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# Morphophysiological aspects of bean plants cultivated with natural reactive phosphate and solubilizing and growth promoting microorganisms

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**Abstract.** Beans (*Phaseolus vulgaris* L.) are an economically important crop, being part of the daily meal of a large part of the Brazilian population. One of the most common problems in bean cultivation is the low availability and low mobility of phosphorus. Among the strategies to improve the acquisition of phosphorus from the soil, the association of plants with microorganisms that promote growth and/or phosphate solubilizers stands out, since the efficiency of phosphate fertilizers depends directly on the microbial action in their cycle. Thus, the objective of this study was to evaluate physiological, morphological and biochemical characteristics in bean plants cultivated with the presence or absence of reactive natural phosphate associated or not with seed inoculation with different efficient microorganisms. The experiment was carried out in a greenhouse with a completely randomized design, consisting of eight treatments and four replications. The treatments were composed of: i) control, and application of ii) *Azospirillum brasilense*, iii) *Bacillus subtilis* and *Bacillus megaterium*, iv) efficient microorganisms (ME), v) reactive natural phosphate (RNP), vi) RNP + *A. brasilense*, vii) RNP + *Bacillus*, and viii) RNP + ME. Gas exchange and material collection to determine height, stem diameter, dry matter, acid phosphatase activity and P content were performed 55 days after sowing. The treatment RNP + *A. brasilense* and other microorganisms contributed to a higher photosynthetic rate and transpiration in bean plants. The treatments RNP + ME and RNP + *Bacillus* promoted greater plant height and stem diameter, respectively. The shoot dry matter was higher in the RNP + ME treatment, in relation to the other treatments. Acid phosphatase activity was higher in the area of bean plants exposed to reactive natural phosphate, and in the roots of plants exposed to treatment with species of the genus *Bacillus*. It was possible to verify that the application of microorganisms with natural phosphate, despite not causing an increase in the P content of the shoot, favored the conditions of growth and production of bean plants.

**Keywords:** Phosphorous, microorganisms, photosynthesis, *Phaseolus vulgaris*.

## Introduction

Beans are a legume that stands out for being an important source of protein, phosphorus, iron, vitamin B1 and fiber in human food (HEINEMANN *et al.*, 2009). Brazil is among the world's largest producers of beans (FAOSTAT, 2022), with production of 2,856.1 thousand t in the 2020/21 harvest (CONAB, 2022). Brazil has adequate climate and soil conditions for the cultivation of this crop (COELHO, 2018), in addition to the technological advances that have contributed to the development of agriculture. Also, Elias *et al.* (2012) reported that around 65% of the national bean production comes from family farming, and the southern region of Brazil extends its cultivation in three annual harvests between August and April. The same authors highlighted that this crop is demanding in macro and micronutrients, and that phosphorus is directly associated with bean yield.

Although production conditions are potentially favorable, crops with inadequate soil management, by disregarding sustainable ecological bases, have resulted in the degradation of more than two billion hectares of soil worldwide (UNEP, 2000). The current agricultural production model with intensive use of inputs disregards the exploitation of biological components of the soil and nutrient cycling (COLA, 2012).

Phosphorus (P) availability in soil is naturally low and, in degraded areas, this condition can become even more critical. In this context, the adoption of production systems using low-solubility phosphorus sources associated with the use of phosphorus-solubilizing microorganisms can contribute to environmental sustainability and improve the productive potential of the crop. Phosphorus is present in the soil solution in the forms of orthophosphoric acid ( $H_3PO_4$ ), phosphoric acid ( $H_2PO_4$ ,  $HPO_4^{2-}$ ), and phosphate ( $PO_4^{3-}$ ), and the concentrations of these anions are pH dependent (MENDES *et al.*, 2003). In the solid phase, phosphorus can be in organic and mineral form. Deficiency of this nutrient can impair respiration, photosynthesis and generate the accumulation of carbohydrates, in addition to affecting the synthesis of nucleic acids and proteins, inducing the accumulation of soluble/nitrogenated compounds in plant tissue (RAVEN, 2013; MENG *et al.*, 2021) and reduced plant growth (SILVA *et al.*, 2014).

The increment of P in the roots promotes a greater exploration of the soil, in order to improve the  $HPO_4^{2-}$  uptake (HENDRICKSON *et al.*, 2004). However, uptake is also dependent on the soil's ability to replenish the solution with nutrients (FERNANDES, 2000). Brazilian soils have low cation exchange capacity (CTC) and high ionic adsorption, thus reducing base saturation with increased retention of anions, such as phosphate (PIMENTEL, 2005). Thus, reactive natural phosphate, due to its slow availability and being

accepted in organic crops, becomes an economically and environmentally advantageous option.

Microorganisms represent on average about 70% of the living and active fraction of soil organic matter. In agricultural ecosystems, phosphorus requires a symbiotic relationship between the plant and mycorrhizal fungi, the action of phosphorus solubilizing and mineralizing microorganisms and phosphatase producers to become available to plants in the biogeochemical cycle (ALVES, 2003; OLIVEIRA *et al.*, 2021).

