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Soybean inoculated with phosphorus-solubilizing bacteria and co-inoculated with arbuscular mycorrhizal fungi in clay and sandy soils

Oriel Tiago Kölln[(1)*], Hebert Teixeira Cândido[(1)], Marcos Yassuhiro Inoue[(1)], Carlos Botelho Pereira Osipi[(1)], Luis Eduardo Azevedo Marques Lescano[(1)] and Arthur Michalowski Filho[(1)]

[(1)] Universidade Estadual do Norte do Paraná, Departamento de Agronomia, Programa de Pós-Graduação em Agronomia, Bandeirantes, Paraná, Brasil.

*** Corresponding author:**
E-mail: oriel.kolln@uenp.edu.br

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ABSTRACT: Although Brazilian soils contain considerable reserves of total P, accumulated by constant phosphorus (P) application, most of it is preserved in non-labile and moderately labile fractions, which could not be efficiently exploited so far. This study aimed to evaluate microorganisms capable of accessing less available P fractions in two soils with different textures. A greenhouse experiment in a completely randomized design, in a factorial arrangement (2 × 5) with four replications, was carried out in the municipality of Bandeirantes, Paraná. The regional climate was classified as humid subtropical (Cfa) and the average temperature during the experimental cycle was 25.6 °C (max. 40 °C, min. 15 °C). Water was added to maintain humidity at around 60 % of the maximum water retention capacity (MWRC). The first factor consisted of two different-textured soils (sandy and clayey), taken from areas with little human interference. The second factor comprised the soybean treatments: T1 – control (no inoculation nor P fertilization); T2 – mineral fertilization (triple superphosphate); T3 – seeds inoculation with *Bacillus* strains; T4 – inoculation with arbuscular mycorrhizal fungi (AMF); T5 – co-inoculation with *Bacillus* and AMF strains. Response variables related to the agronomic performance of soybean were analyzed (shoot and root dry weight; number of pods per plant; nodulation; P content and P accumulation in shoot and roots), as well as variables related directly with microbial performance (degree of AMF colonization and soil available P content (Mehlich-1), an indicator of the P desorption dynamics of the microorganisms). In the sandy soil, AMF proved more effective in raising levels of available P (Mehlich-1) than the control. However, under the experimental conditions, the microorganisms did not significantly increase P availability in clayey soils. Nevertheless, AMF inoculation proved to be a promising possibility of improving P availability in soybean. Regardless of the soil texture studied — sandy or clayey —, the results of inoculation or co-inoculation were equivalent or superior to those of triple superphosphate fertilization and consistently better than those of the control, for most response variables.

Keywords: microorganisms, greenhouse, clayey soil, sandy soil.

INTRODUCTION

Due to the strong interaction of phosphorus with clay minerals, mainly with kaolinite, iron and aluminum oxides and hydroxides, the efficiency of phosphate fertilization is significantly impaired. The reason is that, to a greater or lesser extent, soil and plants compete for the applied phosphorus, and the soil-to-plant drain is frequently much stronger than the plant-to-soil drain (Novais and Smith, 1999; Rheinheimer et al., 2008).

In soils with a high P sink potential, adsorption can exceed 4,000 kg ha^{-1} (Rogeri et al., 2016), whereas annual crops, e.g., soybean, extract approximately 25 kg ha^{-1} of P for their entire biomass (Pauletti and Motta, 2019). The sink potential and binding energy of phosphorus to the adsorption sites increase proportionally with the soil clay content (Oliveira et al., 2014). On the other hand, in soils with less than 20 % clay, the P sink capacity is significantly lower (Mumbach et al., 2021).

Although the constant addition of phosphate fertilizers has left considerable total P reserves, most of the nutrient is preserved in non-labile and moderately labile fractions, which have not yet become effectively exploitable (Rodrigues et al., 2015; Withers et al., 2018). However, P fixation declines as the most avid P adsorption sites become saturated, leading to decreased sink capacity and increased P use efficiency index (Barrow and Debnath, 2014). This suggests that a reduction of P inputs in already consolidated cultivation areas might be possible (Cunha et al., 2014).

Management systems can alter the dynamics of phosphorus in the soil, particularly those that promote a greater input of organic matter into the soil, which increases P availability (Pereira et al., 2010; Bezerra et al., 2015). This can occur through mineralization of soil organic matter (SOM) (Silva and Mendonça, 2007) or the adsorption capacity of organic acids of the soil, which causes competition with specific P adsorption sites (Haynes, 1984), thus decreasing P retention capacity (Barbieri et al., 2014).

In addition to mineral fertilization and SOM mineralization, microorganism-mediated solubilization can be a viable alternative to increase P availability. Soil biota harbors a diversity of microorganisms, called phosphorus solubilizers, capable of releasing P through mineralization and solubilization (Alori et al., 2017). Arbuscular mycorrhizal fungi not only effectively increase the volume of soil explored, which allows a greater uptake of water and nutrients, but are also capable of accessing readily available and less labile P fractions (Sanchez, 2022).

The search for management methods and combinations by which less labile P forms can be exploited, P use efficiency can be improved, and the dependence on P from mineral fertilizers could be reduced is key to address the current economic and environmental challenges. Among these practices, inoculation with microorganisms, such as *Bacillus* sp., has proven to be a promising tool, enabling increases or maintenance of productivity in an environmentally sustainable manner (Li et al., 2022). Similarly, the association with arbuscular mycorrhizal fungi can, in certain situations, provide up to 100 % of the P absorbed by plants (Smith et al., 2003), reinforcing the importance of this symbiosis in plant nutrition, especially in soils with low P content. This characteristic is fundamental for the sustainability of agricultural systems, due to the possibility of reducing the dependence on chemical fertilizers.

