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Influence of phosphorus-solubilizing microorganisms and phosphate amendments on pearl millet growth and nutrient use efficiency in different soils types

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Two greenhouse growth trials were performed to assess inoculation with phosphorus (P)-solubilizing microorganisms (PSM) in combination with alternative sources of phosphate in pearl millet (*Pennisetum glaucum*) cultivation: One using sandy soil and the other using clayey soil. The treatments comprised five P sources, with or without inoculation with PSM B119 (*Bacillus megaterium*) and B2084 (*Bacillus subtilis*) strains. Amendment of alternative sources (granulated, branned organomineral, and Bayovar rock) of P along with PSM inoculation produced more plant dry mass on sandy soil, which was not observed on clayey soil. Phosphorus use efficiency (PUE) did not differ between inoculated and non-inoculated treatments, and it was higher with the alternative P sources, compared to triple superphosphate (TSP) treatments. Available P content in the soil was higher with TSP, in sandy soil, and with PSM inoculation. Overall, acid and alkaline phosphatases and β-glucosidase activity was higher in clayey soil, compared to sandy soil, which contains little organic matter; it was also higher with alternative P sources, compared to TSP, and in inoculation treatments, showing the potential of using PSM inoculation and alternative P sources to achieve higher sustainability and productivity in agriculture.

Key words: *Pennisetum glaucum,* alternative phosphorus sources, fertilization, organominerals.

INTRODUCTION

Phosphorus (P) is closely related to several soil characteristics, particularly weathering degree, buffering potential, mineralogy, and organic matter (OM) content. P is an essential macronutrient to plants as nitrogen (N) and potassium (K). Soils with high-buffer-capacity soils are characterized by high clay content, iron and aluminum oxides and 1:1 clay mineral (Batjes, 2011; Du et al., 2020). This results in strong P adsorption and low P uptake (approximately 10 to 25%) by plants growing on tropical soils (Hanyabui et al., 2020). Therefore, P

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fertilization in dosages exceeding the demand of agricultural crops in tropical soils is necessary to overcome adsorption losses. These factors, combined with the important role of P in the development and yield of several agricultural crops, have attracted research interest to optimize soil P management to achieve optimal use by plants.

Currently, Brazil is a major consumer and importer of phosphate fertilizer (55% of consumed P), due to the characteristics of local soils and absence of high-quality phosphate rock mines (Lapido-Loureiro and Melamed, 2009). Fertilizer import reached the highest level in the country's history in the first half of 2020, accounting for 29.4 million tons. This contributed to the rising cost of grain production and added this issue to the most relevant aspects in the Brazilian agribusiness (Globalfert, 2021). Meanwhile, studies conducted by Embrapa indicated that almost half of the P applied as inorganic agricultural fertilizer in the past 50 years remains fixed in the soil where it is unavailable to plants, amounting to approximately 30 Tg and corresponding to a reserve of over US$ 40 billion (Withers et al., 2018).

The main phosphate fertilizers used in Brazil are soluble P sources such as superphosphates (single and triple) and monoammonium and diammonium phosphates. The national sources include deposits essentially of igneous origin, associated with carbonatites; beneficiation of these rocks is difficult, due to their complex mineralogy, low uniformity, and low apatite and high carbonate content, which entail challenges regarding industrial utilization. By contrast, rock phosphates from other countries, such as those from Tunisia (Gafsa), Israel (Arad), Peru (Bayovar), and Morocco (Benguerir) are of sedimentary origin, easy to mine, and highly reactive as they are composed of open poorly consolidated aggregates of microcrystals with a large specific surface area (Lapido-Loureiro and Ribeiro, 2009).To improve the use of less soluble P sources, alternatives for P use and management are required to achieve a more sustainable, productive, and economically viable form of agriculture by using alternative nutrient sources, organomineral fertilizers, and biological materials.

