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EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ON THE GROWTH OF *AGAVE ANGUSTIFOLIA* HAW. (MAGUEY ESPADÍN)

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Abstract

Experiments were conducted to evaluate the potential effects of phosphate solubilizing bacteria (PSB) on the growth of maguey espadín (*Agave angustifolia* Haw.). A total of nine strains of PSB, previously isolated in the maguey rhizosphere and identified as PSBVa, PSBVb, PSBVf, PSBHa, PSBHc, PSBHd, PSBMg, PSBMh and PSBMi were tested on maguey plants grown in phosphorus-deficient sterilized and unsterilized soils of three sites where maguey is grown (valley, hill and mountain) under greenhouse conditions. Aerial dry weight and the total weight of maguey plants inoculated with PSBMh in unsterile mountain soil increased by 12.14% and 10.99% respectively, compared with the uninoculated control. Total root dry weight increased by 17.85% with PSBMh in sterile mountain soils and by 11.64% with PSBMg in unsterile mountain soils, with respect to the uninoculated sterile and unsterile control respectively. Plant stem diameter increased by 13.61% with PSBVb in unsterile valley soil, compared to the uninoculated control. Root length was 56.84% higher with PSBMi in unsterile mountain soils than the uninoculated control. Plant leaf number increased by 35% with PSBHd in unsterile hill soil, with respect to the uninoculated control. In all treatments, the available phosphorus at harvest was higher than pre-planting levels. This study suggests that isolated PSBMh, PSBMg and PSBMi are the most efficient biofertilizers for maguey espadín cultivation.

Key words: Biofertilizers, Maguey plants, Phosphorus-deficient soils, Plant growth promotion.

Introduction

Free-living or associative rhizosphere bacteria can stimulate plant growth through nutrient solubilization, synthesis of plant growth regulators, nitrogen fixation, production of siderophore compounds and control of plant pathogens (Loredo-Osti *et al*., 2004). These microorganisms are known for promoting plant growth (Kloepper & Schroth, 1978) and can be used as biofertilizers in crops (Vessey, 2003). Some rhizobacteria, such as phosphate solubilizing bacteria (PSB) produce organic acids capable of solubilizing phosphates that form soil-based insoluble compounds (Goldstein, 2007), whereas others are more active in the phosphate liberation of organic compounds through the production of phosphatase enzymes (Osorio-Vega, 2007). Phosphorus is one of the principal macronutrients responsible for plant growth and root development and it is essential for many metabolic processes including photosynthesis, sugar decomposition and the transference of energy and nutrients (Begon *et al*., 1990; Ehrlich, 1990). Although phosphorus is found in soil at concentrations of 400-1200 mg kg^{-1} (Begon *et al*., 1990; Fernández & Novo, 1988), its availability is very low, approximately 1 mg kg^{-1} or less (Beever & Burns, 1980; Goldstein, 1994). The use of PSB as inoculants can increase phosphorus availability for plants (Rodríguez & Fraga, 1999). Some studies note that inoculation with *Rhizobium leguminosarum* improved root colonization, promoted plant growth and significantly increased phosphorus concentration in lettuce and corn (Chabot *et al*., 1993, 1996).

Agave angustifolia Haw. known regionally in Oaxaca (Mexico) as "maguey espadín" is a cultivated specie of great socio-economic value in this entity. It is estimated in more than 15500 ha planted of the species in the "mezcal region" of Oaxaca, the highest production, more than 50%, is concentrated in the Tlacolula district (Chagoya-Méndez, 2004). However, research focused on the agricultural management of this maguey is scarce; little information has been generated in respect to the management of soil fertility and plant nutrition (Robles-Martínez *et al*., 2013). Bautista-Cruz *et al*. (2007) reported the fertility conditions of soil cultivated with maguey espadín in the Tlacolula district in accordance with topographic variations from agroecosystems and plant age, concluding that in general, soils are poor in organic matter, nitrogen and phosphorus, conditions in which to expect a positive response to PSB inoculation. The objective of this study was to evaluate the effects of the inoculation of native PSB strains on the growth of maguey espadín under greenhouse conditions.