Plant growth-promoting bacteria such as *Bacillus* and *Azospirillum* can convert insoluble P into soluble forms, which can be taken up by plants (RAMAKRISHNA *et al.*, 2019). In addition, a group of microorganisms, known as efficient microorganisms (EM), have also been used recently as bioinputs in agriculture. EMs are made up of bacteria, fermented products and photosynthetic products of lactic acid, formed from the metabolic activity of fermented foods and bacteria (enzymes and vitamins). The EMs act as plant growth promoters, as they optimize the physiological processes of plants such as photosynthesis, respiration, transpiration and enzymatic systems (ALLAVERDIYEV *et al.*, 2014). In this way, both growth-promoting bacteria and EM stand out as a sustainable alternative in promoting plant growth, especially in conditions of nutritional deficiency (MA *et al.*, 2019; ZHANG *et al.*, 2019).

Organic production with the use of growth-promoting microorganisms allows the reduction of impacts linked to soil degradation, working from basic ecological principles of agroecosystems such as mineralization and solubilization of phosphates, in order to preserve natural resources in a socially fair way, and economically sustainable (ALTIERI, 1987).

The hypothesis of the present study was that phosphate-solubilizing microorganisms increase P availability in bean plants and improve physiological, morphological and biochemical responses. Thus, this study aimed to evaluate morphophysiological and biochemical characteristics in bean plants cultivated with the presence or absence of reactive natural phosphate associated with or not with seed inoculation with different efficient microorganisms.

## Material and Methods

The study was carried out in a greenhouse and in the Entomology and Biochemistry and Soils laboratories at the Federal University of Fronteira Sul (UFFS), *Campus Erechim*.

### *Plant material and growth conditions*

The Carioca bean cultivar used in this study was obtained from family farmers in the municipality of Cacique Doble, RS, Brazil (SISGEN number A1D7F2E). Three seeds were sown in

each plastic pot with a capacity of 8 dm<sup>3</sup> of soil on 04/06/2021. The soil used as substrate was classified as a typical Aluminoferric Red Latosol (EMBRAPA, 2013) and collected in the experimental area at UFFS, Erechim, RS, Brazil (27.728681° S; 52.285852° W), with the following characteristics: clay content 30%; organic matter 1.1%; pH in water 4.4; phosphorus content 2.7 mg dm<sup>-3</sup>; potassium 28.8 mg dm<sup>-3</sup>; calcium 0.8 cmol dm<sup>-3</sup>; magnesium 0.4 cmol dm<sup>-3</sup>; sulfur 63.3 mg dm<sup>-3</sup>; exchangeable aluminum 12.7 cmolc dm<sup>-3</sup>; zinc 0.3 mg dm<sup>-3</sup>; copper 6.7 mg dm<sup>-3</sup>; manganese 15.0 mg dm<sup>-3</sup>; boron 0.1 mg dm<sup>-3</sup>; CTC at pH 7.0: 14.0; and effective CTC: 4.0.

The soil fertility was corrected 15 days before sowing according to the Rio Grande do Sul Liming and Fertilization Manual (SILVA et al., 2016), based on the results of the chemical analysis. Also, potassium sulfate (60 kg ha<sup>-1</sup>) and urea (30 kg ha<sup>-1</sup>) were added in two applications: at sowing and post-emergence of the plants.

From the vegetative stage V2, which occurs 21 days after sowing (DAS), the plants were submitted weekly to applications of Neem oil (8 mL L<sup>-1</sup>) to control *Diabrotica speciosa* and the homeopathy *Silicea terra* 18 CH (1mL L<sup>-1</sup>). 1) for the *Corinespora cassiicola* control.

**Table 1.** Treatments used in the present study, sources of phosphorus and inoculants.

Treatment	Phosphorous source	Seed inoculation
Control	-	-
<i>A. brasilense</i>	-	<i>Azospirillum brasilense</i> <sup>1</sup>
<i>Bacillus</i>	-	<i>Bacillus subtilis</i> e <i>B. megaterium</i> <sup>2</sup>
EM	-	Efficient microorganisms <sup>3</sup>
RNP	Reactive natural phosphate <sup>4</sup>	-
RNP + <i>A. brasilense</i>	Reactive natural phosphate <sup>4</sup>	<i>Azospirillum brasilense</i> <sup>1</sup>
RNP + <i>Bacillus</i>	Reactive natural phosphate <sup>4</sup>	<i>Bacillus subtilis</i> e <i>B. megaterium</i> <sup>2</sup>
RNP + EM	Reactive natural phosphate <sup>4</sup>	Efficient microorganisms <sup>3</sup>

<sup>1</sup>Strains ABV5/ABV6 (6 mL Kg<sup>-1</sup>); <sup>2</sup>StrainBRM 2084 (1 mL kg<sup>-1</sup>); <sup>3</sup>No dilution (1 mL kg<sup>-1</sup>); <sup>4</sup>20% total P<sub>2</sub>O<sub>5</sub> (140 Kg ha<sup>-1</sup>)

The bean seeds were inoculated with microorganisms at the sowing time, considering the following doses: *Azospirillum brasilense* 6 mL Kg<sup>-1</sup>, Efficient Microorganisms (EM) 1 mL kg<sup>-1</sup>, and a mix of *Bacillus subtilis* and *Bacillus megaterium* 1 mL Kg<sup>-1</sup>. Also, a treatment with reactive natural phosphate (RNP) (20% of total P<sub>2</sub>O<sub>5</sub>; 140 Kg ha<sup>-1</sup>), and the combination of RNP with the other treatments with microorganisms (*A. brasilense*, EM and *Bacillus*) were also carried out, as detailed in table 1.

