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Effect of phosphate-solubilizing bacteria isolated from semiarid soils on pitahaya seedlings (*Hylocereus undatus*)

*Efecto de bacterias solubilizadoras de fosfato aisladas de suelos semiáridos en plántulas de pitahaya (*Hylocereus undatus*)*

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ABSTRACT

Phosphate-solubilizing bacteria (PSB) are a group of organisms that solubilize fixed forms of phosphorus, making it available for the plant growth. The effect of three PSB strains, called PSBMi, PSBHc and PSBVa, on growth of pitahaya seedlings (*Hylocereus undatus*) was studied in a growth chamber. The results indicated that plant stem diameter, plant height, plant total dry weight and root length were greatest in pitahaya seedlings inoculated with PSBMi. The increase in these plant growth variables was 52.40, 34.71, 23.31 and 30.14% with respect to the control pitahaya seedlings not inoculated. The present study showed the potential of P-solubilizers on pitahaya seedling growth. However, further research is needed to understand the specific mechanisms involved in the positive effects of PSB on pitahaya plant growth and to verify these results under field conditions.

Key words: Biofertilizer, climbing cacti, growth improvement, peat moss.

RESUMEN

*Las bacterias solubilizadoras de fosfato (PSB) son un grupo de organismos que solubilizan formas insolubles de fósforo haciéndolas disponibles para el desarrollo vegetal. Se estudió el efecto de tres cepas PSB denominadas PSBMi, PSBHc y PSBVa sobre el desarrollo de plántulas de pitahaya (*Hylocereus undatus*), bajo condiciones de cámara de crecimiento. Los resultados indicaron que el mayor diámetro del tallo, altura de planta, peso seco y longitud de raíz se encontraron en las plántulas de pitahaya inoculadas con PSBMi, el incremento en estas variables fue de 52.40, 34.71, 23.31 y 30.14% respecto del control no inoculado. Este estudio mostró el potencial de las PSB sobre el desarrollo de plántulas de pitahaya. Sin embargo, se necesita más investigación para entender los mecanismos específicos involucrados en el efecto positivo de las PSB sobre el desarrollo de plantas de pitahaya así como verificar estos resultados bajo condiciones de campo.*

Palabras clave: *Biofertilizante, cactácea trepadora, incremento del desarrollo vegetal, peat moss.*

Introduction

Free-living or associative rhizosphere bacteria can stimulate plant growth through nutrient solubilizing, 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 (Jong-Soo *et al*., 2003) and can be used as biofertilizers in crops (Vessey, 2003). Some rhizobacteria, known as phosphate-solubilizing bacteria (PSB), produce organic acids capable of solubilizing phosphates that form soil-based insoluble compounds (Goldstein 2007; Bhattacharyya and Jha, 2012), whereas others are more active in the phosphate liberation from 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; it is essential for many metabolic processes, including photosynthesis, sugar decomposition and the transfer of energy and nutrients (Begon *et al*., 1990; Ehrlich, 1990; Lynch and Brown, 2008). The use of PSB as inoculants can increase phosphorus availability for plants (Rodríguez and Fraga, 1999). Some studies reported that inoculation with *Rhizobium leguminosarum* improved root colonization, promoted plant growth

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and significantly increased phosphorus concentration in lettuce and corn (Chabot *et al*., 1993, 1996).

Pitahaya species (*Hylocereus* spp.) are native to America. The natural distribution of *Hylocereus* ranges from low deciduous to high evergreen forests (Cálix de Dios, 2004). In the last two decades, the cultivation area of pitahaya (genus *Hylocereus*) has increased around the world due to the attributes of its fruit and economic returns. The plant's stems, fruit, and flowers are used in food as well as in medicine (Ortíz-Hernández *et al*., 2012). The objective of this study was to evaluate the effects of the inoculation of PSB strains on the growth of pitahaya seedlings (*Hylocereus undatus*) cultivated in peat moss as substrate under growth chamber conditions.