The P availability from rock phosphates can be improved during crop cultivation, for example, through soil microorganisms (Coutinho et al., 1991; Silva et al., 2017; Ribeiro et al., 2018; Mattos et al., 2020). Applying P solubilizing microorganisms (PSMs) together with different P sources may help optimize the use of P and other fertilizers; therefore, this approach constitutes a promising alternative with low environmental impact and with the potential to increase crop productivity (Spolaor et al., 2016; Oliveira- Paiva et al., 2020; Rosa et al., 2020; de Sousa et al., 2020). This is possible due to processes elicited by microorganisms, such as biosynthesis of phytohormones and metabolites (organic acids, phosphatase enzymes, etc.) (Duca et al., 2014; Tahir et al., 2017; Oliveira-Paiva et al., 2020), induction of tolerance to biotic and abiotic stresses (Yan et al., 2016; Takishita et al., 2018), production of siderophores (Ali et al., 2014), and solubilization of soil nutrients such as P and K (Gupta et al., 2015; Shen et al., 2016; Patel and Archana, 2017). Production of organic acids by microorganisms (Mendes et al., 2014; Abreu et al., 2017) and the release of protons in $NH4^+$ assimilation reactions or other proton-releasing metabolic reactions (Prabhu et al., 2019) stand out among P solubilization mechanisms. In addition to the mechanisms of mineralization of soil organic P by the production of phosphatases, especially of the phytase group, chelating agents are produced by plants and microorganisms (Sharma et al., 2013).

Promising results regarding interactions between microorganisms and phosphate rocks have already been described for different crops, including maize (Manzoor et al., 2016; Silva et al., 2017), wheat (Kaur and Reddy, 2015), millet (Ribeiro et al., 2018), sorghum (Ehteshami et al., 2018; Mattos et al., 2020), and forage legumes (Zineb et al., 2020). In maize, the presence of PSMs, combined with the benefits of organomineral sources, resulted in increased availability of P to plants. This positive effect can also be proven by the available P content in the soil, which is higher in treatments with organominerals and microorganisms and similar to treatments with triple superphosphate (TSP), with comparable results for millet (Almeida et al., 2016). Therefore, the objective of this study was to evaluate the effect of inoculation of PSM on millet plants grown on different soils fertilized with different P sources.

MATERIALS AND METHODS

Two experimental setups were established in the greenhouse of Embrapa Milho and Sorgo, in Sete Lagoas, MG (19°28' S, 44°15' W) from May/2018 until January/2019 using two soil types: one with a very clayey texture from the Experimental Farm of Embrapa Milho and Sorgo (typical Dystrophic Red Latosol) with the following chemical and physical characteristics before any treatment: pH H_2O = 5.2, aluminum (Al) = 0.56; calcium (Ca) = 1.1; magnesium (Mg) = 0.1 ($cmol_C$ dm^{-3}); total cation exchange capacity (T) = 9.7 ($cmol_C$ dm^{-3}); P-Mehlich 1 = 1.2; potassium (K) = 15.1 (mg dm^{-3}); base saturation (V) = 12.7%; organic matter (OM) = 3.64%, and clay content = 74%; the other soil type was sandy and originated from the Trijunção Farm (Dystrophic argisolic Red-Yellow Latosol), with the following characteristics before any treatment: pH H_2O = 6.2, Al = 0.04; Ca = 1.2; Mg = 0.3 ($cmol_C$ dm^{-3}); T = 3.1 ($cmol_C$ dm^{-3}); P-Mehlich 1 = 3.4; K = 11.3 (mg dm^{-3}); V = 49.3%; OM content = 0.91%, and clay content = 14.0%. Pots capable to support 5 kg filled with 4 kg of soil were used. Requirement for liming and fertilization (apart from P) was calculated to reach V = 70% and to meet the demand of the crop in a greenhouse test (Resende et al., 2020), respectively, with application of poor analysis (p.a.) reagents one month before the experiment. During this preliminary period and during the experiments, the pots were irrigated to maintain humidity at 80% field capacity.

The experimental design was entirely randomized in a 5 ×2 factorial design with five P treatments, that is, (1) control without P fertilization, (2) granulated organominerals (GO; mixture of 45% poultry litter, 5% additives to improve granulation, and 50% Bayovar phosphate), (3) branned organominerals (BO; mixture of 45% poultry litter, 5% additives to improve granulation, and 50% Bayovar phosphate), (4) Bayovar phosphate rock, and (5) triple superphosphate (TSP); with or without PSM inoculation, thus comprising 10 treatments. Each treatment was performed using four replicates. The P_2O_5 dosage was 458 mg dm^{-3} per pot, and the P_2O_5 contents in P sources utilized to calculate fertilization rates were 27% of total P_2O_5 for Bayovar, 16% of total P_2O_5 for organominerals, and 41% of citric acid soluble P_2O_5 for TSP.