The experimental design was completely randomized, consisting of eight treatments, with 4 replications.

At 55 DAS, gas exchange was evaluated and then the plants were collected and separated into shoots and roots for biochemical, morphological and nutritional analyses.

#### Physiological Analysis

Gas exchange evaluations were performed on a fully expanded leaf to determine photosynthetic

#### Treatments and experimental design

Efficient microorganisms (EM) were obtained in an area of virgin forest, in the city of Cacique Doble, RS, Brazil (-27.815751° S, 51.723833° W) (SISGEN registration number: AE7F435). Initially, 700 grams of uncooked rice were placed in a plastic tray covered with a screen. The tray was positioned at the edge of the forest after the litter was removed. After 15 days, the microorganisms present in the rice were sent to the Laboratory of Entomology and Biochemistry at the Federal University of Fronteira Sul, Erechim, RS, Brazil. The organisms with pink, blue, yellow and orange coloration were selected for the study, these being the regenerating microorganisms. For the multiplication and activation of microorganisms, the collected material was added to 2 L containers containing 200 mL of molasses as a culture medium, for a period of 30 days. The gases produced were released daily (ANDRADE, 2020). *Azospirillum brasilense* (BiomaMais®, Bioma, Brazil) and a mix of *Bacillus subtilis* and *Bacillus megaterium* (BiomaPhos®, Bioma, Brazil) were obtained commercially.

rate ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol m}^{-1} \text{s}^{-1}$ ), transpiration ( $E$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ), and the relationship between the internal and external CO<sub>2</sub> concentration ( $C_i/C_a$ ). From these data it was possible to calculate the water use efficiency ( $WUE = A/E$ ). The evaluations were carried out between 8 and 10 h under photosynthetically active radiation ( $\sim 1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), and environmental CO<sub>2</sub> concentration ( $C_a$ ,  $\sim 430 \mu\text{mol mol}^{-1}$ ) and temperature ( $\sim 20 \text{ }^\circ\text{C}$ ). For these determinations, an infrared gas analyzer (IRGA; LCA PRO Analytical Development Co. Ltd, Hoddesdon, UK) was used.

#### Morphological Analysis

Plant height was determined by measuring with a ruler. The stem diameter was measured with a digital caliper (Stainless Hardened, Jamarca) at 5 cm from the soil base. Subsequently, the plants were separated into shoots and roots and dried in an oven with forced air circulation at 60° C, until

constant matter. Dry matter was determined using an analytical balance.

#### Biochemical Analysis

To determine the activity of the acid phosphatase enzyme, samples of leaves and roots were collected and immediately frozen in liquid nitrogen, and stored at -20 °C until the moment of analysis.

Acid phosphatase activity was determined according to Tabaldi *et al.* (2007). Samples of leaves and roots (~500 mg) were homogenized in 3 mL of citrate solution (100 mM) and centrifuged at 20,000 x g for 30 min at 4° C. The supernatant was collected and homogenized in a reaction solution containing citrate buffer (100 mM, pH 5.5), sodium azide (3.5 mM) and calcium chloride (2.5 mM), and incubated at 35°C for 10 min. The reaction started by the addition of the organic pyrophosphate substrate (PPi, 3.0 mM) and stopped with the addition of trichloroacetic acid (TCA, 5%). Inorganic phosphate was quantified at 630 nm using a spectrophotometer (700 Plus, FEMTO Indústria e Comércio de Instrumentos, São Paulo, Brazil), using malachite green as a colorimetric reagent. Acid phosphatase enzyme activity was determined based on a standard curve of KH<sub>2</sub>PO<sub>4</sub> (10 mM) and expressed as fresh weight.

#### Nutritional analysis

Phosphorus content was determined in the aerial part of bean plants, previously dried and

ground, according to the methodology described by Tedesco *et al.* (1995). Initially, the samples (~0.2 g) were digested with H<sub>2</sub>O<sub>2</sub> (30%) and H<sub>2</sub>SO<sub>4</sub> in a digester block at 160-180°C until the water evaporated. Subsequently, the samples were kept at 350-375°C for 1 h. After cooling the flasks, the material was resuspended in distilled water and stored in a refrigerator. Samples were analysed at 660 nm in a spectrophotometer (Halogen Lamp, Nova).

#### Statistical analysis

Data were submitted for analysis of variance and the means were compared by Tukey's test at 5% probability, using the *easynova* package in the R statistical software (version 4.0.4). For the multivariate analysis, the data were transformed by cube root and scaled by centering the mean and analyzed using the *MetaboAnalyst* software (version 5.0).

#### Results and Discussions

The natural phosphate treatment promoted the highest photosynthetic rate in bean plants, followed by the treatments RNP+A. *brasilense*, *Bacillus*, RNP+*Bacillus* and RNP+EM (Table 2). In general, transpiration was higher in treatments containing RNP, while stomatal conductance, water use efficiency and Ci/Ca ratio did not differ statistically between treatments (Table 2).