The significance of this study is directly related to the relevance of soybean for the Brazilian Gross Domestic Product (GDP) and agribusiness (Montoya et al., 2019; Andrade Neto and Raiher, 2024). In addition, it sheds light on the problem of phosphorus in tropical soils (Novais and Smith, 1999; Martinez et al., 2021; Sanchez, 2022), which has led to a dependence of the Brazilian agriculture on external markets, to a large extent, to meet the high demand for phosphate fertilizers (Sasabuchi et al., 2023; Brasil, 2024). In this context, the proposed approach may represent an important solution to increase the economic profitability of agricultural activity, while reducing dependence on mineral fertilizers (Leite et al., 2022).

We hypothesized that inoculation and co-inoculation with beneficial microorganisms can increase the P desorption capacity and P recovery by soybean plants, adding increments to the response variables related to agronomic performance. Although phosphorus retention is greater in clayey soils due to the interaction with the mineral fraction, evaluating sandy soils is also relevant, considering their lower P adsorption capacity and the potential to make the element available more efficiently to plants. This study aimed to evaluate the performance of *Bacillus* sp. strains and arbuscular mycorrhizal fungi compared with soybean phosphate fertilizer, to test the P desorption and recovery capacity in soils with two different textures.

MATERIALS AND METHODS

Description of the experiment: location, design and duration

The experiment was carried out in a greenhouse of the Universidade Estadual do Norte do Paraná, Campus Luiz Meneghel, in the municipality of Bandeirantes, PR, (23° 06′ S and 50° 21′ W), and 420 m above sea level. The predominant climate in the region is humid subtropical (Cfa), according to the Köppen climate classification system. The experiment lasted from soybean sowing (63IX65R IBERÁ seeds), on 11/07/2022, until the cutting of the plant material on 03/07/2023, for final evaluations.

Before the installation of the experiment, the soil material was exposed to solarization by being left in the sun between layers of plastic tarpaulin for seven days, to reduce or eliminate the native microbial population. Thereafter, preliminary chemical and physical soil analyses were performed (Table 1). Initial phosphorus (P) levels were classified as "high" for the clayey and "low" for the sandy soil, according to the Pauletti and Motta classification (2019).

The trial was arranged in a completely randomized design, in a factorial scheme (2 × 5), with four replications and two plants per pot. The first factor ("soil") consisted of the use of two different textured soils: a clayey and a sandy soil, both taken from the surface layer (0.00-0.20 m) of areas with low anthropogenic interference. Clayey soil was collected in the municipality of Bandeirantes/PR, while the sandy soil was taken in the municipality of Assis/SP.

The second factor, called "inoculation", consisted of five different levels. The first level, called "control", did not include inoculation of microorganisms or addition of phosphorus. The second level consisted of phosphorus application, in the form of triple superphosphate (TSP), at a rate of 30 mg kg^{-1} of P. The third level consisted of inoculation with two strains of phosphorus-solubilizing bacteria (PSB): *Bacillus subtilis* (CNPMS B2084, BRM034840) and *Bacillus megaterium* (CNPMS B119, BRM033112), with a solution containing 4 × 10^{9} CFU mL^{-1} (viable cells). The fourth level consisted of the inoculation with arbuscular mycorrhizal fungi (AMF) propagules. Finally, the last level was a combined inoculation of PSB and AMF propagules.

Table 1. Characterization of the soils used in the experiment

Solos	pH ($CaCl_2$)	P[(1)]	K[(1)]	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	CEC[(2)]	BS[(3)]	Clay	Silt	Sand	O.M[(4)]
		mg dm^{-3}				cmol$_c$ dm^{-3}				%		g kg^{-1}		g kg^{-1}
Clayey	5.2	11.1	0.12	5.7	0.8	0.0	6.07	6.62	12.69	52.2	720	100	180	21.1
Sandy	4.9	9.3	0.07	4.2	0.8	0.0	5.3	5.07	10.3	49.2	140	110	750	12.8

Characterization based on the methodology described by Raij et al. (2001). [(1)] P and K extracted by Mehlich-1 (Teixeira et al., 2017). [(2)] Cation exchange capacity. [(3)] Base saturation. [(4)] Organic matter.

For PSB inoculation, the seeds were treated with a solution containing the bacterial strains at a rate of 2 mL of solution per kg of seeds. The AMF propagules, previously cultivated for four months in pots with *Urochloa decumbens*, were applied at a rate of 100 mL of soil, containing approximately 100 spores, placed below the soybean seeds in the respective treatments. The soil used for multiplication of the AMF propagules was collected in an area of organic cultivation on the campus, which contained approximately 100 spores per 100 mL of soil, after four months.

Experimental setup and procedure

Each experimental unit consisted of one 5-L pot that contained 3.5 kg sifted soil. Pots were lined internally with a transparent plastic bag to prevent water and nutrient losses by leaching during irrigation. Soybean was sown on 11/07/2022, and the seedlings emerged on 11/13/2022.

The amount of water added to each pot was defined according to the maximum water retention capacity (MWRC) of each soil. Until the first pair of true leaves (V1) was developed, 50 % of the MWRC was applied, and thereafter 60 %.