The granulation process involved mixing the raw materials in a mass: mass ratio, homogenizing and placing them in a pelletizer disc with constant speed and inclination, and adding water manually. After granulation, the fertilizer was sieved to segregate granules with diameters of 2-4 mm in order to meet the granulometric requirements specified by Brazilian Department of Agriculture, Livestock and Food Supply (MAPA), in accordance with the Normative Instruction No. 23 of August 31, 2005. Then, the granules were dried to constant mass at 40°C using an oven with forced air circulation.

Phosphate fertilization was conducted one month after soil incubation by producing “cross-shaped” furrows in the soil. The inoculant contained the P-solubilizing bacteria B119 (*Bacillus megaterium*) and B2084 (*Bacillus subtilis*) were obtained from the Microorganism Collection of Embrapa Maize and Sorghum. One isolated colony of each strain grown on BDA plates (200 g L^{-1} potato, 20 g L^{-1} dextrose and 15 g L^{-1} agar) was transferred to TSB medium (Trypticase Soy Broth) and incubated overnight at 28° C. The concentration of bacteria was determined by measurement of absorbance at 560 nm (10^8 cells mL^{-1}) using a spectrophotometer UV/Vis UV1800 (Shimadzu, Japan). Liquid inoculant was applied on top of the fertilizers or on the soil (control treatment) at 10 ml pot^{-1}. The applied fertilizers and inoculant were covered by thin layer of soil, after which 20 pearl millet seeds (*Pennisetum glaucum*) ADR500 obtained from a breeding company was sown. After germination the seedlings were thinned to six plants per pot. The plants were grown for three successive crop cycles until the budding stage. Phosphate fertilization was performed only in the first crop to assess residual effects on the following crop cycles. Inoculation with PSM was executed on the first crop and was repeated before planting the third crop. N fertilizer was applied at three times weekly, 15 days after sow (50 mg dm^{-3} per pot) were provided per crop cycle.

Plants of each crop cycle were harvested at the booting stage by cutting the shoot part close to the ground. After the third crop cycle, the roots were collected in addition. The collected material was weighed, green mass was determined, and samples were placed in a forced circulation oven at 65°C. After reaching constant mass, dry mass and macronutrients in shoot part (and roots, in case of the third crop) were determined (Silva, 2009). The data were used to calculate extraction and use efficiency (UE) of N (NUE), P (PUE), and K (KUE), according to the ratio between plant biomass and total nutrient accumulation (Tomaz and Amaral, 2008). Soil samples were collected at each harvest to determine available P using a sodium bicarbonate extraction solution ($NaHCO_3$), 0.5 N at pH 8.5, according to Olsen et al. (1954).

Soil microbial activity was evaluated based on acid and alkaline phosphatases (Tabatabai, 1994) and β-glucosidase (Eivazi and Tabatabai, 1988) through colorimetric determination of released p-nitrophenol after incubation at 37°C with buffered solution of p-nitrophenyl phosphate and p-nitrophenyl-β-D-glycopyranoside, respectively.

Data were checked for normality and analyzed using One-way Analysis of Variance, and means of treatments were compared using Tukey's test at 5% probability, as implemented in Sisvar software (Ferreira, 2011). Data were analyzed comprehensively, considering the crops as subplots in order to assess differences between crops. Correlation analyses were performed according to Hair Jr. et al. (2006).

RESULTS AND DISCUSSION

Production of dry mass of shoot parts and roots

Dry mass production in shoot parts of millet plants was affected by P source and by PSM inoculation in all crop cycles on sandy soil (Figure 1), with no significant effect of the interaction (data not shown). Dry mass in clayey soil was affected by the P sources in all crops, but by the microorganisms only in the third crop and by the Bayovar source (Figure 1); no interaction effect was observed.

The first crop exhibited higher production of dry mass of shoot parts in the TSP treatment, with or without PSM inoculation, followed by GO and BO and by the control without addition of P and Bayovar (Figure 1). In the first crop on sandy soil, higher production of dry mass was observed in the TSP treatment with no PSM inoculation.