Materials and Methods

Strains: The PSB strains used in this study, designated as PSBVa, PSBVb, PSBVf, PSBHa, PSBHc, PSBHd, PSBMg, PSBMh and PSBMi, were isolated from the rhizosphere of maguey espadín cultivated in valley (V), hill (H), and mountain (M) sites respectively, in the Tlacolula district of Oaxaca after serial dilution of soil solution on Sundara Rao and Shina (SRS) agar medium (Sundara & Sinha, 1963). These strains were previously selected for their high phosphate solubilizing ability. To evaluate the effect of PSB inoculation on the growth of maguey espadín plants, a microbial suspension was prepared with a scraping from each strain. Each sample was transferred to a tube with 10 ml of a 0.85% saline solution and was adjusted to a cellular concentration of 15 x 10^8, using Mc Farland's scale as a reference. 10 ml of SRS liquid medium without indicator was added to 1ml of the microbial suspension, thus obtaining a pre-inoculant. 1 ml of the pre-inoculant for each strain was added to 45 ml of the SRS liquid medium and incubated at 30°C, for 12 h at 150 opm. To produce the inoculant the bacteria were separated from the liquid medium by centrifugation at 3000 rpm for five minutes. They were then diluted in 0.85% sterile saline solution to a final concentration of 1.5×10^8 cells ml^{-1}, using Mc Farland's scale as a reference.

Experimental design: The maguey espadín seedlings were obtained from rhizomatous tillers cultivated in the Tlacolula district. The roots of the rhizomatous tillers were washed with sterile water to eliminate any adhered soil, disinfected by immersion in 0.624% sodium hypochlorite for five minutes, washed with distilled sterile water and then placed in a container filled with sterile river sand for one week. The experimental unit was a maguey espadín seedling planted in a rigid polyethylene pot with a capacity of 1200 g of substrate. The substrate was a mixture of native soil from the sites where maguey espadín is grown (valley, hill and mountain) and sterile river sand in a 1:1 proportion (v/v). The assay included sterile and unsterile substrate. In the last condition, the sterilization was carried out by autoclaving for 1 h at 121°C for three consecutive days.

A randomized block design was used with four replications for a total of 20 treatments and 144 experimental units (Table 1). PSB inoculants were applied at a dose of 5 ml per plant pot. The growth of the inoculated plants occurred entirely in a greenhouse (day temperature 26-30°C; night temperature 18-20°C; photoperiod 14 h light, 10 h dark). Irrigation was performed weekly with a Hoagland solution, diminishing the phosphorus concentration by ten. The phosphorus concentration was only varied by changing the volume of KH_2PO_4 (Hershley, 1994; Maynard & Hochmuth, 2007). The growth period evaluated was 16 weeks after transplantation.

Response variables: Plant height, stem diameter, leaf number and plant-available soil phosphorus content were measured monthly. At the end of the evaluation period, the solid soluble content of the stem (°Bx), root length, plant aerial dry weight, total root dry weight and total weight of plant were determined. The plant-available soil phosphorus content was extracted with a 0.5 M $NaHCO_3$ solution adjusted to pH 8.5. The extracted phosphorus was quantified with the procedure modified by Murphy-Riley (Olsen & Sommers, 1982).

Statistical analysis: The data was submitted to a variance analysis and a multiple range test for mean separation (Tukey $p \leq 0.05$). The registered data was transformed, prior to analysis, with the arcCos procedure, in order to satisfy the requirement of normal distribution. A Pearson correlation analysis was used to determine the relation between the analyzed variables. In all cases the statistical software JMP 7.0® (JMP 7.0 for windows 7) was used.

