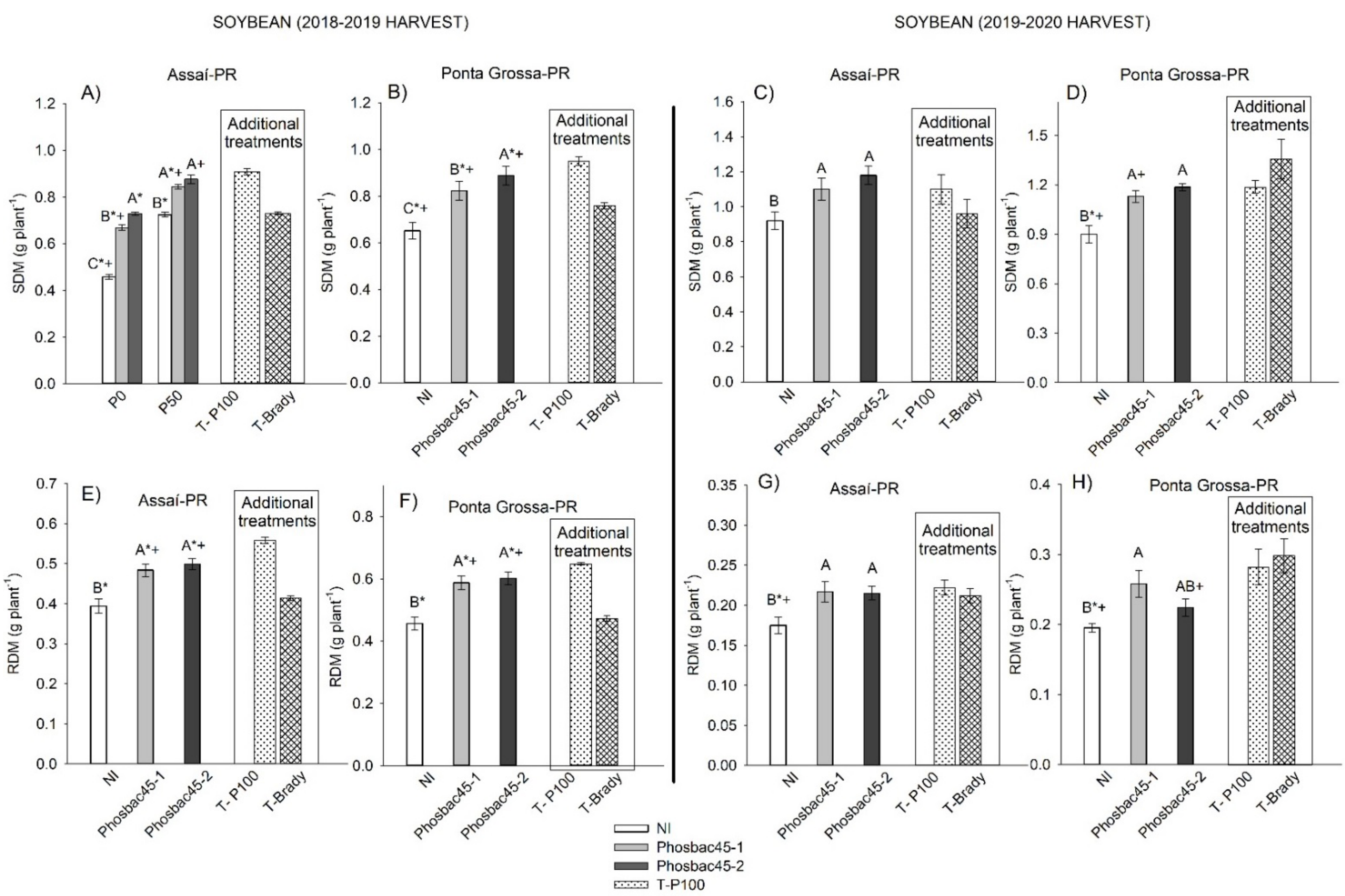
Figure 2. Shoot dry mass (SDM) and root dry mass (RDM) of corn plants 40 days after seed inoculation with Phosbac-45 (NI non-inoculated seeds; 1.0 and 2.0 mL kg^{-1} of seeds) and phosphate fertilization (0 and 50% of P) in the 2018-2019 and 2019-2020 harvests.

treatments with Phosbac-45 increased shoot biomass production by an average of 34 and 7.3% compared to the NI treatment at dose P_0 and P_{50}, respectively. Additionally, the Phosbac-45-2 treatment at the P_0 dose showed significantly higher SDM than the additional T-Azo treatment. In RDM production, the benefits of Phosbac-45 inoculation were also evidenced for the corn crop in the 2018-2019 harvest, with most inoculation treatments being significantly superior to the additional T-Azo treatment.