Bars bearing the same capital letter for P sources and the same lowercase letter for microorganism inoculation do not differ significantly at $p > 0.05$ (Tukey’s test), for each soil type. The second and third crops accumulated more shoot dry mass in treatments with GO, BO, and Bayovar rock, compared to the TSP and control treatments, on both soil types, as well as compared to the PSM treatments, especially on sandy soil (Figure 1). These results highlight the importance of residual effects of organomineral sources containing rock phosphate, and even Bayovar, compared to TSP because in addition to the improvement in dry mass production throughout the crops (Figure 1), organomineral sources and Bayovar also showed lower depletion of available P in soil compared to TSP (data not shown). Furthermore, the importance of PSM, especially in sandy soil and for alternative P sources (organominerals and Bayovar) is shown in Figure 1, and confirms the positive effects of phosphate rock-PSM association observed in previous studies (Mattos et al., 2020). Maize grown on soil fertilized with rock phosphate for three years showed biomass production and grain yield comparable to those of plants fertilized with TSP (Silva et al., 2017). Based on metataxonomic data, Silva et al. (2017) hypothesized that, in the long term, rock phosphate fertilization promoted higher relative abundance of microbial taxa related to P solubilization/acquisition in the soil and, consequently, increased P release and availability to plants.

In general, the performance regarding shoot dry mass production was very similar among organomineral sources, as well as between these and Bayovar, with the exception of crop 1, where the Bayovar treatment

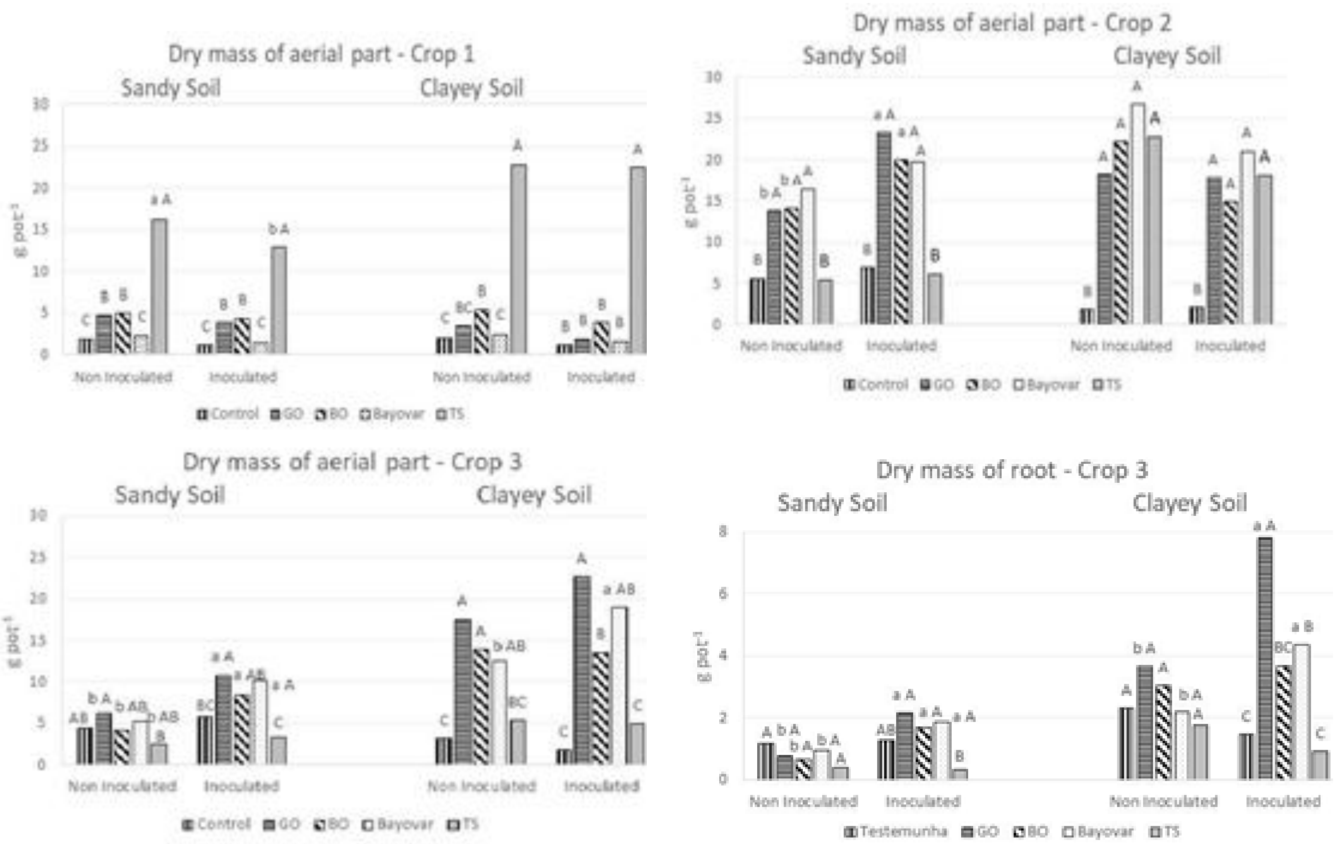

Figure 1. Dry mass of shoot of the three crop cycles and root dry mass of the third crop grown on sandy and clayey soils as a function of P sources and inoculation with phosphate (P) solubilizing microorganisms.