**Table 2.** Photosynthetic rate (*A*, μmol m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance (*g<sub>s</sub>*, mol m<sup>-1</sup> s<sup>-1</sup>), transpiration (*E*, mol m<sup>-2</sup> s<sup>-1</sup>), the relationship between the internal and external CO<sub>2</sub> concentration (Ci/Ca), and water use efficiency (WUE) of bean plants collected 55 days after sowing and cultivated after seed treatment application with *Azospirillum brasilense* (6 mL Kg<sup>-1</sup>), efficient microorganisms (EM; 1mL kg<sup>-1</sup>), *Bacillus subtilis* and *B. megaterium* (1mL Kg<sup>-1</sup>), fertilizer containing reactive natural phosphate (RNP; 140g ha<sup>-1</sup>) and the combination of RNP with the different microorganisms.

Treatment <sup>o</sup>	<i>A</i> (μmol m <sup>-2</sup> s <sup>-1</sup> )	<i>g<sub>s</sub></i> (mol m <sup>-2</sup> s <sup>-1</sup> )	<i>E</i> (mol m <sup>-2</sup> s <sup>-1</sup> )	WUE	Ci/Ca
Control	1.22 b	0.042 a	0.460 b	2.380 a	0.875 a
<i>Azospirillum brasilense</i>	1.23 b	0.035 a	0.445 b	2.485 a	0.860 a
EM	1.31 b	0.040 a	0.498 ab	2.107 a	0.869 a
<i>Bacillus</i>	2.06 ab	0.035 a	0.475 ab	3.055 a	0.815 a
RNP	3.61 a	0.045 a	0.630 ab	4.075 a	0.737 a
RNP + <i>Azospirillum brasilense</i>	2.40 ab	0.048 a	0.705 a	2.424 a	0.828 a
RNP + EM	1.78 ab	0.043 a	0.630 ab	2.345 a	0.831 a
RNP + <i>Bacillus</i>	1.82 ab	0.035 a	0.553 ab	2.447 a	0.822 a

Means of treatment followed by the same letter did not differ by Tukey's test (p<0.05).

In plants, phosphate is a constituent of adenosine triphosphate (ATP), a molecule that represents the metabolic energy of cells and is essential for carrying out processes such as photosynthesis, respiration, glycolysis, starch biosynthesis and ion absorption (HAWKESFORD *et al.*, 2011; VENEKLAAS *et al.*, 2012; CARSTENSEN *et al.*, 2018). Despite being an essential nutrient for plants, the concentration of P in the soil solution, at the root-soil interface, is often present at submicromolar levels, with a low rate of diffusion and mobility (JHORI *et al.*, 2015). Thus, plants with adequate phosphorus supply are

able to maintain higher photosynthetic rates as a consequence of maintaining ATP production (SILVA *et al.*, 2006; LIN *et al.*, 2009; WARREN, 2011).

Furthermore, bacteria of the genus *Azospirillum* are described for promoting plant growth, stimulating the production of phytohormones (auxins) that act on root development, allowing the mineralization of organic phosphate and solubilization of inorganic phosphate. In studies with corn, *Azospirillum* also contributed to the increase in gas exchange in plants (RODRÍGUEZ & FRAGA, 1999).

The natural phosphate associated with EM contributed to the higher plant height and root dry matter in relation to the treatment with natural phosphate plus *A. brasilense* (Table 3). The EMs act by decreasing compaction and increasing soil aggregation/porosity, which directly affects water availability and rooting depth. They also act in the decomposition of organic matter and in the mineralization and availability of nutrients for plants (BONFIM *et al.*, 2011; OLIVEIRA *et al.*, 2021). The synergistic effect of the plant with the microorganisms can be expressed by the plant growth rates, since the increase in the efficiency of phosphorus use indicates the solubilization of aluminum, calcium and iron metal-cation complexes precipitated and fixed in the soil (OLIVEIRA *et al.* 2019; ESTESAMI, 2020). Most of these effects are due to the presence of arbuscular mycorrhizal fungi, which increase the contact area of plant roots with the soil, allowing greater P absorption (WANG *et al.*, 2012), and, consequently, greater growth and growth. plant productivity, as already described for maize (ALMAGRABI & ABDELMONEIM, 2012), chickpeas (PELLEGRINO & BEDINI, 2014), soybean (OLIVEIRA *et al.*, 2019) and cotton (GAO *et al.*, 2020) plants. In addition, the interaction between bacterial and fungal microorganisms with phosphate sources allowed an increase in the total dry matter of sugarcane and greater efficiency of reactive phosphate (GUIMIERE *et al.*, 2019). This occurs when there is a good symbiotic performance of microorganisms with the host

plants, as in legumes, root growth is potentiated as the root contact surface increases, thus generating an increase in the bioavailability of nutrients, mainly of phosphorus (TALAAT *et al.*, 2015).

The largest stem diameter (Table 3) was observed in bean plants exposed to natural phosphate associated with *B. subtilis* and *B. megaterium*. Species of the genus *Bacillus* are described for being growth-promoting bacteria, promoting an increase in the root surface (SOUZA *et al.*, 2015). In addition, *B. subtilis* and *B. megaterium* act directly on the solubilization of phosphorus and/or the release of soluble phosphates (KALAYU, 2019), in order to increase the efficiency use of this nutrient, which makes them economically important, since it allows reducing the doses of fertilizers used and maintaining crop productivity (ABREU *et al.*, 2017).