In the 2019-2020 harvest, mean SDM yield of the corn crop was influenced only by inoculation treatments, with average increments of 24% compared to the NI treatment (Figure 2C-D). On the other hand, in this same harvest, there were no significant effects of the evaluated factors on RDM production (Figure 2G-H).

In soybean it was found that there was interaction between the factors evaluated for SDM production ($p < 0.05$) in the municipality of Assaí (PR) in the 2018-2019 harvest. Inoculation with Phosbac-45 increased SDM yield on average by 52 and 19% compared to the NI treatment at doses P_0 and P_{50}, respectively. Additionally, the SDM yield of Phosbac-45 inoculated seeds was higher than the additional T-Brady treatment (Figures 3A). For the municipality of Ponta Grossa (PR) in the 2018-2019 harvest and for both locations in the 2019-2020 harvest, a significant effect was found only for the inoculation factor on soybean SDM yield (Figures 3B, 3C and 3D), with average increases of 24 and 33% being found for the Phosbac-45-1 and Phosbac-45-2 treatments compared to the NI treatment, respectively. Similarly, RDM yield in both harvests was influenced only by inoculation treatments, with inoculation with Phosbac-45 providing higher values than the NI treatment, but lower than the additional T-P_{100} treatment.

In general, seed inoculation with Phosbac-45 increased both corn and soybean grain yields in the two harvests evaluated (Figure 4). In the municipality of Assaí (PR), inoculation with Phosbac-45 resulted in higher corn yields, with the highlight being the Phosbac-45-2 treatment, which provided average increments of 27% compared to the NI treatment (Figures 4A and 4C). Additionally, inoculation with Phosbac-45 resulted in equal or significantly higher yields than the additional T-Azo treatment. For the municipality of Ponta Grossa (PR), in both harvests, the contribution of Phosbac-45 inoculation on corn grain yield was most evident in the P_0 dose, finding values 23



T-P_{100} = additional treatment corresponding to 100% of P; T-Brady = additional treatment corresponding to the standard inoculation with *Bradyrhizobium*; * = significant difference between inoculation and T-P_{100} treatments; + = significant difference between inoculation and T-Brady treatments. Means followed by the same letter do not differ by the Tukey test at 5% probability.

Figure 3. Shoot dry mass (SDM) and root dry mass (RDM) of soybean plants at 40 days after seed inoculation with Phosbac-45 (NI non-inoculated seeds; 1.0 and 2.0 mL kg^{-1} of seeds) and phosphate fertilization (0 and 50% of P) in the 2018-2019 and 2019-2020 harvests.