The MWRC was determined by placing a sieved soil sample in a volumetric ring (diameter 5 cm, height 5 cm). The bottom of the cylinder was sealed with filter paper and placed on a water-filled container. After soil saturation by capillarity, the excess water was drained by gravity for 2 h. Then, the weight of the moist soil was measured and, subsequently, the soil was heated in an oven to 105 °C, for 48 h, to dry completely. The MWRC was determined by the difference between the weight of the saturated and that of the dry soil, divided by the weight of the dry soil.

Water rates were calibrated indirectly (by touch), based on a dry soil sample as a reference. Water quantities were measured to correspond to 50 and 60 % of the MWRC of each soil type.

Evaluations

Plant height was measured 18, 32 and 115 days after emergence (DAE). Final height (115 DAE) was used as a parameter for comparing the performances of the treatments.

To evaluate mycorrhizal colonization (in the control treatments, isolated AMF inoculation and AMF and PSB co-inoculation), a root sample was taken from one plant per treatment at the beginning of the R1 stage. The percentage of mycorrhizal colonization was determined by staining the AMF structures on the roots, according to the methodology described by Phillips and Hayman (1970). The process included bleaching with 10 % KOH, acidification with 1 % HCl, and staining of the fungal structures with 0.05 % trypan blue. The percentage of mycorrhizal colonization was determined by the quadrant intersection method (McGonigle et al., 1990) in 100 root fragments, where the presence of coenocytic hyphae, arbuscules, and vesicles in the cortex was analyzed under 100 × magnification.

At the end of the test, at 115 DAE, the plant material was harvested for the final evaluations of dry weight, P content, and P accumulation in shoot and roots, number of pods per plant, number of nodules, and soil P content (Mehlich-1).

Statistical analysis

Statistical analyses were performed using RStudio® software, version 4.4.1. The normality test of residues (Shapiro-Wilk) and the homoscedasticity test of variances (Levene) were applied at the 5 % significance level. Once the criteria for parametric tests were met, the data were subjected to analysis of variance using the F test and, when significant, to the multiple comparison test of means (Tukey, $\alpha = 5$ %). Correlation analysis was performed using Pearson coefficient to assess the degree of association between the parameters, at a significance level of 5 % ($\alpha = 0.05$). No data transformation was required. The graphs were constructed using SigmaPlot® software, version 12.5.

RESULTS

The percentage of colonized roots was influenced only by the "inoculation" factor (Table 2). Co-inoculation with PSB and AMF did not significantly alter the percentage of colonization compared with only AMF inoculation, indicating an absence of suppression of colonization by PSB. In the control treatment, as expected, the percentage of colonization was lower (63 %) than in response to only AMF inoculation (94 %) and to PSB and AMF co-inoculation (96 %) (Figure 1).

Plant growth rates in sandy soil increased constantly, without the sharp breaking point observed in clay soil (Figures 2a and 2b). The effect of the interaction between the factors "inoculation" and "soil" was significant for plant height (Table 2). In clay soil, AMF and PSB co-inoculation showed superior performance ($p<0.05$) to plants inoculated only with PSB, increasing to 0.09 m. In sandy soil, plants inoculated with AMF (0.75 m) grew to significantly taller heights ($p<0.05$) than those treated with STP (0.60 m) and the control (0.54 m) (Figure 2c).

Table 2. Descriptive statistics, p-values, and variation coefficient obtained from the analysis of variance (ANOVA), of biometric variables, P content and accumulation in soil and plants tissues as a function of inoculation phosphorus-solubilizing bacteria and co-inoculation with arbuscular mycorrhizal fungi

Variable	CV	Inoculation (I)	Soil (S)	I × S
	%			
Plant height (cm)	10.8	0.0083	0.0002	0.0132
Stem diameter (mm)	12.3	0.3103	0.1192	0.2985
Pods (number plant^{-1})	9.48	0.3700	<0.0001	0.0586
Shoot dry mass (g plant^{-1})	8.28	0.5183	0.0548	0.0881
Root dry mass (g plant^{-1})	20.8	0.1234	<0.0001	0.8665
Nodulation (number plant^{-1})	22.3	0.0002	<0.0001	0.0064
Shoot P content (g kg^{-1})	22.1	0.0059	0.0016	0.9089
Shoot P accumulation (mg plant^{-1})	13.8	0.0047	0.2090	0.5678
Root P content (g kg^{-1})	16.8	0.0187	0.0001	0.8977
Root P accumulation (mg plant^{-1})	26,1	0.0586	<0.0001	0.9921
Soil P content (mg dm^{-3})	11.3	<0.0001	<0.0001	0.0001

CV: coefficient of variation.

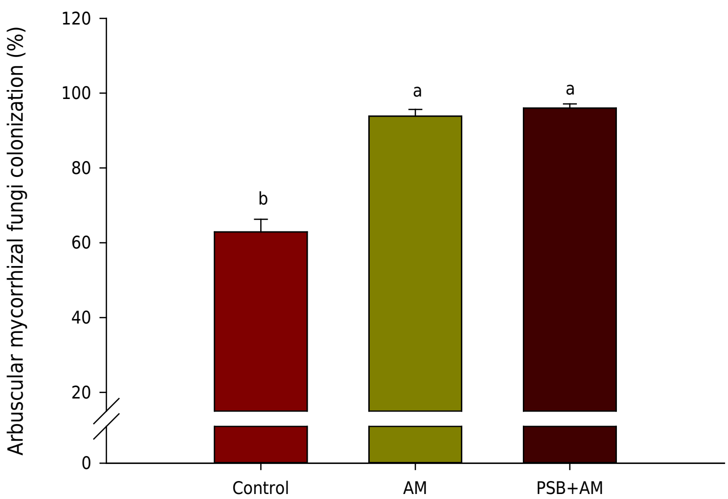

Figure 1. Percentage of soybean root colonization by arbuscular mycorrhizal fungi (AMF) under different inoculation treatments: single inoculation with AMF (AM) and co-inoculation with phosphorus-solubilizing bacteria (PSB + AM). Different letters above the standard error bars indicate means that differ significantly from each other according to Tukey test ($p\leq0.05$).

