Botanical Sciences 100 (1): 56-66. 2022
DOI: 10.17129/botsci.2841

Received: January 24, 2021, Accepted: April 29, 2021
First online: September 22, 2021

Ecology / Ecología

Plant growth-promoting bacteria belonging to the genera *Pseudomonas* and *Bacillus* improve the growth of sorghum seedings in a low-nutrient soil

Bacterias promotoras del crecimiento vegetal de los géneros *Pseudomonas* y *Bacillus* mejoran el crecimiento de plántulas de sorgo en un suelo con nutrientes escasos

Enriqueta Amora-Lazcano[1], Héctor J. Quiroz-González[1], Cristofer I. Osornio-Ortega[1], Juan A. Cruz-Maya[2], Janet Jan-Roblero[1]*

[1] Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, México.
[2] Unidad Profesional Interdisciplinaria en Ingeniería y Tecnologías Avanzadas, Instituto Politécnico Nacional, Ciudad de México, México.
*Corresponding author: jjan_r@yahoo.com.mx

Abstract

Background: Deficiency in sorghum growth in ecosystems of low-nutrient soils has been scarcely studied. This soil deficiency can be overcome by the addition of plant growth-promoting bacteria which increase sorghum growth.
Questions and/or Hypotheses: indole acetic acid (IAA) producing and phosphate solubilizing bacteria can promote sorghum growth under nutritional stress.
Studied species: *Sorghum bicolor* (L.) Moench.
Study site and dates: Mexico City, 2018.
Methods: Of the twelve bacterial strains utilized, three produce IAA (group BI), two strains produce IAA and siderophores (BIS group), four strains produce IAA and solubilize phosphate (BIP group), and three strains produce IAA, solubilize phosphate, and produce siderophores (BIPS group). Hydroponic bioassays and low-nutrient soil bioassay were used.
Results: In hydroponic bioassays, for BI and BIS groups, five strains significantly increased the growth parameters with respect to the control, and for the BIP and BIPS groups, two strains promoted stem development and shoot dry weight. In a low-nutrient soil bioassay, *Pseudomonas* sp. BI-1 (from BI group) was the one that presented the highest percentages 32, 48, 140 and 79 % in stem diameter, height and dry weight of the shoot and dry weight of the root, respectively, followed by the *P. mohnii* BIPS-10 strain (from BIPS group) that exhibited similar results.
Conclusions: IAA producing *Pseudomonas* strains improve the sorghum growth in a low-nutrient soil and suggest that *Pseudomonas* sp. BI-1 and *P. mohnii* BIPS-10 could be used as potential bioinoculants for sorghum.
Keywords: indole acetic acid, low-nutrient soil, *Pseudomonas*, soil, sorghum.

Resumen

Antecedentes: La deficiencia en el crecimiento del sorgo en suelos con nutrientes escasos es poco estudiada. Esta deficiencia puede superarse mediante la adición de bacterias promotoras del crecimiento vegetal que aumentan el crecimiento del sorgo.
Preguntas y/o hipótesis: bacterias que producen ácido indol acético (IAA) y solubilizan fosfato promueven el crecimiento del sorgo bajo estrés nutricional.
Especies de estudio: *Sorghum bicolor* (L.) Moench.
Sitio y años de estudio: Ciudad de México, 2018.
Métodos: de doce bacterias utilizadas, tres producen IAA (grupo BI), dos producen IAA y sideróforos (grupo BIS), cuatro producen IAA y solubilizan fosfato (grupo BIP), y tres producen IAA, solubilizan fosfato y producen sideróforos (grupo BIPS). Bioensayos hidropónicos y bioensayos en suelo con nutrientes escasos fueron empleados.
Resultados: en los bioensayos hidropónicos, para los grupos BI y BIS, cinco cepas incrementaron significativamente los parámetros de crecimiento con respecto al control, y para los grupos BIP y BIPS, dos cepas promovieron el desarrollo del tallo y el peso seco del brote. En los bioensayos con suelo con nutrientes escasos, *Pseudomonas* sp. BI-1 (del grupo BI) presentó los porcentajes más altos 32, 48, 140 y 79 % en el diámetro del tallo, altura y peso seco del brote y peso seco de la raíz, respectivamente, seguido de *P. mohnii* BIPS-10 (del grupo BIPS).
Conclusiones: *Pseudomonas* productoras de IAA mejoran el crecimiento del sorgo en un suelo con nutrientes escasos, sugiriendo que *Pseudomonas* sp. BI-1 y *P. mohnii* BIPS-10 podrían usarse como bioinoculantes potenciales para el sorgo.
Palabras claves: ácido acético indol, suelo con nutrientes escasos, *Pseudomonas*, sorgo, suelo.