Table 1. Biofertilizer treatments of native strain of phosphate solubilizing bacteria (PSB) inoculated in maguey espadín plants (*Agave angustifolia* Haw.) grown in mountain (M), hill (H) and valley (V) soils under greenhouse conditions.

Biofertilizer treatment	Unsterile soil (T1) number of replicates	Sterile soil (T2) number of replicates
PSBVa	4	4
PSBVb	4	4
PSBVf	4	4
PSBHa	4	4
PSBHc	4	4
PSBHd	4	4
PSBMg	4	4
PSBMh	4	4
PSBMi	4	4
Uninoculated sterile control, SC	0	36
Uninoculated unsterile control, C	36	0

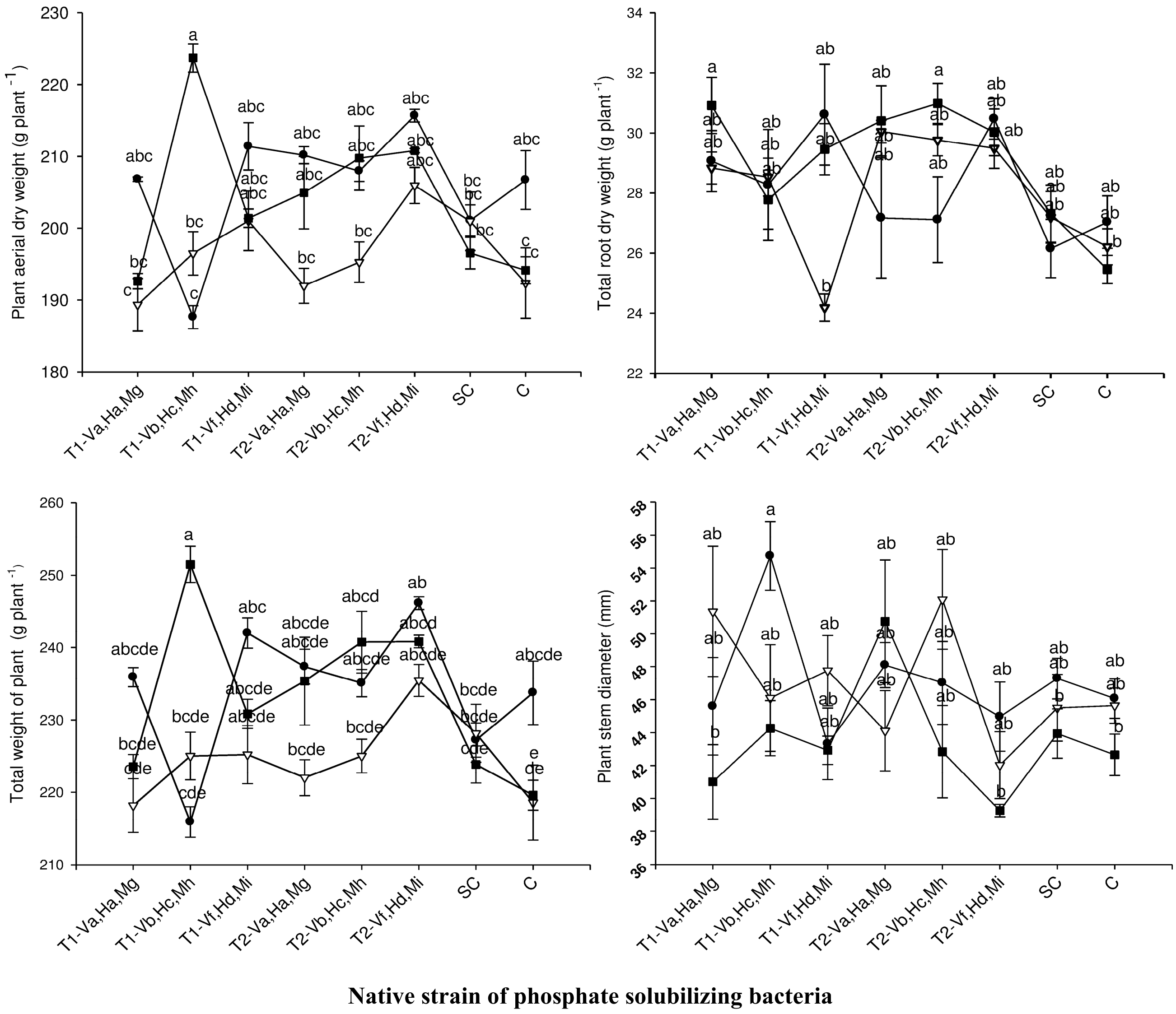

Fig. 1. Influence of native strain of phosphate solubilizing bacteria isolated from the rhizosphere of maguey espadín (*Agave angustifolia* Haw.) grown in mountain (M), hill (H) and valley (V) soils on plant aerial dry weight, total root dry weight, total weight of plant and plant stem diameter under greenhouse conditions. SC, uninoculated sterile control; C, uninoculated unsterile control; T1, inoculated unsterile soil, T2 inoculated sterile soil. Different lowercase letters indicate the biofertilizer treatment effect at 0.05-probability levels based on Tukey test.

Results and Discussion

Microbial processes such as biological nitrogen fixation, phosphate solubilization and cellulose degradation, among others have the potential to supplement the nutrient requirements of crops. The contributions of these microbial processes have been improved with the introduction of efficient microorganisms to the rhizosphere (Messele & Pant, 2012). In this study, 