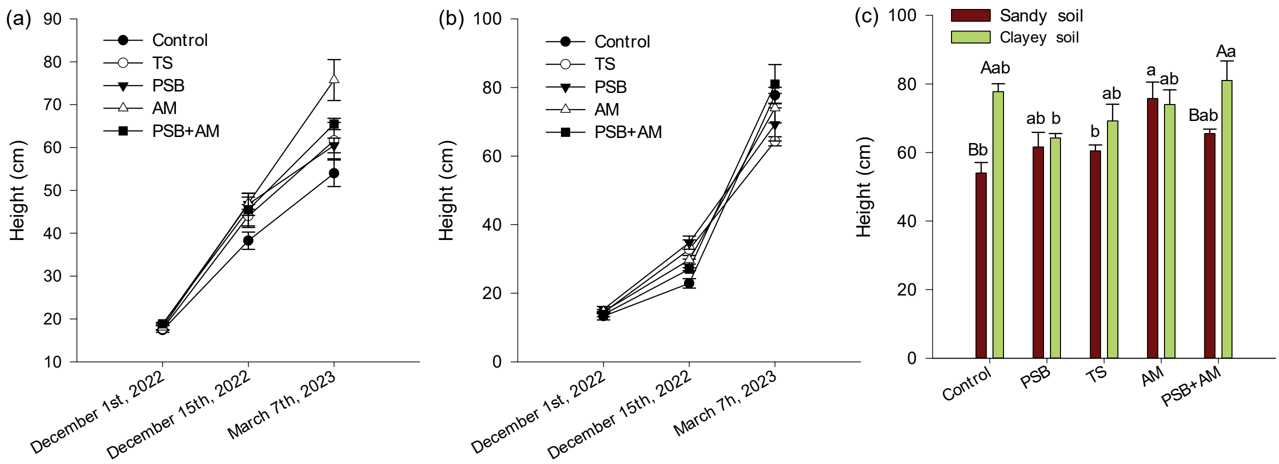

Figure 2. Mean and standard error of plant height over time in sandy soil (a), mean and standard error of plant height over time in clayey soil (b), and the effect of inoculation and soil texture on plant height at the end of the experiment (c). Different lowercase letters above the standard error bars within the same soil type, and different uppercase letters within the same inoculation treatment, indicate means that differ significantly according to Tukey test (p≤0.05).

The effect of the interaction between the factors was significant for the variable nodulation (Table 2). In sandy soil, the experimental units subjected to co-inoculation with AMF and PSB (21.2 nodules plant^{-1}) or isolated AMF inoculation (16.3 nodules plant^{-1}) produced a lower number of nodules per plant ($p<0.05$) compared with the control (41 nodules plant^{-1}), PSB (41.3 nodules plant^{-1}) and STP (53.7 nodules plant^{-1}) treatments (Figure 3a). The effects of the "inoculation" factor on nodulation were not significant ($p>0.05$) in clayey soil. Nodulation correlated positively with soil phosphorus concentration (0.496; p = 0.001), with the shoot (0.331; p = 0.037) and root levels (0.481; p = 0.002), and with root dry weight (0.555; p = 0.000) (Figure 4).

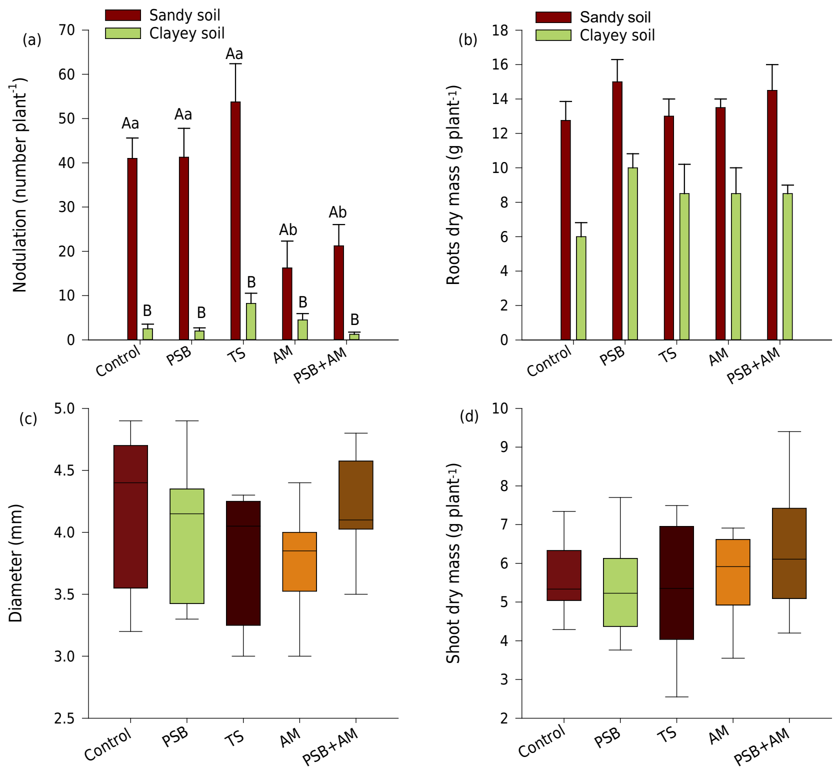

Figure 3. Effects of inoculation and soil texture on nodulation (a), distribution of means and standard errors of root dry mass under different inoculation treatments and soil textures (b), and distribution of stem diameter (c) and shoot dry mass (d). Different lowercase letters above the standard error bars within the same soil type, and different uppercase letters within the same inoculation treatment, indicate means that differ significantly according to Tukey test (p≤0.05).