The highest activity of acid phosphatase in the aerial part of bean plants was observed in the treatment with RNP, followed by the treatments RNP+*Bacillus*, RNP+EM and RNP+*A. brasilense* (Table 4). The acid phosphatase enzyme acts by hydrolyzing phosphate esters into soluble P, which allows the cycling and availability of this nutrient and the maintenance of metabolic activities in plants (VENEKLAAS, 2012). The availability of P in the medium favors the growth of microorganisms, which, in adequate and not excessive concentration, stimulates soil microorganisms in the production and secretion of phosphatases for greater organic hydrolysis of P (NAHAS, 2015).

**Table 3.** Plant height (PH), stem diameter (SD), shoot dry matter (SDM) and root dry matter (RDM) of bean plants collected 55 days after sowing and cultivated after seed treatment application with *Azospirillum brasilense* (6 mL Kg<sup>-1</sup>), efficient microorganisms (EM; 1mL kg<sup>-1</sup>), *Bacillus subtilis* and *B. megaterium* (1mL Kg<sup>-1</sup>), fertilizer containing reactive natural phosphate (RNP; 140g ha<sup>-1</sup>) and the combination of RNP with the different microorganisms.

Treatment	PH (cm plant <sup>-1</sup> )	SD (mm plant <sup>-1</sup> )	SDM (g plant <sup>-1</sup> )	RDM (g plant <sup>-1</sup> )
Control	10.33 ab	3.36 ab	0.282 a	0.380 a
<i>A. brasilense</i>	9.63 ab	3.16 ab	0.376 a	0.227 ab
EM	10.15 ab	3.07 ab	0.238 a	0.184 b
<i>Bacillus</i>	9.40 ab	3.28 ab	0.229 a	0.238 ab
RNP	10.15 ab	3.37 ab	0.261 a	0.154 b
RNP + <i>A. brasilense</i>	8.98 b	2.82 b	0.223 a	0.268 ab
RNP + EM	10.70 a	3.08 ab	0.297 a	0.372 a
RNP + <i>Bacillus</i>	10.00 ab	3.60 a	0.350 a	0.230 ab

Means of treatment followed by the same letter did not differ by Tukey's test (p<0.05).

Thus, microorganisms, in addition to increasing the surface area of the roots by the extension of the root system with the mycorrhizae, they release phytohormones, favoring the displacement of the uptake balance through the transfer of phosphate ions to the soil solution. This increases P mobility, and stimulates metabolic processes related to the P cycle, such as the production of phosphatases, enzymes that hydrolyze organic phosphorus, release organic acids and excrete hydrogen ions (MENDES *et al.*, 2003).

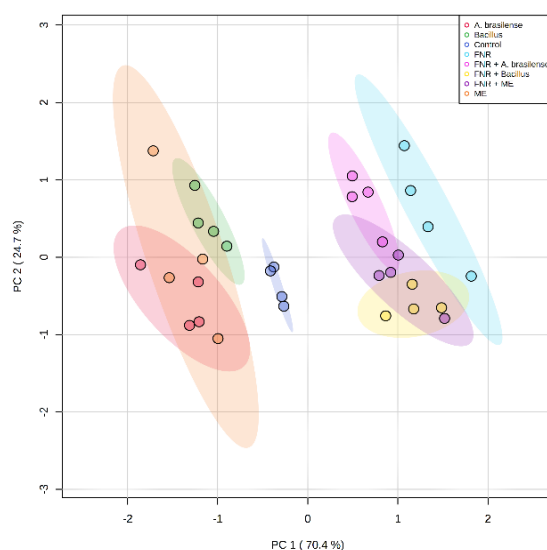
Although the P content in the aerial part of the bean plants did not differ between the

treatments evaluated (Table 4), the treatments based on RNP associated with different microorganisms, promoted, in general, higher values for gas exchange and production of dry matter in bean plants. It should also be noted that the genetic potential of cultivars to uptake P depends on factors such as morphology, presence of root hairs, mycorrhizal colonization and association with phosphorus-solubilizing microorganisms, secretion of phosphatase enzymes and high photosynthetic efficiency. This occurs since the responses may vary depending on the genotype used and the soil and climate conditions of cultivation (HUNGRIA *et al.*, 2010).

**Table 4.** Acid phosphatase activity (APase. U mg<sup>-1</sup> protein) in shoot (Sh) and roots (R), and total phosphorus content (P, %) in shoot of bean plants collected 55 days after sowing and cultivated after seed treatment application with *Azospirillum brasilense* (6 mL Kg<sup>-1</sup>), efficient microorganisms (EM; 1mL kg<sup>-1</sup>), *Bacillus subtilis* and *B. megaterium* (1mL Kg<sup>-1</sup>), fertilizer containing reactive natural phosphate (RNP; 140g ha<sup>-1</sup>) and the combination of RNP with the different microorganisms.

Treatment	Apase (Sh)	Apase (R)	P
Control	189.5 c	192.0 ab	0.236 a
<i>A. brasilense</i>	102.4 d	209.3 ab	0.211 a
EM	116.4 d	279.6 ab	0.212 a
<i>Bacillus</i>	143.6 cd	312.8 a	0.216 a
RNP	438.6 a	242.4 ab	0.222 a
RNP + <i>A. brasilense</i>	337.4 b	275.2 ab	0.212 a
RNP + EM	355.7 b	158.7 ab	0.224 a
RNP + <i>Bacillus</i>	358.0 b	130.1 b	0.209 a

Means of treatment followed by the same letter did not differ by Tukey's test (p<0.05).