Materials and Methods

The PSB strains used in this study, designated as PSBMi, PSBHc and PSBVa, were isolated from the rhizosphere of maguey espadín (*Agave angustifolia* Haw.) cultivated in semiarid soils in the Tlacolula district of Oaxaca, Mexico, after serial dilution of soil solution on Sundara Rao and Sinha (SRS) agar medium (Sundara and Sinha, 1963). These strains were previously selected for their high phosphate solubilizing ability *in vitro*. To evaluate the effect of PSB inoculation on the growth of pitahaya seedlings, 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 cell concentration of 15 x 10^8, using McFarland's scale as a reference. 10 ml of SRS liquid medium without indicator was added to 1 ml of the microbial suspension, thus obtaining a pre-inoculant. One ml of the pre-inoculant of each strain was added to 45 ml of the SRS liquid medium and incubated at 30 °C for 12 h at 150 oscillations per minute (opm). To produce the inoculant, the bacteria were separated from the liquid medium by centrifugation at 3000 revolutions per minute (rpm) for five minutes. They were then diluted in 0.85% sterile saline solution to a final concentration of 1.5 $\times 10^8$ cells ml^{-1}, using McFarland's scale as a reference.

One-month old pitahaya seedlings were obtained from seeds germinated in Petri dishes. The roots of the pitahaya seedlings were disinfected by immersion in 0.624% sodium hypochlorite for five minutes, washed with distilled sterile water and then placed in polyethylene containers filled with 20 g of wet peat moss. The experimental unit was a pitahaya seedling planted in a polyethylene pot.

A randomized blocks design was used with 38 replications, giving a total of 4 treatments and 152 experimental units. PSB inoculants were applied at a dose of 10 ml per plant pot. The growth of the pitahaya seedlings occurred entirely in a Biotronette Mark III Lab-Line® (Melrose Park, IL USA) growth chamber, under continuous photosynthetically active radiation of 70 µmol m^{-2} s^{-1} (PAR measured with LI-190SA quantum sensor and LI-189 quantum meter, LI-COR, Lincoln NE USA) provided by four Philips cool white fluorescent lamps (TL40W/54RS), at a temperature of 25 °C ± 2 °C, 85-90% relative humidity and a 12 h light:12 h dark photoperiod for four months. Irrigation was performed weekly with a Hoagland solution, reducing the phosphorus concentration by a factor of ten. The phosphorus concentration was varied only by changing the volume of KH_2PO_4 (Maynard and Hochmuth, 2007). The growth was evaluated 16 weeks after transplantation. Plant height and stem diameter were measured monthly, while plant total dry weight and root length were determined at the end of the evaluation period.

Statistical analysis

The data was submitted to a variance analysis and a multiple range test to detect differences among means (Tukey $p \leq 0.05$). Prior to analysis the data were transformed with the arc cosine procedure in order to satisfy the requirement of normal distribution. In all cases the statistical software JMP 7.0® (JMP 7.0 for windows 7) was used.

Results and Discussion

All plant growth variables analyzed were greatest in pitahaya seedlings inoculated with PSBMi (Figure 1). This increase in the plant growth parameters measured was probably due to two factors: 1) an increase in cell elongation and multiplication due to enhanced nutrient uptake, particularly of phosphorus, promoted by PSB inoculation (Bhattacharyya and Jha, 2012); 2) the production of plant growth-promoting substances by the PSBs in the rhizosphere zone, as indicated by Podile and Kishore (2006) and (Appanna, 2007). Koo and Cho (2009) and Bhattacharyya and Jha (2012) mentioned that in addition to P-solubilizing, PSB could also have produced other secondary metabolites such as siderophore, which makes PSB more suitable as a biofertilizer. Stem diameter ranged