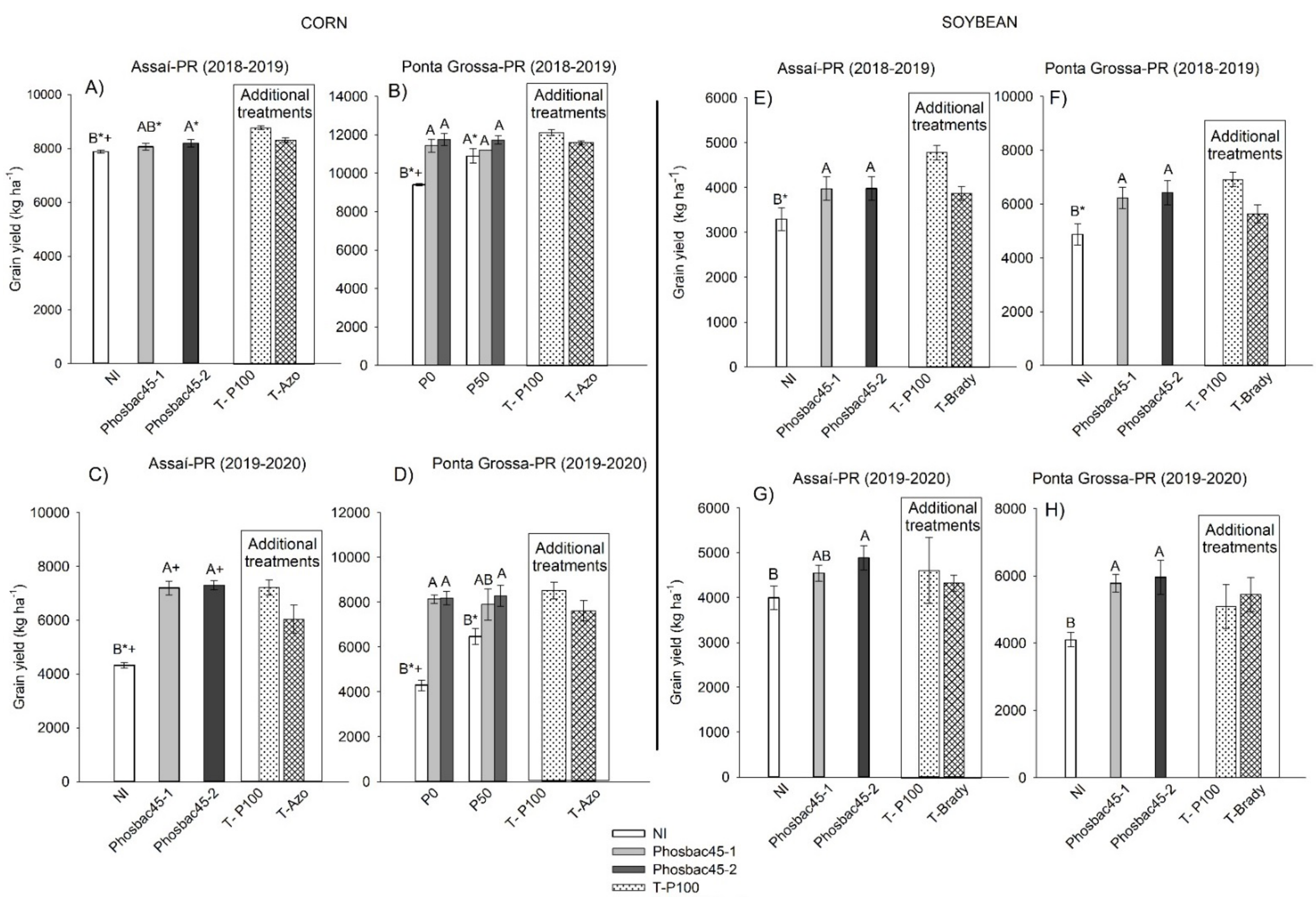
and 90% higher than the NI treatment in the 2018-2019 and 2019-2020 harvests, respectively (Figures 4B and 4D).

For the soybean crop it was found that only inoculation treatments influenced grain yield in the two locations and harvests evaluated (Figure 4E to 4H). On the other hand, there were no significant differences between the inoculant doses, and these treatments showed average increments of 28% in soybean yield compared to the NI treatment (average of the two locations and two harvests). Additionally, it was found that the Phosbac-45 inoculation treatments did not differ from the additional T-P_{100} and T-Brady treatments.

As presented in Figure 5, there was a positive relationship between plant P uptake at 40 DAP (P accumulation) and grain yield, except for the corn crop in the 2018-2019 harvest. Plants and soil microorganisms, especially those present in the rhizosphere, have different strategies to increase the acquisition of P from organic and inorganic fractions in the soil. Organic P comprises 30 to 70% of the total P in agricultural soils and is as important as the inorganic P pool in providing plant available P (Tiecher et al., 2018).

The improved P uptake by corn and soybean plants when inoculated with Phosbac-45 may be associated with the different direct and indirect mechanisms of P availability by the *B. amyloliquefaciens* FZB45 strain that makes up the product (Idriss et al., 2002). Ramirez & Kloepper (2010) demonstrated that the strain *B. amyloliquefaciens* FZB45 is capable of phytase enzyme production and related the activity of this enzyme as a major factor associated with shoot biomass production and P uptake by *Brassica rapa* L. under conditions of high phytate supply in the soil. The phytase enzyme activity has been reported as an important mechanism organic phosphate mineralizing bacteria in mineralizing phytate, the main component of the soil organic fraction, with consequent benefit for plant growth (Ghosh et al., 2021). This strain is also capable of favoring root growth through the production of the phytohormone indole 3-acetic acid (IAA), acting as a direct mechanism in P acquisition. In fact, the benefits of Phosbac-45 inoculation on the root dry mass production of corn and soybean roots were evident in the present study, and this may explain, although partially, the direct relationship between the favorable P uptake by plants at 40 DAP and the grain yield of the crops.