**Figure 1.** Score plot for physiological and morphological traits, and P content of bean plants collected 55 days after sowing and cultivated after seed treatment application with *Azospirillum brasilense* (6 mL Kg<sup>-1</sup>), efficient microorganisms (EM; 1mL kg<sup>-1</sup>), *Bacillus subtilis* and *B. megaterium* (1mL Kg<sup>-1</sup>), fertilizer containing reactive natural phosphate (RNP; 140g ha<sup>-1</sup>) and the combination of RNP with the different microorganisms.

Taken the data together, it was possible to verify a clear separation between treatments containing RNP associated with microorganisms and treatments in the absence of RNP, both separate from the control group (Figure 1). This demonstrates that the association of RNP with microorganisms was advantageous for the physiological activities and growth of bean plants.

## Conclusion

Treatment with reactive natural phosphate associated with *Azospirillum brasilense* and other microorganisms contributed to a higher photosynthetic rate and transpiration in bean plants. The treatments RNP+EM and RNP+*Bacillus* promoted greater plant height and stem diameter, respectively. The shoot dry matter was higher in the RNP+EM treatment, in relation to the other treatments. Acid phosphatase activity was higher in the shoots of bean plants exposed to

reactive natural phosphate, and in the roots of plants subjected to treatment with *Bacillus subtilis* and *B. megaterium*.

It was possible to verify that the application together of microorganisms with natural phosphate, despite not causing an increase in the P content of the shoot, favored the conditions of growth and production of bean plants. Thus, it can be concluded that seed inoculation with growth-promoting microorganisms and phosphorus solubilizers, associated with fertilization with reactive natural phosphate, benefits the physiological, biochemical and morphological aspects of bean plants.

## References

ABREU, C. S. de; FIGUEIREDO, J. E. F.; OLIVEIRA-PAIVA, C. A.; SANTOS, V. L. dos; GOMES, E. A.; RIBEIRO, V. P.; BARROS, B. de A.; LANA, U. G. de P.; MARRIEL, I. E. Maize endophytic bacteria as mineral