produced less than the organominerals in both soil types, and of crop 3 on clayey soil where the BO treatment produced less (Figure 1).

Higher production of dry matter of shoot parts only in the TS treatment on clayey soil in the first cycle was probably due to rapid P availability as this fertilizer is a soluble phosphate with high P concentration (45% of total P_2O_5). This characteristic leads to marked differences in the speed of P release to plants, as well as in the potential for fixation of this element in the soil (Korndörfer et al., 1999) and reduced availability to subsequent crops.

Similarly, the organomineral fertilizers and the combination of Bayovar rock with PSM inoculation resulted in higher production of dry masses of shoot parts, especially in the second and third crop cycles, compared to the TSP treatment. As soluble P in TSP is released faster to the soil, plants, may consume it largely during the first crop cycle and was thus less available thereafter. Furthermore, the fast initial release of P in TSP may also favor its adsorption to the soil and thereby reducing its availability to plants (Novais et al., 2007). Thus, because organomineral phosphates are less soluble, P release is slower, favoring plant uptake rather than soil adsorption (Novais et al., 2007; Almeida et al., 2012). These characteristics of organomineral fertilizers may have helped overcome the limitations of soil P dynamics, particularly regarding P availability in second and third crop cycles allowing increased P uptake by plants, thus resulting in higher dry mass production. Silva et al. (2021) observed that the combination of rock phosphate with P- solubilizing bacteria helped to increase the supply of P to millet, especially in management systems where chemical fertilizers such as TSP are not admissible. Indeed, the slow release of P from rock phosphate may be an advantage in tropical soils, as it minimizes the loss of soluble P by adsorption to soil particles.

The combination of natural phosphates with PSM may be a viable alternative to improve P availability and recycling. These microorganisms can solubilize and mineralize P from inorganic and organic sources and thereby increase its availability to plants (Richardson, 2001); additionally, they produce other metabolites which promote plant growth, which also explains the observed larger dry mass of shoot parts in the inoculation treatments, especially in the second and third crop cycles. Re-inoculation before the third crop showed the importance of repeated application to ensure the desired effects to achieve higher crop yields, especially regarding sandy soil where biological activity is lower due to more limiting conditions for growth and survival of soil microbiota (Sessitsch et al., 2001).

Root dry mass production was significantly affected by P sources and by PSM inoculation, as well as by the interaction, with the organomineral and Bayovar treatments producing higher root mass, compared to the TSP treatments. Moreover, PSM inoculation led to higher production of root mass, especially in sandy soil (Figure 1). The bacteria used in the present study favorably affect plant root development (Ribeiro et al., 2018; de Sousa et al., 2020), and de Sousa et al. (2020) observed that, under controlled conditions, inoculation of maize seedlings with these *Bacillus* strains resulted in increased biomass and nutrient content of shoot parts and root surface area, compared to the non-inoculated control. Under field conditions, the same authors noticed that inoculation with these two *Bacillus* strains resulted in increased grain yield of maize plants in soils fertilized with TSP. For example, inoculation with *B. megaterium* B119 resulted in 26% increase in the production and accumulation of P in grains, compared to non-inoculated plants and to 23% increase in P accumulation in grains, compared to plants that received only phosphate fertilization (de Sousa et al., 2020). In millet plants, positive effects of inoculation with endophytic *Bacillus* strains on dry weight of shoot parts and roots, in addition to plant N, P, and K content, were reported, reaching increments of 55% in the shoot part biomass, and N, P, and K content increased by 30, 50, and 70%, respectively (Ribeiro et al., 2018).