The ability of microorganisms to solubilize phosphate is also a mechanism that favors the acquisition of P by



T-P_{100} = additional treatment corresponding to 100% of P; T-Azo = additional treatment corresponding to the standard inoculation with *Azospirillum brasilense*; T-Brady = additional treatment corresponding to the standard inoculation with *Bradyrhizobium*; * = significant difference between inoculation and T-P_{100} treatments; + = significant difference between inoculation and T-Azo treatments. Means followed by the same letter do not differ by the Tukey test at 5% probability.

Figure 4. Corn and soybean grain yield after seed inoculation with Phosbac-45 (NI non-inoculated seeds; 1.0 and 2.0 mL kg^{-1} of seeds) and phosphate fertilization (0 and 50% of P) in the 2018-2019 and 2019-2020 harvests.

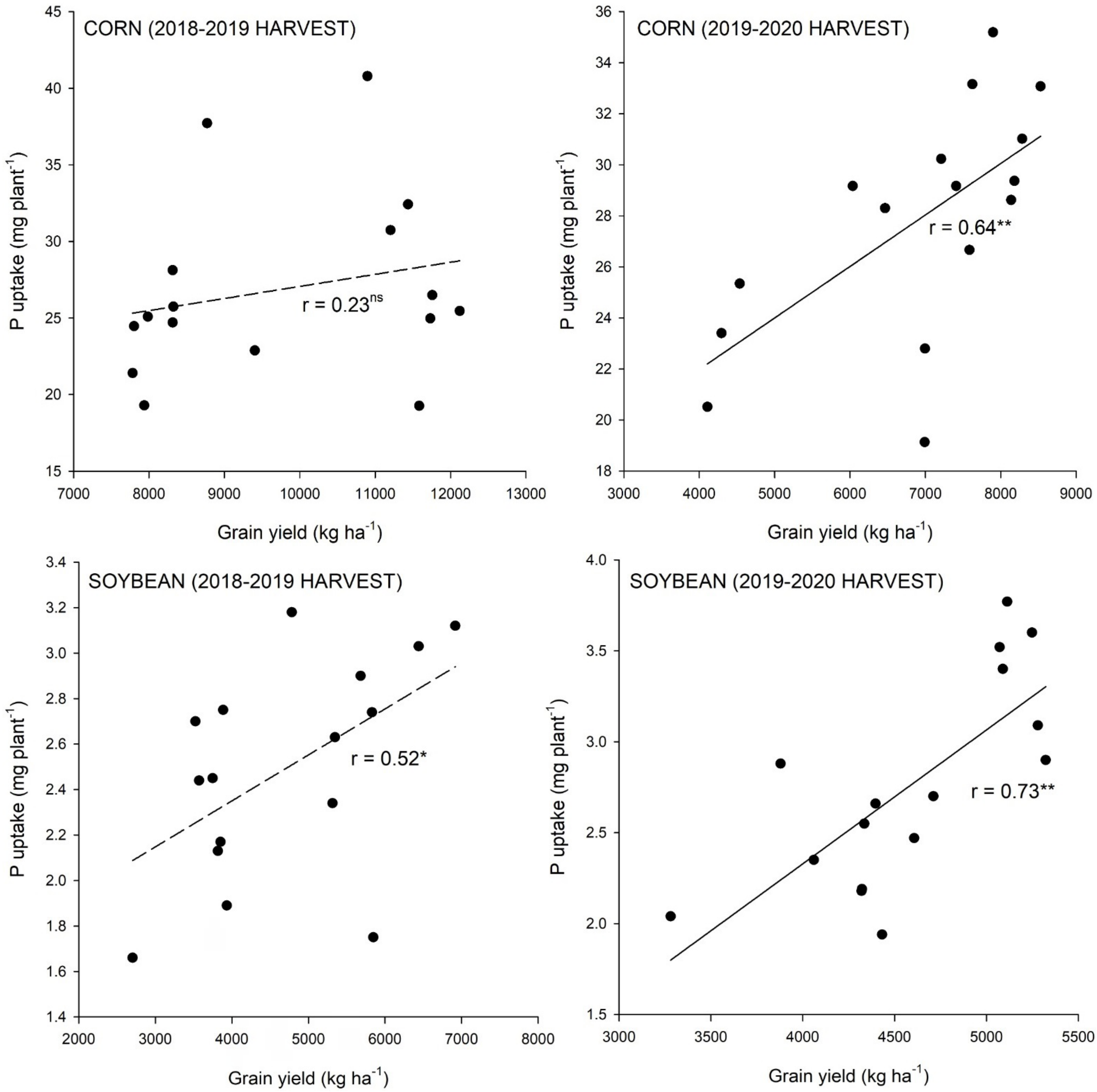


Figure 5. Pearson correlation between P uptake in corn and soybean plants at 40 DAP and grain yield in the 2018-2019 and 2019-2020 harvests.

plants. Several studies have verified the positive effects of P solubilizing bacteria (PSB) inoculation (Ribeiro et al., 2018), and a positive correlation has been found between PSB density in the rhizosphere and P uptake and grain yield. Solubilization of insoluble inorganic phosphates involves the production of low molecular weight organic acids (e.g. gluconic acid and citric acid) that act on calcium (Ca), iron (Fe), and aluminum (Al) bound forms of P in soil (Jha & Saraf, 2015). Indeed, *in vitro* studies have demonstrated the ability of *B. amyloliquefaciens* strain FZB45 to efficiently solubilize $Ca_3(PO_4)_2$, $AlPO_4$, and $FePO_4$ added individually to NBRIP culture medium (unpublished data). In this study, solubilizing capacity increments of 133, 1,534, and 3,082% compared to a commercial product containing PSB were found.