- phosphate solubilizers. *Genetics and Molecular Research*. v. 16, n. 1, p. 1-13, 2017.
- ALLAHVERDIYEV, S. R.; KIRDAR, E.; GUNDUZ, G.; KADIMALIEV, D.; REVIN, V.; FILONENKO, V.; RASULOVA, D. A.; ABBASOVA, Z. I.; GANI-ZADE, S. I.; ZEYNALOVA, E. M.; Effective microorganisms (EM) technology in plants. *Technology*. Bardin. Turquia. ed. 14, p. 103- 106, 2014.
- ALMAGRABI, O. A., ABDELMONEIM, T. S. Using of arbuscular mycorrhizal fungi to reduce the deficiency effect of phosphorous fertilization on maize plants. p. 1648, 2012.
- ALTIERI, M. A. *Agroecology: the scientific basis of alternative agriculture*. Boulder: Westview Press, 1987.
- ALVES, R. T. *et al.* Microrganismo e disponibilidade de fósforo (P) nos solos: uma análise crítica. *Embrapa Cerrados*. Planaltina. Distrito Federal. ed. 1, 2003.
- ANDRADE, F. M. C. *Caderno dos microrganismos eficientes (EM). Instruções práticas sobre uso ecológico e social do EM*. UFV, Viçosa, MG, ed. 3, 2020.
- CARSTENSEN, A., HERDEAN, A., SCHMIDT, S., SHARMA, A., SPETEA, C., PRIBIL, M., HUSTEND, S. The impacts of phosphorus deficiency on the photosynthetic electron transport chain. *Plant Physiology*. v. 177, p. 271- 184, 2018.
- COELHO, J. D. *Produção de grãos – feijão, milho e soja*. *Caderno setorial ETENE*. Fortaleza- CE, 2018. Disponível em: [https://www.bnb.gov.br/documents/80223/3585904/grao\\_s\\_33-2018.pdf/ed76744b-3ae6-ef50-43f2-f4e72c457f10](https://www.bnb.gov.br/documents/80223/3585904/grao_s_33-2018.pdf/ed76744b-3ae6-ef50-43f2-f4e72c457f10). Acesso em: 31 mar. 2020.
- COLA, G. P. A.; SIMÃO, B. P. Rochagem como forma alternativa de suplementação de potássio na agricultura agroecológica. *Revisão de literatura*. *Revista Verde de Agroecologia e Desenvolvimento Sustentável Grupo Verde de Agricultura Alternativa (GVAA)*. Mossoró. Rio Grande do Norte. v. 7, n. 1, p. 1- 8. jan- mar. 2012.
- CONA Acompanhamento da Safra Brasileira. v. 1, n. 1, 2021.
- BONFIM, F.P.G. *et al.* *Caderno dos microrganismos eficientes (EM). Instruções práticas sobre uso ecológico e social do EM*. UFV, Viçosa, MG, 2011.
- DINIZ, P. F. A.; OLIVEIRA, L. E. M.; GOMES, M. P.; CASTRO, E. M.; MESQUITA, A. C.; BONONE, L. T. S.; SILVA, L. Crescimento, parâmetros biofísicos e aspectos anatômicos de plantas jovens de seringueira inoculadas com fungo micorrízico arbuscular *Glomus clarum*. *Acta Botanica Brasílica*. p. 65- 72, 2010.
- EMBRAPA. *System of Soil Classification*. Brasília: Embrapa Solos, ed. 3, p, 353, 2013.
- ELIAS, H. T. *Informações técnicas para o cultivo do feijão na Região Sul brasileira*. Comissão Técnica Sul-Brasileira de feijão. Epagri. Florianópolis. Santa Catarina. ed. 2, p. 157, 2012.
- ETESAMI, H. Enhanced phosphorus fertilizer use efficiency with microorganisms. In: Meena R. (eds) *Nutrient Dynamics for Sustainable Crop Production*. p. 215- 245, 2020.
- FAOSTAT. *Crops*. Disponível em: <http://www.fao.org/faostat/en/#data/QC>. Acesso em: 30 mar. 2022.
- FERNANDES, L. A.; FAQUIN, V.; FURTINI, A. E. N.; CURI, N. Frações de fósforo e atividade da fosfatase ácida em plantas de feijoeiro cultivadas em solos de várzea. *Revista Brasileira de Ciência do Solo*. p. 561-571, 2000.
- GAO, X., GUO, H., ZHANG, Q., GUO, H., ZHANG, L., ZHANG, C., GOU, Z., LIU, Y., WEI, J., CHEN, A., CHU, Z. Fungos micorrízicos arbusculares (FMA) melhoraram o crescimento, a produtividade, a qualidade da fibra e a regulação do fósforo em algodoeiro de terras altas (*Gossypium hirsutum* L.). *Cientific Reports*. p. 12, 2020.
- GUIMIERE, T.; ROUSSEAU, A. N.; COSTA, D. P.; CASSETARI, A.; COTTA, S. R.; ANDREOTE, F. D.; GUIMIERE, S. J.; PAVINATO, P. S. Phosphorus source driving the soil microbial interactions and improving sugarcane development. *Scientific Reports*. p. 1- 9, 2019.
- HAWKESFORD, M.; HORST, W.; KICHEY, T.; LAMBERS, H.; SCHJOERRING, J.; MØLLER, I.S.; WHITE, P.; *Functions of macronutrients*. *Marschner's Mineral Nutrition of Higher Plants*. 3rd Ed. Elsevier Inc. 2011.
- HERDRICKSON, L.; CHOW, W. S.; FURBANK, R. T. Low temperature effects on grapevine photosynthesis: the role of inorganic phosphate. *Functional Plant Biology*. v. 31, p. 789- 801, 2004.
- HEINEMANN, A. L. Feijão. cap. 11, pg. 185, In: *Agrometeorologia dos cultivos*. Instituto nacional de meteorologia – INMET. Brasília. Distrito Federal. p. 530, 2009.
- HUNGRIA, M.; CAMPO, R. J.; PEDROSA, F. O. Inoculation with selected strains of *Azospirillum brasilense* and *Azospirillum lipoferum* improves yields of maize and wheat. *Brazil Plant and Soil*. p.413-425, 2010.
- JOHRI, A.K.; OELMÜLLER, R.; DUA, M.; YADAV, V.; KUMAR, M.; TUTEA, N.; VARMA, A.; BONFANTE, P.; PERSSON, B.L.; STROUD, R.M.; Fungal association and utilization of phosphate by plants: success, limitations, and future prospects. *Front. Microbiol.* 2015, 6, 984.
- KALAYU, G. Phosphate solubilizing microorganisms: promising approach as biofertilizers. *International Journal of Agronomy*, v. 2019, p.7, 2019.
- LIN, Z. H.; CHEN, L.; CHEN, R.; ZHANG, F.; JIANG. H.; TANG, N. CO<sub>2</sub> assimilation, ribulose-1,5-bisphosphate carboxylase/oxygenase, carbohydrates and photosynthetic electron transport probed by the JIP-test, of tea leaves in response to phosphorus supply. *Plant biology*, p. 43-55, 2009.
- MA. Y.; VOSATKA, M.; FREITAS, H. Beneficial Microbes Alleviate Climatic Stresses in Plants. *Plant Sci*. p. 595, 2019.