75% of the evaluated growth variables responded positively to PSB inoculation, whereas plant height and solid soluble content of the stem (°Bx) were not significantly affected by the biofertilization treatments. The aerial dry weight and total weight of the maguey plants inoculated with the isolated PSBMh in unsterile mountain soil increased by 12.14% and 10.99%, respectively, in comparison to the uninoculated control (Fig. 1). The root dry weight increased by 17.85% in plants that received the PSBMh inoculant in sterile mountain soil and by 11.64% in plants inoculated with the isolated PSBMg in unsterile mountain soil in comparison with the respective uninoculated controls (Fig. 1). The increase in these parameters can be attributed to two factors: 1) an increase in cellular elongation and multiplication, due to enhanced nutrient uptake, particularly of phosphorus, promoted by PSB inoculation; 2) the production of plant growth promoting substances such as auxins, cytokinins, gibberellins and some volatile compounds, due to the PSBs in the rhizosphere zone (Podile & Kishore, 2006; Appanna, 2007). Previous studies report an increase in the total biomass content as a consequence of PSB inoculation in lettuce (Chabot *et al*., 1996), wheat (Appanna, 2007), corn (Hameeda *et al*., 2006; Hussain *et al*., 2013), peanut (Pandey *et al*., 2006) and bean (*Vicia faba* L.) plants. Walpola & Yoon (2013) also found that the radical biomass of mung bean seeds (*Vigna radiata*) inoculated with PSB increased.