In the present study, we also evaluated the effect of Phosbac-45 inoculation on nodule dry mass (NDM) in soybean crop (Table 2). Studies have shown that the higher efficiency of rhizobia-legume symbiosis in N_2 fixation depends more on nodule size and mass than number of nodules produced (Hernández et al., 2017). In general, the increase in the Phosbac-45 dose caused average increases of 37% in NDM compared to the NI treatment, considering all harvests and planting locations. Table 2 shows no differences between the additional treatments evaluated and the Phosbac-45

Table 2. Nodule dry mass (NDM) of soybean plants 40 days after seed inoculation with Phosbac-45 (NI non-inoculated seeds; 1.0 and 2.0 mL kg^{-1} of seeds) and phosphate fertilization (0 and 50% of P) in the 2018-2019 and 2019-2020 harvests.

Inoculation treatments	Assaí-PR 2018-2019 (mg $plant^{-1}$)		Ponta Grossa-PR 2018-2019 (mg $plant^{-1}$)
	P_0	P_{50}	$\bar{x}$ P
NI	64.4 B*+	113.2 A	102.1 B*+
Phosbac-45-1	146.4 A	117.4 A	154.7 A
Phosbac-45-2	142.6 A	123 A	162.8 A
$T\text{-}P_{100}$	139.00		165.00
T-Brady	135.60		146.80
Inoculation treatments	**Assaí-PR 2019-2020 (mg $plant^{-1}$)**		**Ponta Grossa-PR 2019-2020 (mg $plant^{-1}$)**
	$\bar{x}$ P		$\bar{x}$ P
NI	129.67 B+		139.02 B+
Phosbac-45-1	144.93 AB+		158.37AB+
Phosbac-45-2	150.72 A+		173.26 A+
$T\text{-}P_{100}$	147.16		159.62
T-Brady	190.74		216.18

$T\text{-}P_{100}$ = additional treatment corresponding to 100% of P; T-Brady = additional treatment corresponding to the standard inoculation with *Bradyrhizobium*; * = significant difference between inoculation and $T\text{-}P_{100}$ treatments; + = significant difference between inoculation and T-Brady treatments. Means followed by the same letter do not differ by the Tukey test at 5% probability.

inoculation treatments in the 2018-2019 harvest, while the T-Brady treatment showed significantly higher NDM values in the 2019-2020 harvest. Adequate P supply has a direct influence on nodule initiation, growth, and function, increasing their activity, consequently stimulating symbiont growth (Mirriam et al., 2022). Thus, P supply mediated by inoculation with Phosbac-45 could act on the development of nodulation in soybean at the early stages of infection and nodule formation under conditions of low P availability.