The positive effects of inoculation with *Bacillus* strains may be related to the synergistic effects of multiple growth-promoting factors, such as production of phytohormones and solubilization of plant nutrient sources. The bacterial strains used in the current study are considered effective for the production of the phytohormone indoleacetic acid (IAA), with strains B119 and B2084 producing 61.67 and 30.16 μg mL^{-1} IAA, respectively (de Sousa et al., 2020). Solubilization of rock phosphate by B119 has been reported (Gomes et al., 2014), whereas B2084 is an endophytic bacterium which can solubilize tricalcium phosphate ($Ca_3(PO_4)_2$), in addition to producing high concentrations of organic acids *in vitro* (Abreu et al., 2017).

A larger root system increases the plant's efficiency to acquire nutrients and water from the soil. The production of IAA increases the root surface, allowing the penetration of larger volumes of soil, which leads to increased absorption of nutrients, especially those of low mobility in soil, such as P. In addition, the increase of root mass allows higher exudation of carbon compounds, which stimulates microbial activity in the rhizosphere.

Combined analysis of the three crop cycles

The overall means of the three crop cycles (Table 1) showed that the alternative sources GO, BO, and Bayovar inoculated with PSM stimulated the production of dry mass of the shoot part of plants on sandy soil. Such effect was not observed in plants on clayey soil (Table 1). This indicated a major effect of PSM inoculation in sandy soil with low clay and OM content, that is, where conditions for plant growth are more limiting. Increased root growth in the inoculation treatment in sandy soil (Figure 1) shows the potential of this approach which may help make use of a bigger soil volume and thereby increase water and nutrient absorption and plant productivity.

No effect of PSM inoculation on NUE, PUE, and KUE was observed, however, between P source treatments, a difference was observed regarding P and K (Table 1). Considering P and the two soil types, although there was no statistical difference between the treatments with and without inoculation, the PUE values of the inoculated treatments were higher, except for the TSP treatment. These results suggest a beneficial effect of PSM to increase UE by plants. The higher PUE of the control may be irrelevant, as dry mass production was not increased (Figure 1), that is, the plants absorbed P more efficiently but were less productive, which is not of agronomic interest.

Regarding P sources in both soil types, plants receiving GO, BO, and Bayovar were more efficient in absorbing P than those receiving TSP. This suggests the potential of alternative sources of phosphate nutrients. The low performance of the TSP source stands out because of the low PUE values, even though it resulted in good dry mass production, especially in clayey soil (Figure 1 and Table 1).

PUE was lower in sandy than in clayey soil (Table 1) which has lower buffering capacity and thus facilitates higher nutrient accumulation by plants (Novais et al., 2007).This suggests higher P export by plants in sandy soils. PSM inoculation may help increase P uptake, which is important regarding sandy soils in which biological activity is lower due to the low OM content (typically < 10 g kg^{-1}) and, in general, enzyme activity increases with increasing soil OM content, thus suggesting higher stability of enzymes adsorbed to humic materials and growth of microbial communities in the soil (Marinari and Antisari, 2010; Oliveira et al., 2015). This influence is evidenced by the results in Table1, as enzymatic activity in clay soil was higher than in sandy soil, regarding the three evaluated enzymes.

Significant correlation at 5% probability occurred in plants on clayey soil between DM and UEN (r = 0.91), DM and PUE (r = 0.81) as well as DM and UE of K (r = 0.80). OM is an important source of N for plants, and higher OM content in clayey soil (36.4 g kg^{-1}) may explain for this significant correlation.

The Olsen extractor (Olsen et al., 1954) was used to analyze soil P availability, as acid extractors such as

Table 1. Mean values of three crop cycles regarding shoot part dry mass, utilization efficiency of nitrogen (NUE), phosphorus (PUE), and potassium (KUE), soil available phosphorus (P) by Olsen extraction, and activity of acid phosphatase, alkaline phosphatase, and β-glucosidase as a function of P source and inoculation with phosphate solubilizing microorganisms.