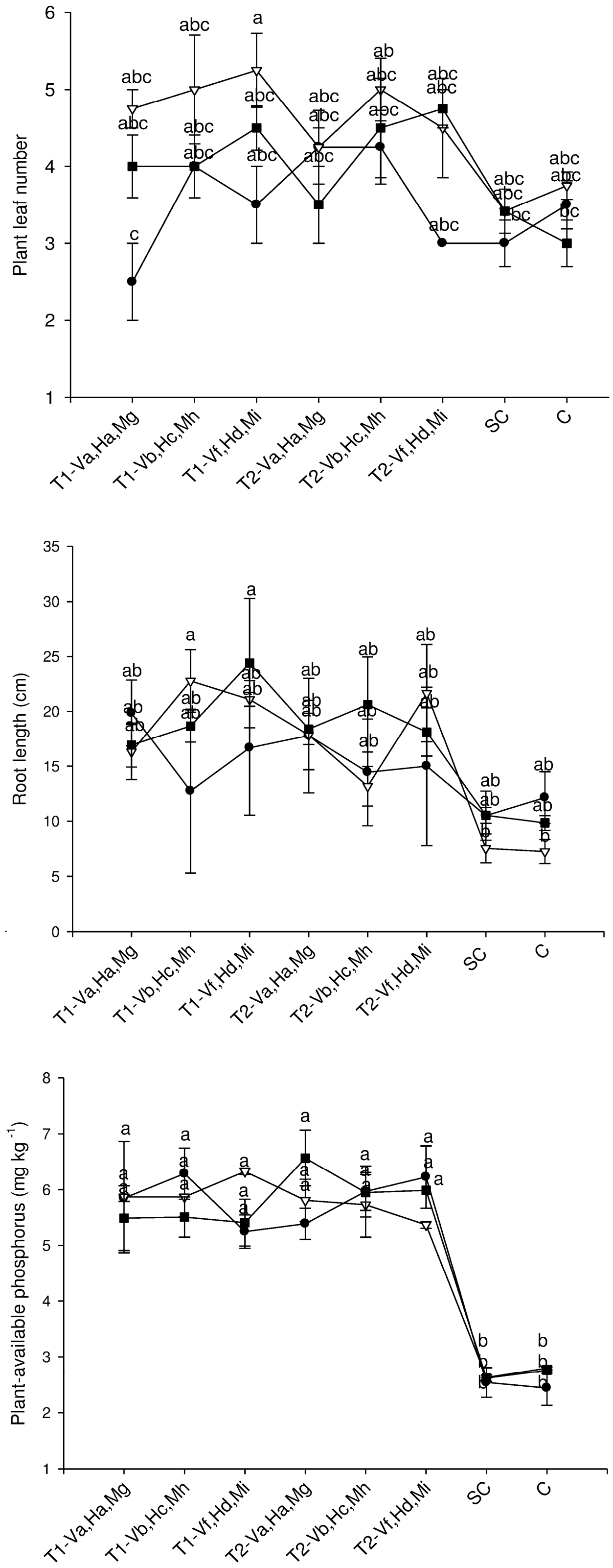

Fig. 2. Influence of native strain of phosphate solubilizing bacteria isolated from the rhizosphere of maguey espadín (*Agave angustifolia* Haw.) grown in mountain (M) —■— , hill (H) —▽— and valley (V) —●— soils on plant leaf number, root length and soil plant-available phosphorus under greenhouse conditions. SC, uninoculated sterile control; C, uninoculated unsterile control; T1, inoculated unsterile soil, T2 inoculated sterile soil. Different lowercase letters indicate the biofertilizer treatment effect at 0.05-probability levels based on Tukey test.

Stem diameter increased by 13.61% in maguey plants with isolated PSBVb in unsterile valley soil (Fig. 1). Root length increased by 56.84% in plants grown in unsterile mountain soil with the PSBMi inoculant (Fig. 2). Similarly, an increase in root length has been observed in sorghum (Appana, 2007), corn (Hussain *et al*., 2013), alfalfa (Li *et al*., 2013) and rice (*Oryza sativa*) (Ravicumar *et al*., 2013) inoculated with PSB. Iqbal *et al.,* (2010) and Walpola & Yoon (2013) found an increased root length in mung bean seeds when they were inoculated with PSB and Demissie *et al.* (2013) also reported an increase in the root length of bean plants when inoculated with PSB. The increase in the root length of maguey plants may be attributed to the positive effect of the PSB inoculations, which could promote a greater accumulation of nutrients in the roots, as well as to the production of plant growth regulating hormones that improved root development. These two processes may conjointly improve water and nutrient uptake and in this way contribute to improved root growth (Demissie *et al*., 2013). Dowling & O'Gara (1994) indicated that many strains of *Pseudomonas* produce indole-3-acetic acid, which promotes root elongation by stimulating the development of root hairs.