Although the potential of plant growth promoting microorganisms is recognized worldwide, their contribution in Brazilian agriculture may be even more relevant. As an example, the registration of new biobased products has grown from three in 2011 to 106 in 2018, suggesting that this is an area with potential for expansion. Recently, the product BiomaPhos® based on *B. subtilis* (BRM 119) and *B. megaterium* (BRM 2084) was registered in Brazil. These strains have several mechanisms for promoting plant growth, but have been marketed for their recognized role in increasing P availability, more specifically, as solubilizers of P in soil (Oliveira-Paiva et al., 2020). Evaluations carried out in corn and soybean production areas, where P fertilizers were applied according to local recommendations, the application of BiomaPhos® resulted in an average yield gain of 8.9% for corn and 6.3% for soybean (Oliveira-Paiva et al., 2020). Later studies were conducted on sugarcane (SolubPhos Cana®) increasing the dosage (700 mL ha^{-1}) and reducing the application of P (50% recommended dose), enabling 20% increases in crop productivity (Cançado et al., 2021).

In the present study it was shown that the seed inoculation technology with the product Phosbac-45 provided minimum 20% gains in corn and soybean productivity. Additionally, the inoculation with Phobac-45 and the reduction of phosphate fertilization allowed for average grain yields of corn and soybeans close to those obtained in the $T\text{-}P_{100}$ treatment, demonstrating that the application of this product is a promising technology for agricultural production. This aspect is very relevant at the present moment, when the Brazilian agricultural sector high dependence on imported phosphate fertilizers has been verified, which has caused an increase in production costs (Oliveira-Paiva et al., 2020).

Based on the phosphate fertilizer recommendation of the present study, the fertilizer cost reduction in the 50% of P (P_{50}) inoculated treatments for corn and soybean is US$34.00 and US$15.00 ha^{-1}, respectively (Supersimples fertilizer price at https://www.conab.gov.br in March/2022; 1 US$ = R$ 5.11 quoted on 08/24/2022). On the other hand, considering the dosages of Phosbac-45 of 1.0 and 2.0 mL kg^{-1} of seeds, the investment required to adopt the inoculation technology in corn culture varies from US$ 2.40 to US$ 4.80 ha^{-1}, while for soybean the investment required varies from US$ 6.00 to US$ 12.00 ha^{-1} (reference value of Phosbac-45 in the market of US$ 118.00 L^{-1}). In this context, the adoption of this technology shows itself to be a sustainable alternative to guarantee productivity in areas with corn and soybean cultivation in soils with lower P supply.

Conclusion

The product Phosbac-45 based on *Bacillus amyloliquefaciens* FZB45 presents agronomic efficiency for corn and soybean crops at a dosage of 2.0 mL kg^{-1} of seeds, contributing to the production of plant biomass, grain yield and increased phosphorus absorption.

Compliance with Ethical Standards

Author contributions: Conceptualization: MLM; Data curation: MVRM; Formal analysis: CRFSS; Investigation: MVRM; Methodology: MLM; Supervision: MVRM; Visualization: CRFSS; Writing – original draft: MVRM; Writing – review & editing: MP, DSS; CRFS S, MLM.

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Literature Cited

Cançado, G.M.A.; Vasconcelos, J.C.S.; Oliveira-Paiva, C.A.; Christofoletti, D.; Severino, F.J.; Pinto Junior, A.S.; Medeiros, G.; Barbosa, L A.F.; Speranza, E.A. Antunes, J.F.G. Utilização de inoculante líquido solubilizador de fosfato formulado a base dos isolados de *Bacillus megaterium* (b119) e *Bacillus subitilis* (b2084) no plantio da cana-de-açúcar. Campinas: Embrapa Agricultura Digital, 2021. 25p. (Embrapa Agricultura Digital. Boletim de Pesquisa e Desenvolvimento, 49). http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1137862. 02 Aug. 2022.

Companhia Nacional de Abastecimento - CONAB. Acompanhamento da safra brasileira de grãos. Brasília: CONAB, 2022. 88p. (v.9 – Safra 2021/22, n.10 - Décimo levantamento). https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos/item/download/43195_4877b01240feea94340214d6c9e37afa. 13 May. 2022.