Variables	Nodulation	Pods	Height	Diameter	Shoot dry mass	Shoot P content	Shoot P accumulation	Roots dry mass	Roots P content	Roots P accumulation	Grain mass pot-1	Soil phosphorous
Nodulation	**1**	**-0.631**	**-0.608**	0.107	**-0.406**	**0.331**	0.001	**0.555**	**0.481**	**0.615**	**-0.604**	**0.496**
Pods	**-0.631**	**1**	**0.637**	-0.041	**0.554**	-0.309	0.080	**-0.653**	-0.234	**-0.565**	**0.566**	**-0.316**
Height	**-0.608**	**0.637**	**1**	-0.004	**0.415**	-0.043	0.221	**-0.389**	-0.026	-0.283	**0.454**	-0.162
Diameter	0.107	-0.041	-0.004	**1**	0.193	0.107	0.185	0.230	-0.079	0.133	-0.072	-0.110
Shoot dry mass	**-0.406**	**0.554**	**0.415**	0.193	**1**	-0.033	**0.572**	-0.177	-0.212	-0.202	**0.431**	-0.245
Shoot P content	**0.331**	-0.309	-0.043	0.107	-0.033	**1**	**0.789**	**0.477**	**0.432**	**0.550**	-0.031	**0.426**
Shoot P accumulation	0.001	0.080	0.221	0.185	**0.572**	**0.789**	**1**	0.264	0.225	**0.317**	0.227	0.161
Roots dry mass	**0.555**	**-0.653**	**-0.389**	0.230	-0.177	**0.477**	0.264	**1**	**0.407**	**0.897**	**-0.364**	**0.359**
Roots P content	**0.481**	-0.234	-0.026	-0.079	-0.212	**0.432**	0.225	**0.407**	**1**	**0.752**	-0.156	**0.599**
Roots P accumulation	**0.615**	**-0.565**	-0.283	0.133	-0.202	**0.550**	**0.317**	**0.897**	**0.752**	**1**	**-0.325**	**0.548**
Grain mass pot-1	**-0.604**	**0.566**	**0.454**	-0.072	**0.431**	-0.031	0.227	**-0.364**	-0.156	**-0.325**	**1**	-0.206
Soil phosphorous	**0.496**	**-0.316**	-0.162	-0.110	-0.245	**0.426**	0.161	**0.359**	**0.599**	**0.548**	-0.206	**1**

Figure 4. Pearson correlation matrix of the variables analyzed as a function of inoculation and co-inoculation of microorganisms in soybean cultivation. Values in bold are different form 0 with a significance level alpha=0.055.

Root dry matter and number of pods were significantly influenced only by soil texture (Table 2). Plants grown in sandy soil had higher root dry matter content (13.8 g plant^{-1}) than those grown in clayey soil (8.30 plant^{-1}) (Figure 3b). In contrast, plants grown in clayey soil produced a significantly higher number of pods (20.6 pods plant^{-1}) than those grown in sandy soil (13.1 pods plant^{-1}) (Figure 5). The evaluated factors did not influence the stem diameter and shoot dry matter (Table 2; Figures 3c and 3d). Number of pods per plant was positively correlated with height (0.637; $p = 0.000$) and shoot dry weight (0.554; $p = 0.000$), but negatively with root dry weight (-0.653; $p = 0.000$) and soil phosphorus concentration (-0.316; $p = 0.047$) (Figure 4).

The main effects of the studied factors on P content (g kg^{-1}) and shoot P accumulation (g plant^{-1}) were analyzed separately (Table 2). Inoculation with AMF (3.12 g kg^{-1}) and co-inoculation with PSB and AMF (3.45 g kg^{-1}) resulted in higher shoot P contents compared with the control (2.16 g kg^{-1}) (Figure 6a). Regarding soil texture, plants grown in sandy soil (3.28 g kg^{-1}) had higher P contents than those grown in clayey soil (2.58 g kg^{-1}) (Figure 6b). The levels of shoot P accumulation increased more by co-inoculation with PSB and AMF (21.4 and 11.3 g plant^{-1}, respectively) than in the control treatment (Figure 6c).