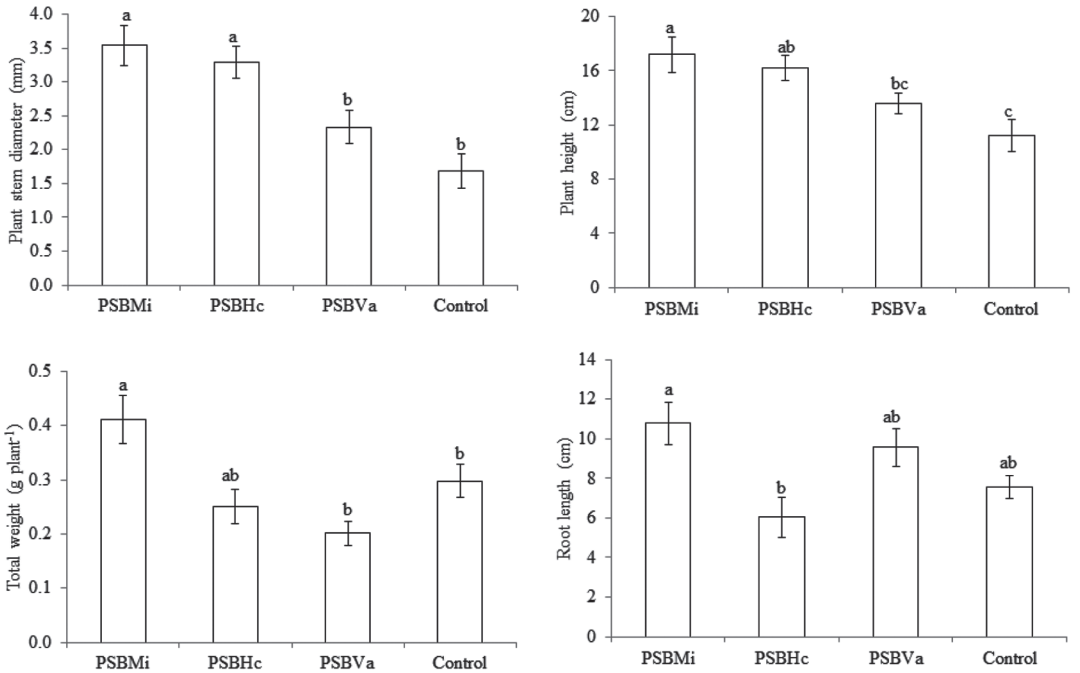

Figure 1. Influence of phosphate-solubilizing bacteria (PSB) on the growth of pitahaya seedlings (*Hylocereus undatus*). Different lower case letters indicate the biofertilizer treatment effect at 0.05 probability levels based on a Tukey test.

from 1.68 mm to 3.53 mm, this variable increased by 52.40% compared to the not inoculated control pitahaya seedlings (Figure 1). Plant height ranged from 11.21 cm to 17.17 cm, this variable increased 34.71% with respect to the not inoculated control seedlings (Figure 1). Ghanem and Abbas (2009) observed an increase in plant height, branch number, number of pods, grain weight and yield in mung beans following inoculation with *B. megaterium* in salt-affected soils. Other researchers have also reported an increase in plant height due to use of the *P. fluorescens* (Hameedaa *et al*., 2008; Gandi and Sivakumar, 2010).

Plant total dry weight ranged from 0.201 g plant^{-1} to 0.410 g plant^{-1}, this variable increased 23.31% compared to the not inoculated control pitahaya seedlings (Figure 1). The results of Piromyou *et al*. (2011) showed that maize seed inoculation with inoculants of PSB *Pseudomonas* sp. and *Brevibacillus* along with compost as nutrient material for the bacteria significantly increased growth, shoot and root dry weight compared to control plants. Root length ranged from 6.03 cm to 10.78 cm; this variable increased 30.14% compared to the not inoculated control pitahaya seedlings (Figure 1). Gupta *et al.* (2012) found the maximum increase in dry rind weight (51.8%) in plants treated with *P. synxantha*, whereas in case of root length, maximum stimulatory effect was seen in *S. marcescens*- treated plants.

Conclusions

The effects of PSB on growth of pitahaya species such as *H. undatus* are little known. The results suggest that PSBMi was the most effective strain to improve the pitahaya seedling growth parameters. The present study clearly indicates the potential of P-solubilizers on pitahaya seedling growth under chamber conditions. However, further research is needed to understand the specific mechanisms involved in the positive effects of PSB on pitahaya plant growth and to verify these results under field conditions.

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