Ghosh, S.; Goswami, A.; Ghosh, G.K.; Pramanik, P. Characterization of potent phytate solubilizing bacterial strains of tea garden soils as futuristic potent bio-inoculant. International Journal of Current Microbiology and Applied Sciences, v. 10, n. 4, p. 470-484, 2021. https://doi.org/10.20546/ijcmas.2021.1004.049.

Gong, A.; Wang, G.; Sun, Y.; Song, M.; Dimuna, C.; Gao, Z.; Wang, H.; Yang, P. Dual activity of *Serratia marcescens* Pt-3 in phosphate-solubilizing and production of antifungal volatiles. BMC Microbiology, v. 22, n. 1, p. 1-11, 2022. https://doi.org/10.1186/s12866-021-02434-5.

González, A.H.; Morales Londoño, D.; Pille da Silva, E.; Nascimento, F.X.I.; Souza, L.F.; Silva, B.G.; Soares, C.R.F.S. *Bradyrhizobium* and *Pseudomonas* strains obtained from coal-mining areas nodulate and promote the growth of *Calopogonium muconoides* plants used in the reclamation of degraded areas. Journal of Applied Microbiology, v.126, n.2, p.523-533, 2019. https://doi.org/10.1111/jam.14117.

Grant, A; Gay, C.G.; Lillehoj, H.S. *Bacillus* spp. as direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry. Avian Pathology, v. 47, n. 4, p. 339-351, 2018. https://doi.org/10.1080/03079457.2018.1464117.

Guang-Can, T.A.O.; Shu-Jun, T.I. A.N.; Miao-Ying, C. A. I.; Guang-Hui, X.I.E. Phosphate-solubilizing and-mineralizing abilities of bacteria isolated from soils. Pedosphere, v. 18, n. 4, p. 515-523, 2008. https://doi.org/10.1016/S1002-0160(08)60042-9.

Hernández, A.G.; de Moura, G.D.; Binati, R.L.; Nascimento, F. X. I.; Londoño, D.M.; Mamede, A.C.P., Soares, C.R.F.S. Selection and characterization of coal mine autochthonous rhizobia for the inoculation of herbaceous legumes. Archives of microbiology, v.199, n.7, p.991-1001, 2017. https://doi.org/10.1007/s00203-017-1373-2.

Idriss, E.E.; Makarewicz, O.; Farouk, A.; Rosner, K.; Greiner, R.; Bochow, H.; Richter, T.; Borriss, R. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiology, v. 148, n. 7, p. 2097-2109, 2002. https://doi.org/10.1099/00221287-148-7-2097.

Jha, C.K.; Saraf, M. Plant growth promoting rhizobacteria (PGPR): a review. Journal of Agricultural Research and Development, v.5, n.2, p.108-119, 2015. https://www.e3journals.org/cms/articles/1434021343_Chaitanya%20and%20Meenu.pdf. 02 Aug. 2022.

Krebs, B.; Höding, B.; Kübart, S.; Workie, M.A.; Junge, H.; Schmiedeknecht, G.; Grosch, R.; Bochow, H.; Hevesi, M. Use of *Bacillus subtilis* as biocontrol agent. I. Activities and characterization of *Bacillus subtilis* strains. Journal of Plant Diseases and Protection, v.1o5, n.2, p. 181-197, 1998. https://www.jstor.org/stable/43215232. 28 Jul. 2022.

Malavolta, E.; Vitti, G.C.; Oliveira, S.A. Avaliação do estado nutricional das plantas: princípios e aplicações. Piracicaba: POTAFOS, 1989. 201p.

Mehnaz, S. An overview of globally available bioformulations. In: In: Arora, N.; Mehnaz, S.; Balestrini, R. (Eds.) Bioformulations: for sustainable agriculture. New Delhi: Springer, 2016. p. 267-281. https://doi.org/10.1007/978-81-322-2779-3_15.

Mirriam, A.; Mugwe, J.; Raza, M.A.; Seleiman, M.F., Maitra, S.; Gitari, H.H. Aggrandizing soybean yield, phosphorus use efficiency and economic returns under phosphatic fertilizer application and inoculation with *Bradyrhizobium*. Journal of Soil Science and Plant Nutrition, v.22, n.4, p.5086-5098, 2022. https://doi.org/10.1007/s42729-022-00985-8.