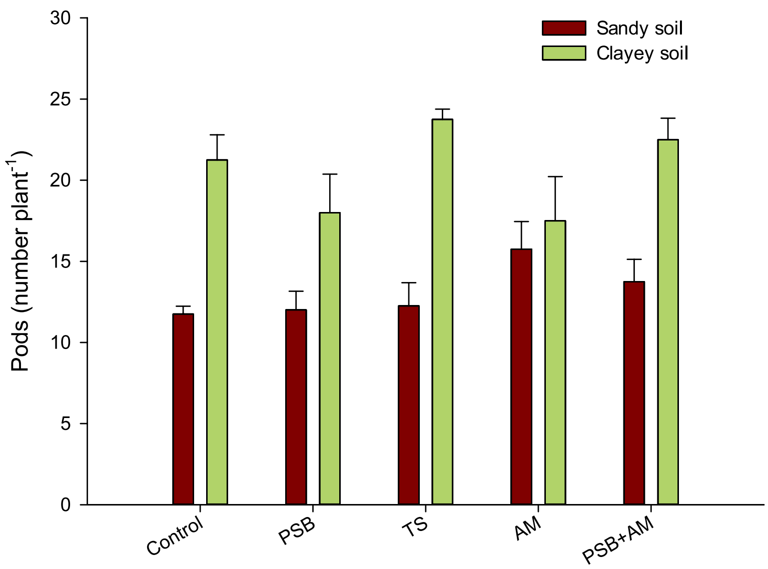

Figure 5. Distribution of means and standard errors of the number of pods under different inoculation treatments and soil textures.

The main effects of the factors on the P content (g kg^{-1}) and P accumulated in roots (g plant^{-1}) were analyzed (Table 2). Inoculation with AMF (1.75 g kg^{-1}) induced a significant increase (0.42 g kg^{-1}) in root P content over the control plants (1.33 g kg^{-1}) (Figure 7a). In soybean plants grown in sandy soil, the root P content was higher (1.72 g kg^{-1}) than in plants grown in clay soil (1.36 g kg^{-1}) (Figure 7b). The "inoculation" factor did not significantly affect root P accumulation. In plants grown in sandy soil, cumulative P was higher (23.7 g plant^{-1}) than in those grown in clay soil (11.2 g plant^{-1}) (Figure 7c).

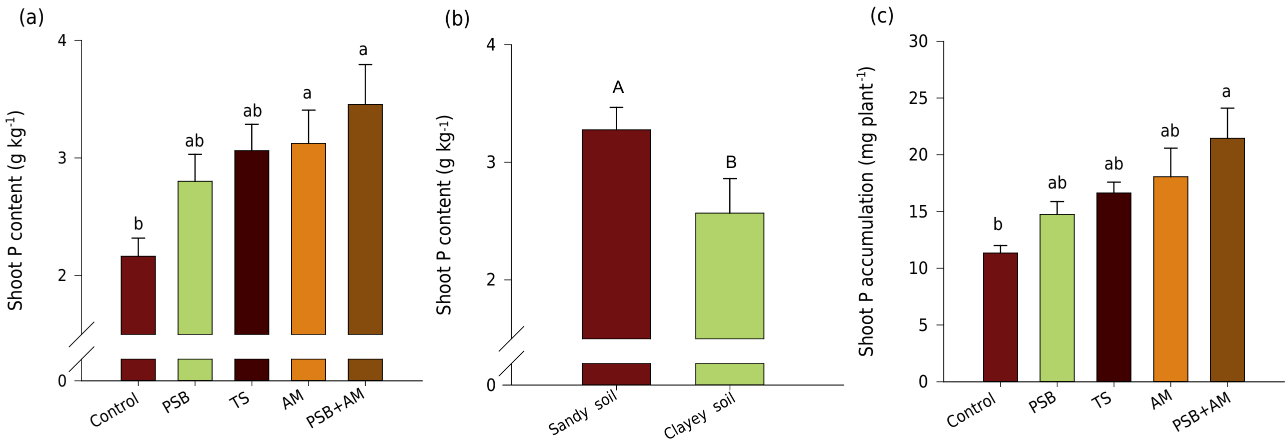

Figure 6. Phosphorus content in the shoot under different inoculation treatments (a) and soil textures (b), and the effect of inoculation on phosphorus accumulation in the shoot (c).