Soil	Source	DM	NUE	PUE	KUE	P-OLSEN	ACI PHOS	ALK PHOS	β-GLUCOS
		g pot^{-1}	g shoot /g nutrient accumulated in the shoot			mg dm^{-3}	µg p-nitrophenol h^{-1}g^{-1} soil	µg p-nitrophenol h^{-1} g^{-1} soil	µg p-nitrophenol h^{-1} g^{-1} soil
Sandy	Test*	3.94^{f**}	30.45^{a}	995.94^{a}	138.43^{a}	1.58^{c}	126.99^{bc}	27.99^{e}	17.65^{c}
	Test +Inoc	4.61^{ef}	29.67^{a}	1178.62^{a}	125.65^{a}	2.21^{c}	132.20^{abc}	55.80^{cd}	21.81^{bc}
	GO	8.20^{bcd}	33.59^{a}	517.49^{bc}	166.96^{a}	9.08^{c}	158.56^{ab}	58.42^{cd}	25.89^{ab}
	GO +Inoc	12.64^{a}	42.91^{a}	614.26^{bc}	136.79^{a}	5.89^{c}	187.69^{a}	79.92^{ab}	32.00^{a}
	BO	7.76^{cd}	28.01^{a}	474.30^{c}	153.39^{a}	9.73^{c}	158.04^{ab}	56.65^{cd}	23.47^{bc}
	BO +Inoc	10.93^{ab}	39.62^{a}	613.22^{bc}	122.43^{a}	8.56^{c}	141.60^{abc}	98.02^{a}	27.99^{ab}
	Bayovar	7.94^{cd}	32.83^{a}	500.81^{bc}	141.64^{a}	6.18^{c}	106.78^{bc}	38.03^{de}	21.80^{bc}
	Bayovar +Inoc	10.47^{abc}	47.11^{a}	659.13^{b}	154.43^{a}	3.64^{c}	143.72^{abc}	72.93^{bc}	24.03^{bc}
	TSP	8.03^{bcd}	26.79^{a}	157.73^{d}	134.79^{a}	93.38^{b}	94.40^{c}	29.68^{e}	22.91^{bc}
	TSP +Inoc	7.40^{de}	33.08^{a}	155.19^{d}	137.32^{a}	144.80^{a}	112.75^{bc}	45.14^{de}	23.43^{bc}
Clayey	Test	2.33^{c}	25.12^{a}	1300.12^{a}	23.97^{d}	2.11^{b}	409.56^{c}	275.67^{b}	43.97^{e}
	Test +Inoc	1.67^{c}	41.24^{a}	1330.28^{a}	52.66^{d}	1.80^{b}	664.74^{b}	308.88^{b}	49.23^{de}
	GO	13.05^{ab}	32.35^{a}	766.55^{b}	142.02^{c}	5.41^{b}	715.11^{b}	321.22^{b}	69.54^{bcd}
	GO +Inoc	14.10^{ab}	44.15^{a}	787.75^{b}	134.28^{c}	5.56^{b}	1047.78^{a}	434.88^{a}	114.84^{a}
	BO	13.83^{ab}	27.49^{a}	660.75^{b}	160.21^{bc}	6.16^{b}	681.26^{b}	294.08^{b}	70.98^{bcd}
	BO +Inoc	10.71^{b}	31.11^{a}	684.65^{b}	115.24^{c}	6.87^{b}	1024.74^{a}	444.25^{a}	90.81^{ab}
	Bayovar	13.92^{ab}	28.67^{a}	677.56^{b}	147.33^{c}	6.58^{b}	631.31^{b}	259.86^{b}	62.55^{cde}
	Bayovar +Inoc	13.85^{ab}	35.71^{a}	771.39^{b}	143.53^{c}	5.72^{b}	946.21^{a}	423.32^{a}	76.62^{bc}
	TSP	16.95^{ab}	31.88^{a}	385.28^{c}	225.50^{a}	56.53^{a}	564.07^{bc}	243.10^{b}	68.23^{bcde}
	TSP +Inoc	15.10^{ab}	41.30^{a}	374.19^{c}	208.82^{ab}	47.55^{a}	655.63^{b}	330.07^{b}	78.01^{bc}

*Test: non-inoculated control; GO: granulated organomineral; BO: branned organomineral; Inoc.: inoculated; TSP: triple superphosphate.**Same lower-case letter in the row does not differ at 5% probability level (Tukey's test) in each soil type. Dry mass (DM), soil available phosphorus (P) by Olsen extraction (P- Olsen), activity of acid phosphatase (ACI PHOS), alkaline phosphatase (ALK PHOS), β-glucosidase (β-GLUCOS).

Mehlich 1 are not indicated for phosphate rock P sources. P availability in the two soils was higher in the TSP treatment and with PSM inoculation in sandy soil (Table 1), showing the potential of solubilizing microorganisms to increase soil P availability.