Nascimento, F.X.; Hernandez, A.G.; Glick, B.R.; Rossi, M.J. The extreme plant-growth-promoting properties of *Pantoea phytobeneficialis* MSR2 revealed by functional and genomic analysis. Environmental Microbiology, v.22, n.4, p. 1341-1355, 2020a. https://doi.org/10.1111/1462-2920.14946.

Nascimento, F.X.; Hernández, A.G.; Glick, B.R.; Rossi, M.J. Plant growth-promoting activities and genomic analysis of the stress-resistant *Bacillus megaterium* STB1, a bacterium of agricultural and biotechnological interest. Biotechnology Reports, v.25, e00406., 2020b. https://doi.org/10.1016/j.btre.2019.e00406.

Novo, L.A.B.; Castro, P.M.L.; Alvarenga, P.; Silva, E.F. Plant growth–promoting rhizobacteria-assisted phytoremediation of mine soils. In: Prasad, M. N. V.; Favas, P. J. C.; Maiti, S. K. (Eds.). Bio-geotechnologies for mine site rehabilitation. Oxford: Elsevier, 2018. p. 281–295. https://doi.org/10.1016/B978-0-12-812986-9.00016-6.

Oliveira-Paiva, C. A.; Cota, L. V.; Marriel, I. E.; Gomes, E. A.; Sousa, S. M.; Lana, U. G. de P.; Santos, F. C.; Pinto Junior, A. S.; Alves, V. M. C. Viabilidade técnica e econômica do Biomaphos® (*Bacillus subtilis* CNPMS B2084 e *Bacillus megaterium* CNPMS B119) nas culturas de milho e soja. Sete Lagoas: Embrapa Milho e Sorgo, 2020. 20p. (Embrapa Milho e Sorgo. Boletim de Pesquisa e Desenvolvimento, 210). https://ainfo.cnptia.embrapa.br/digital/bitstream/item/217542/1/Bol-210.pdf. 22 Jun. 2022.

Pertot, I.; Alabouvette, C.; Esteve, E.H.; Franca, S. The use of microbial biocontrol agents against soil-borne diseases. Brussels: European Commission; The Agricultural European Innovation Partnership - EIP-AGRI, 2015. 11p. https://ec.europa.eu/eip/agriculture/sites/default/files/8_eip_sbd_mp_biocontrol_final.pdf. 31 May 2022

Quintas-Nunes, F.; Rossi, M..; Nascimento, F.X. Genomic insights into the plant-associated lifestyle of *Kosakonia radicincitans* MUSA4, a diazotrophic plant-growth-promoting bacterium. Systematic and Applied Microbiology, v.45, n.2, e126303, 2022. https://doi.org/10.1016/j.syapm.2022.126303.

R Development Core Team. R: A language and environment for statistical computing. Vienna: R Development Core Team, 2009.

Ramírez, C.A.; Kloepper, J.W. Plant growth promotion by *Bacillus amyloliquefaciens* FZB45 depends on inoculum rate and P-related soil properties. Biology and Fertility of Soils, v. 46, n. 8, p. 835-844, 2015. https://doi.org/10.1007/s00374-010-0488-2.

Ribeiro, V.P.; Marriel, I.E., Sousa, S.M.D.; Lana, U.G.D.P.; Mattos, B.B.; Oliveira, C.A.D.; Gomes, E.A. Endophytic *Bacillus* strains enhance pearl millet growth and nutrient uptake under low-P. Brazilian Journal of Microbiology, v.49, suppl. 1, p.40-46, 2018. https://doi.org/10.1016/j.bjm.2018.06.005.

Saath, K.C.D.O.; Fachinello, A.L. Crescimento da demanda mundial de alimentos e restrições do fator terra no Brasil. Revista de Economia e Sociologia Rural, v.56, n.2, p.195-212, 2018. https://doi.org/10.1590/1234-56781806-94790560201.

Tiecher, T.; Gomes, M.V; Ambrosini, V.G.; Amorim, M.B.; Bayer, C. Assessing linkage between soil phosphorus forms in contrasting tillage systems by path analysis. Soil and Tillage Research, v.175, p.276-280, 2018. https://doi.org/10.1016/j.still.2017.09.015.