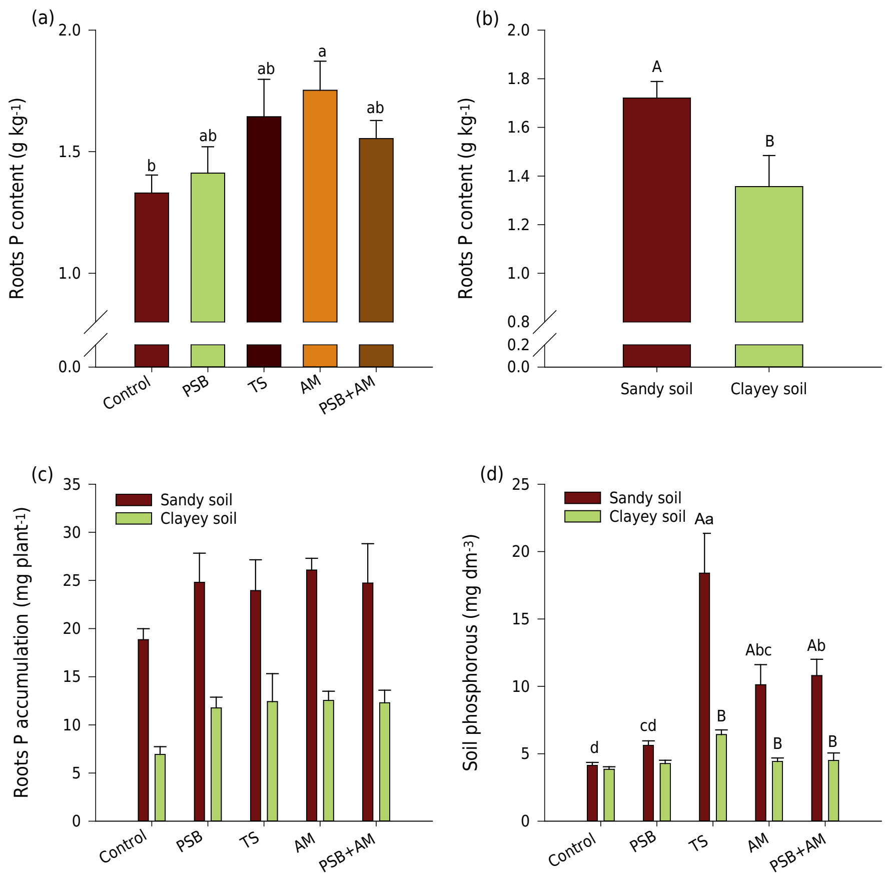

Figura 7. Phosphorus content in roots under different inoculation treatments (a) and soil textures (b); distribution of means and standard errors of phosphorus accumulation in roots under inoculation treatments and soil textures (c); and the effect of inoculation and soil texture on soil phosphorus concentration (d).

The interaction effects between the factors were significant for residual soil P content (mg dm^3) (Table 2). In the clay soil, residual P contents did not shift significantly. In the sandy soil, fertilization with STP (18.4 mg dm^{-3}) promoted a significant increase in residual P content compared with the control (4.13 mg dm^{-3}), PSB (5.65 mg dm^{-3}), AMF (10.1 mg dm^{-3}), and AMF+PSB inoculation (10.8 mg dm^{-3}). Significant positive effects on P desorption potential were observed in the sandy soil units inoculated with AMF alone or associated with PSB, in relation to the control (Figure 7d). These results fortified the thesis that in sandy soils, the P sink potential decreases, and efficiency in P desorption increases in response to AMF inoculation. Soil P concentration correlated positively with soybean shoot P content (0.426; $p = 0.006$), dry weight (0.359; $p = 0.023$) and with root content (0.599; $p = 0.000$) (Figure 6).

DISCUSSION

The higher soil P levels were reflected in higher P concentrations in the shoot and roots, and in higher root dry weight. Among numerous other primary metabolism functions, phosphorus participates in carbohydrate synthesis and transport. In addition, it is used as an energy source by membrane ATP-ases for active nutrient uptake (Fagan et al., 2016).

Although inoculation did not influence the number of pods, which is an important indicator of the yield potential, a positive correlation was observed with the variables shoot height and dry weight, and a negative correlation with soil P concentration. The positive correlation can be explained by the fact that larger plants, with greater leaf area and greater photosynthetic capacity, have more resources to sustain pod and grain formation, i.e., the main photoassimilate sink after the beginning of the reproductive phase (Fávero and Lana, 2014; Bianco et al., 2012; Durli et al., 2020).

On the other hand, the association between the increase in the number of pods and the slight decrease in soil P content may be related, to some extent, to the ability of the plants to manage resources under conditions of P stress. Symbioses between nitrogen (N)-fixing bacteria and legumes are influenced by the P concentration in plant tissues (O'Hara, 2001). In environments with low P availability, plants can enhance the symbiosis by optimizing N fixation, by the expression of genes related to the self-regulation of nodulation, to limit the number of nodules (Isidra-Arellano et al., 2018, 2020). This mechanism can help the plant concentrate the available P towards a reduced number of more efficient nodules (Paries and Gutjahr, 2023). Lower P concentrations in plant tissues and a reduced number of nodules in plants grown in clayey soil suggest a compensatory capacity in the nodulation process, as an adaptation strategy to conditions of lower P availability, which may partially explain the better performance in pod production (Figures 5b, 6b, and 7b).

Another complementary hypothesis, in addition to greater SOM preservation in clayey soils, is associated with another important aspect of the nutritional dynamics of plants: the management of other essential nutrients, e.g., potassium (K). Soils with a high K buffering capacity (KBP) ensure constant K replenishment in the soil solution at adequate concentrations for a prolonged period (Mielniczuk, 1982; Ernani et al., 2007), as is generally the case in clayey soils. In sandy soils, however, KBP is naturally lower, which may lead to faster fluctuations in available K levels. Therefore, despite the lower P recovery by plants, the greater stability of the clay soil may have contributed to the higher pod production in response to the standardized fertilization management in both soil types. Furthermore, the sandy soil had a slightly lower pH than the clay soil, which may have negatively influenced pod production (Table 1).

Although the initial P availability class (Mehlich-1) in the sandy soil was lower than in the clay soil (Pauletti and Motta, 2019), the plant P content as well as the residual soil P content increased more in the sandy soil (Figures 6b, 7b, and 7d), evidencing greater potential for P desorption and recovery in sandy soils. This result can be attributed to the intrinsic characteristics of sandy soils, with naturally low binding energy of phosphate ions to the soil sorption complex (Oliveira et al., 2014). In contrast, typically tropical clayey soils contain high concentrations of iron, aluminum, and kaolinite oxides, which are components that form more stable bonds with phosphate (Rheinheimer et al., 2008; Oliveira et al., 2014), hampering desorption and, consequently, reducing the P recovery capacity of plants.

Compared with the control, the significant increase in residual P content in sandy soil confirms the hypothesis that AMF, isolated or combined with PSB, can promote P desorption. Conversely, no significant differences were observed in clayey soils. These results, in principle, suggest that P desorption is more efficient in sandy soils. However, as the most avid P adsorption sites are saturated by the application of phosphate fertilizers (Barrow and Debnath, 2014), and after the decrease in binding energy with increasing P saturation, the binding energy tends to stabilize, regardless of the soil texture (Oliveira et al., 2014). This suggests that P desorption in clayey soils with consolidated crop areas may be more efficient.