The number of leaves increased by 35% in maguey plants grown in unsterile hill soil with the PSBHd strain (Fig. 2). Ghanem and Abbas (2009) observed an increase in plant height, branch number, number of pods, grain weight and yield in mung bean seeds following inoculation with *B. megaterium* in salt-affected soils. Demissie *et al.* (2013) also reported a greater quantity of leaves in bean plants that inoculated with PSB, in comparison with uninoculated plants. Similarly, Li *et al.* (2013) indicated an increase in the leaf number of alfalfa plants inoculated with PSB.

At the end of the 16-week growth period, the levels of plant-available phosphorus in the rhizosphere of maguey plants inoculated with PSB were higher than pre-planting levels for all treatments (Fig. 2). The positive and highly significant correlations between the amount of plant-available phosphorus in the soil at the end of the established growth period and the root length (r = 0.764; p<0.0001), the leaf number (r = 0.365; p<0.028), the aerial dry weight (r = 0.514; p<0.001) and the total root dry weight (r = 0.542; p<0.0006), suggest that maguey growth was favored by the phosphorus solubilized by the PSB. According to Chabot *et al*. (1996) phosphorus solubilization appears to be the most important mechanism in promoting plant growth. These authors demonstrated that two phosphate solubilizing strains of *R. leguminosarum* increased the phosphorus content in corn and lettuce. The increase in phosphorus availability, as a result of PSB inoculation, and its positive impact on maguey yield can be attributed to the ability of PSB strains to solubilize inorganic insoluble phosphates, as well as the production of plant hormones (Khalid *et al*., 2004; Hameeda *et al*., 2006). Other researchers (Walpola & Yoon, 2013) have also reported an increase in the available phosphorus content in the soil due to PSB inoculation.

Theoretical estimations suggest that the phosphorus accumulated in the soil is enough to sustain crop yields worldwide for approximately 100 years (Goldstein *et al.*, 1993). Phosphate solubilizing microorganisms can help to increase phosphorus availability to plants. This study demonstrates the effectiveness of isolated phosphate solubilizing microorganisms in promoting plant growth in maguey espadín grown in phosphorus-deficient soils in greenhouse conditions. The bacteria evaluated in this study for their potential use as P-biofertilizers are of particular interest for their capacity to increase the amount of phosphorus available to plants and soil. The positive influence of the isolated phosphate solubilizing bacteria on the growth and yield of maguey espadín can be further confirmed by testing its effectiveness in field conditions. However, further research is necessary to achieve a comprehensive understanding of the relationships between different PSB species and the effects on maguey espadín, which may reveal some of the mechanisms of the synergistic interactions that are involved in the promotion of plant growth.

Conclusions

Maguey espadín growth parameters improved with the inoculation of phosphate solubilizing bacteria. Plant aerial dry weight, total weight of plant, total root dry weight, stem diameter, root length and leaf number all increased in maguey plants inoculated with phosphate solubilizing bacteria. The strains PSBMh, PSBMg and PSBMi, isolated from the rhizosphere of maguey cultivated in mountain sites, were the most effective in promoting the growth of maguey espadín in phosphorus-deficient soils under greenhouse conditions. In all of the bio-fertilizer treatments the amount of phosphorus available in the soil at the end of the established growth period was greater than the amount of phosphorus prior to planting. The bacteria evaluated in this study for their potential use as P-biofertilizers are of particular interest for their capacity to increase the amount of phosphorus available to plants and soil. The positive influence of the isolated phosphate solubilizing bacteria on the growth and yield of maguey espadín can be confirmed by testing its effectiveness in field conditions. However, further research is necessary to achieve a deeper understanding of the relationships between different phosphate solubilizing bacteria species and the effects on maguey espadín, which may reveal some of the mechanisms of the synergistic interactions that are involved in the promotion of plant growth.

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