Higher values of available P were observed in sandy soil compared to clayey soil, corroborating the results of Novais et al. (2007) with higher critical levels of P in sandy soils (Table 1). As TSP is a soluble phosphate and, therefore, more rapidly available in the soil (Korndörfer et al., 1999), treatment with this fertilizer resulted in a higher concentration of P in the soil in the first crop and, consequently, it increased the P availability to plants; thus, P incorporation into cellular structures results in an increase in the dry mass of shoot parts of the first crop, that was higher than other sources of P fertilizers (Figure 1 and Table 1). In line with this, the correlation of PUE and available P were stronger in sandy soil (r = 0.86) than in clayey soil (r = 0.76).

The increased effect of inoculation with TSP corroborated the results of other studies showing that inoculant is more effective in the presence of fertilizers (Oliveira-Paiva et al., 2020; de Sousa et al., 2020). The activity of acid and alkaline phosphatases in soil can be a good indicator of organic P mineralization potential of and biological activity (Margalef et al., 2017). Acid phosphatase activity differed significantly between P sources and inoculation treatments. Combined analysis of the three crop cycles revealed that, in sandy soil and with PSM inoculation, acid phosphatase and β-glucosidase activity was highest in the GO treatment, and that of alkaline phosphatase was highest in the BO treatment (Table 1). In clayey soil, high activity of acid and alkaline phosphatases was found in the Bayovar, GO, and BO treatments with PSM inoculation, and β-glucosidase activity occurred only in the GO plus inoculation treatment. These results differed statistically from those of the other treatments; these three sources provided less available P, which may have increased enzyme production in inoculation microorganisms in response to stimuli from the plants. Regarding organominerals, the existence of a carbon source can stimulate microorganisms activity (Zucareli et al., 2018).

PSM inoculation increased the enzyme activity in most treatments, which was expected, as soil enzyme activity is directly linked to the presence and origin of microorganisms (Tabatabai, 1994, Mendes and Reis Junior, 2004). Phosphatases are enzymes released by plants and microorganisms, which contribute to the cleavage of organic P, thereby releasing it into the soil solution to be absorbed by plants (Abou-Baker et al., 2011), and alkaline phosphatase is produced mainly by microorganisms. Approximately 80% of the P in Brazilian soils is organic, thus the role of phosphatases is crucial because organic P is not readily available to plants. Pinho et al. (2016) examined the activity of phosphatases in millet cultivated on latosol under greenhouse conditions and supplied with TSP, Bayovar, and GO, and they observed that inoculation of organomineral fertilizers with PSM contributed to increased cycling activity of available P in the soil, which corroborates our results.

Mendes et al. (2018) emphasized the importance of β-glucosidase in the early detection of soil changes in response to different management practices and its crucial role in improving soil physical properties, in addition to carbon cycling in soil organic matter, which may also influence soil P and N dynamics (Ndossi et al., 2020). Similar results were observed by Silva (2018), who evaluated enzyme activity in PSM-inoculated Yellow Red Acrisol on which sugarcane was grown, and one year after planting, acid phosphatase activity increased in soil fertilized with organominerals, TSP, and Bayovar; alkaline phosphatase activity increased in soils fertilized with organominerals, and β-glucosidase showed higher activity with organominerals and Araxá phosphate. Thus, soil enzyme activity is a potential indicator of soil quality due to its high sensitivity to external factors, compared to physical and chemical attributes, as well as for ease of evaluation (Utobo and Tewari, 2015). Soil biological attributes react rapidly to any changes in the environment and can therefore serve as indicators of soil quality and sustainability of agroecosystems (Schloter et al., 2018).

Sandy soils have low levels of OM, typically below 10 g kg^{-1}, and, in general, enzyme activity increases with increasing soil OM content, suggesting higher stability of enzymes adsorbed to humic materials (Marinari and Antisari, 2010; Oliveira et al., 2015). This influence is evidenced by the results presented in Table 1, showing that the enzyme activity in clay soil is higher than in sandy soil, regarding the three evaluated enzymes. In sandy soil, significant correlations were observed at 5% of probability between the production of dry mass of shoot parts and acid and alkaline phosphatases and β- glucosidase.

Conclusion

PSM inoculation may be used for increasing crop productivity and PUE. Furthermore, the potential for using alternative P sources such as GO, BO, and rock phosphates for improving crop production is also highlighted. However, future studies are required to evaluate the use of alternative P sources and the use of bioinoculants under field conditions and in other crops.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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