Several authors have reported that P depletion stimulates colonization, while P sufficiency may lead to a repression of AMF colonization (Breuillin et al., 2010; Müller et al., 2019). However, under the experimental conditions, colonization was not restrained, despite P sufficiency in the clayey soil, which reinforces the thesis that the binding energy of P with the clay fraction was decisive for the P desorption and recovery rates.

However, regardless of the soil texture, the effects of AMF inoculation, isolated or combined with PSB, promoted similar P recovery rates as in plants in the experimental units fertilized with a mineral source of P in the form of STP. This supports the thesis that P use efficiency can be improved with only AMF or AMF+PSB inoculation (Figures 6c and 7c).

Inoculation with phosphorus-solubilizing bacteria (PSB) did not significantly alter the availability of this nutrient. In both soil types, PSB inoculation induced no significant differences, compared with the control. Similarly, AMF inoculation and co-inoculation with PSB and AMF, despite the better results than in the control treatment, did not differ from each other, which indicates that PSB did not effectively enhance the action of AMF, resulting in similar P contents and agronomic performance. According to Leite et al. (2022), no significant P content increases were observed in soybean leaves grown with or without phosphate fertilization and inoculated with two types of PSB, although PSB inoculation provided other agronomic and economic benefits. As stated by Ordoñez et al. (2016), PSB associated with AMF can promote a synergistic effect, which, however, depends on the solubilization capacity of PSB and the interaction between these microorganisms, varying according to the combination of strains.

Inoculation with mycorrhizal propagules (spores, hyphae and fragments of colonized roots) in the soil led to an increase of up to 52.7 % in intensity of AMF root colonization, in comparison with the control (Figure 1). This resulted in higher P contents in the soybean shoots and roots, compared with the control plants (Figures 6a and 7a). Around 80 % of all plant species form symbiotic associations with AMF, including important crops such as soybean and cotton (Smith and Read, 2008; Cely et al., 2019). Arbuscular mycorrhiza represents an additional pathway of nutrient acquisition. After root colonization, the external mycelium of AMF grows in the soil and transfers nutrients, including phosphorus, which are not readily accessible to the plant roots (Agnolucci et al., 2019; Etesami et al., 2021). In return, AMF receive photoassimilates produced by the plants (Smith and Read, 2008). Inoculation with AMF can also increase microbial enzymatic activity in the soil and make minerals available to plants in complexed forms that are unavailable for root uptake (Burak et al., 2024).

However, although AMF inoculation, alone or combined with PSB, has promoted a performance similar or superior to fertilization with the mineral source of P, these microorganisms should be viewed as a complement to phosphate fertilization, with the reduction of fertilizer rates in mind, rather than as a replacement, as pointed out by Leite et al. (2022).

CONCLUSION

Although most of the phosphorus (P) in Brazilian soils is still preserved in non-labile and moderately labile fractions, inoculation with arbuscular mycorrhizal fungi (AMF), associated or not with phosphorus-solubilizing bacteria (PSB), demonstrated potential to improve the availability and recovery efficiency of this nutrient by soybean plants. On the other hand, inoculation with PSB alone caused no significant effects.

In clayey soil, the positive effect of AMF on P desorption, associated or not with PSB, was limited, without significant increases in P availability. Nevertheless, the performance of AMF treatments was consistent, with results similar to or superior to those induced by TSP application, which suggests a potential for P recovery by soybean plants, regardless of the soil texture. Thus, as a sustainable and potentially complementary approach to mineral fertilization, AMF may represent a breakthrough in improving P use efficiency, especially in management systems designed to reduce the dependence on mineral fertilizers.

SUPPLEMENTARY MATERIALS

Supplementary data to this article can be found online at https://www.rbcsjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-49-spe1-e0240165/1806-9657-rbcs-49-spe1-e0240165-suppl01.pdf.

DATA AVAILABILITY

The data will be provided upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization: Luis Eduardo Azevedo Marques Lescano (equal) and Oriel Tiago Kölln (equal).

Data curation: Carlos Botelho Pereira Osipi (equal), Hebert Teixeira Cândido (equal), Luis Eduardo Azevedo Marques Lescano (equal) and Oriel Tiago Kölln (equal).

Formal analysis: Luis Eduardo Azevedo Marques Lescano (equal) and Oriel Tiago Kölln (equal).

Funding acquisition: Oriel Tiago Kölln (lead).

Investigation: Arthur Michalowski Filho (equal), Carlos Botelho Pereira Osipi (equal), Luis Eduardo Azevedo Marques Lescano (equal), Marcos Yassuhiro Inoue (equal) and Oriel Tiago Kölln (equal).

Methodology: Arthur Michalowski Filho (equal), Carlos Botelho Pereira Osipi (equal), Luis Eduardo Azevedo Marques Lescano (equal) and Oriel Tiago Kölln (equal).

Project administration: Oriel Tiago Kölln (lead).

Resources: Oriel Tiago Kölln (lead).

Supervision: Oriel Tiago Kölln (lead).

Validation: Hebert Teixeira Cândido (lead).

Writing – original draft: Hebert Teixeira Cândido (equal), Marcos Yassuhiro Inoue (equal) and Oriel Tiago Kölln (equal).

Writing – review & editing: Luis Eduardo Azevedo Marques Lescano (equal), Marcos Yassuhiro Inoue (equal) and Oriel Tiago Kölln (equal).

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