- MENDES, I. C.; REIS, F. B. J. Microrganismo e disponibilidade de fósforo (P) nos solos: uma análise crítica. Embrapa Cerrados. Planaltina. Distrito Federal. ed. 1, 2003.
- MENG, X., CHEN, W., WANG, Y., HUANG, Z., CHEN, L., Y, T. Effects of phosphorus deficiency on the absorption of mineral nutrients, photosynthetic system performance and antioxidant metabolism in *Citrus grandis*. Plos One. p. 20, 2021.
- MORENCO, R. Fisiologia Vegetal. Viçosa, MG. Ed. UFV. ed. 3, p. 251. 2009.
- NAHAS, E. Control of acid phosphatases expression from *Aspergillus niger* by Soil Characteristics. Agriculture, Agribusiness and Biotechnology. v. 58, n. 5, p. 658, 2015.
- OLIVEIRA, T. C.; MÜLLER, C.; CABRAL, J. S. R.; TAVARES, G. G.; REZENDE, L. S.; SOUCHIE, E.; MENDES, GISELLE C. O papel das micorrizas na mitigação dos estresses abióticos em plantas cultivadas. o papel das micorrizas na mitigação dos estresses abióticos em plantas cultivadas. ed. 16.: Atena, v. 1, p. 180-190, 2021.
- OLIVEIRA, T. C.; UEHARA, H. M.; DA SILVA, L. D.; TAVARES, G. G.; SANTANA, L. R.; CABRAL, J. S. R.; SOUCHIE, E. L.; MENDES, G. C. Produtividade da soja em associação ao fungo micorrízico arbuscular *Rhizophagus clarus* cultivada em condições de campo. Revista de Ciências Agroveterinárias, Lages, v. 18, n. 4, p. 530-535, 2019. DOI: 10.5965/223811711832019530.
- PELLEGRINO, E., AND BEDINI, S. Enhancing ecosystem services in sustainable agriculture: biofertilization and biofortification of chickpea (*Cicer arietinum* L.) by arbuscular mycorrhizal fungi. Soil Biol. v. 68, p. 429–439, 2014.
- PIMENTEL, C. Leaf protoplasmic tolerance to water Stress in bean genotypes. Physiology and Molecular Biology of Plants. v. 6, p. 15-20, 2005.
- RAMAKRISHNA, W.; YADAV, R.; LI, K. Bactérias promotoras de crescimento de plantas na agricultura: dois lados de uma moeda. Ecologia Aplicada ao solo, v. 138, p. 10- 18, 2019.
- RAVEN, J. A. RNA function and phosphorus use by photosynthetic organisms. Review Article. v. 4, p. 536, 2013.
- RODRÍGUEZ, H.; FRAGA, R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances. p. 319-339. 1999.
- SILVA, A. A.; DELATORRE, C. A. Alterações na arquitetura de raiz em resposta à disponibilidade de fósforo e nitrogênio. Revista de Ciências Agroveterinárias. v. 8, n. 2, p. 152- 163, 2009.
- SILVA, C. E. M.; GONÇALVES, J. F. C.; FELDPAUSCH, T. R.; LUIZÃO, F. J.; MORAIS, R. R.; RIBEIRO, G. Eficiência no uso dos nutrientes por espécies pioneiras crescidas em pastagens degradadas na Amazônia central. Acta Amazonica, 503-512. 2006.
- SILVA, L. S. Manual de calagem e adubação para os estados do Rio Grande do Sul e Santa Catarina.
- Sociedade Brasileira de Ciência do solo- Núcleo Regional Sul. [s. l.]: Comissão de Química e Fertilidade do Solo- RS/ SC, p. 376, 2016.
- SOUZA, R.; AMBROSINI, A.; PASSAGLIA, L. M. P. Plant growth-promoting bacteria as inoculants in agricultural soils. Genetics and Molecular Biology. 2015, v. 38, n. 4, p. 401-419. Disponível em: <https://www.scielo.br/j/gmb/a/qdJ8jSMKJjbszhVDncqpxp/?lang=en#>. Acesso em: 21 jun. 2021.
- TABALDI, L. A.; RUPPENTHAL, R.; CARGNELUTTI, D. Effectes of metal elements on acid phosphatase activity in cucumber (*Cucumis sativus* L.) seedlings. Environmental and Experimental Botany. Amsterdam, v. 59, p. 43-48, 2007.
- TALAAT, N. B.; GHONIEM, A. E.; ABDELHAMID, M. T.; SHAWKY, B. T. Effective microorganisms improve growth performance, alter nutrients acquisition and induce compatible solutes accumulation in common bean (*Phaseolus vulgaris* L.) plants subjected to salinity stress. Plant Growth Regul. pg. 281-295. 2015. Disponível em: [www.researchgate.net/publication/280601351](http://www.researchgate.net/publication/280601351). Acesso em: 05 abr. 2020.
- TAIZ, L.; ZEIGER, E. Fisiologia vegetal. Porto Alegre: Artmed, ed. 4, 2009.
- TEDESCO, M. J.; GIANELLO, C.; BISSANI, C. A.; BOHNEN, H.; VOLKWEISS, S. J. Análise de solo, plantas e outros matérias. Boletim técnico. 2. ed, n. 5, UFRGS. Porto Alegre. 1995.
- UNITED NATIONS ENVIRONMENT PROGRAMME (UNEP). Global. Environment outlook 2000. London: Earthscan Publications. 2000.
- VENEKLAAS, E. J.; LAMBERS, H.; BRAGG, J.; FINNEGAN, P. M.; LOVELOCK, C. E.; PLAXTON, W. C.; PRINCE, C. A.; SCHEIBLE, W.; SHANE, M. W.; WHITE, J. A. R. Opportunities for improving phosphorus-use efficiency in crop plants. New Phytologist, p. 306-320, 2012.
- WANG, Y.; SHI, Y.; LI, B.; SHAN, C.; IBRAHIM, M.; JABEEN, A.; XIE, G. S. Phosphate solubilization of *Paenibacillus polymyxa* and *Paenibacillus macerans* from mycorrhizal and non- mycorrhizal cucumber plants. African Journal of Microbiology Research, v. 6, n.21, p. 4567-4573, 2012.
- WARREN, C. R. How does P affect photosynthesis and metabolite profiles of *Eucalyptus globules*? Tree Physiology, p. 727-739. 2011.
- ZHANG, T. MA, F. H. L. Phosphate-solubilizing bactéria from safflower rhizosphere and their effect on seedling growth. Open Life Sciences. 14, n. 1, p. 246- 254, 2019.