Plant growth-promoting bacteria (PGPB) produce hormones, siderophores, solubilize phosphate and induce plant resistance to biotic and abiotic stress, among other (Santos *et al*. 2019) to improve plant growth. Among these activities, the ability to solubilize phosphates is attractive due to the presence of large amounts of phosphorus in soil, from which the largest portion is insoluble, and therefore, not available to promote plant growth (Kour *et al.* 2021). Plants absorb phosphorus only in two soluble forms: monobasic and dibasic. Insoluble phosphorus is present as inorganic material such as apatite, or in organic forms such as inositol phosphate, phosphomonoesters, and phosphotriesters (Sharma *et al.* 2013). In these scenarios, microorganisms that solubilize inorganic phosphorous play a critical role in providing phosphorous to plants (Castagno *et al*. 2021).

Moreover, the microbial synthesis of the phytohormone auxin (indole acetic acid, IAA) has been known for a long time, it is known that 80 % of the microorganisms isolated from the rhizosphere of various crops present the ability to synthesize and release auxins as secondary metabolites (Keswani *et al.* 2020). IAA plays a critical role in plant growth and development, as well as it can be produced as a defense response in diverse ways (Casanova-Sáez *et al.* 2021). Bacterial IAA increases root surface area and root length, providing the plant with a greater and easier access to soil nutrients. Additionally, rhizobacterial IAA increases the permeability of plant cell walls and, as a result, facilitates an increased amount of root exudation that provides additional nutrients to support bacterial growth (Majda & Robert 2018). Therefore, rhizobacterial IAA is identified as a key molecule in plant-microbe interaction, both in pathogenesis and phytostimulation (Ahemad & Kibret 2014).

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal in the world after wheat, rice, corn, and oats. Sorghum is used in the preparation of balanced foods to make soy sorghum flour or as a compound flour utilized for the manufacture of cookies, biscuits, bread, etc. In the extraction industry it is used to obtain starch, alcohol, and glucose (Nasidi *et al*. 2016, Hao *et al.* 2021). Furthermore, it is used in aceto-butyl fermentation from which solvents such as alcohol, acetone and butanol are produced. In 2016, Mexico was the fourth largest producer of sorghum worldwide. However, only 60 % of national requirements are satisfied with domestic production, which creates a dependence on imports (SAGARPA 2017). For this reason, it is necessary to implement strategies such as the application of biofertilizers during cropping to improve the productivity of this crop.

Since rhizobacteria are considered to have beneficial contributions to plants, our research group has made efforts to isolate PGPB. In a previous work (Flores-Núñez *et al.* 2018), rhizobacteria from agricultural and forest soils from a pine forest were isolated and identified. Several of the isolates presented *in vitro* a profile compatible with plant growth promotion (PGP); in fact, all bacteria are producers of IAA, some solubilize phosphate and/or produce siderophores. Therefore, these isolates could improve the growth of sorghum, in this study, some of these strains were selected to test their growth-promoting activity in sorghum seedlings at greenhouse level. Moreover, considering that climate change and low-nutrient soils are factors that influence the low production of sorghum crops, the incorporation of PGPB in low-nutrient soils represents an eco-friendly alternative to increase the production of this crop. It was also addressed whether the PGPB isolates can contribute to the growth of sorghum seedlings in low-nutrient soil, which would be interesting for the future application of these PGPB as bioinoculants for sorghum crops.

Materials and methods

Microbial resources. A collection of twelve bacteria obtained in a previous work, from an agricultural soil and a pine forest soil, were evaluated as PGPB for their ability to produce *in-vitro* IAA (I), siderophores (S) and /or solubilizing phosphate (P) (Flores-Núñez *et al.* 2018). All bacteria produce IAA, and based on their PGP activities, they were grouped as follows: bacteria that only produce IAA, called as BI group (n = 3); bacteria that also produce siderophores, BIS group (n = 2); bacteria that additionally solubilize phosphate, BIP group (n = 4); and bacteria that solubilize phosphate and produce siderophores, BIPS group (n = 3). Table 1 shows the bacteria used in this study.

Bacterial inoculum. Bacteria were inoculated onto nutrient agar plates and incubated for 24 h at 28 °C. Subsequently, a suspension of each bacterial species was prepared with sterile saline solution and adjusted to OD_{600nm} = 0.5, then 1 mL of this suspension was inoculated into flasks containing 49 mL of nutrient broth and was incubated at 20 ºC overnight.

Table 1. Characteristics that Promote the Growth of Sorghum Seedlings.

Group	Isolation source	Bacterial strain	Plant growth promoting		
			I	S	P
			(µg mL^{-1})	(mm)	
BI	**F**	*Pseudomonas* sp. BI-1	11.83 ± 0.36	-	-
(n=3)	**A**	*Pseudomonas mohnii* BI-2	12.42 ± 1.86	-	-
	A	*Bacillus subtilis* BI-3	10.75 ± 0.16	-	-
BIS	**F**	*Bacillus simplex* BIS-4	9.20 ± 0.86	1.50 ± 0.71	-
(n=2)	**A**	*Bacillus drentensis* BIS-5	0.61 ± 0.61	1.4 ± 0.55	-
BIP	**F**	*Pseudomonas mohnii* BIP-6	7.43 ± 0.42	-	+
(n=4)	**F**	*Burkholderia phytofirmans* BIP-7	4.97 ± 0.90	-	+
	A	*Phylobacterium* sp. BIP-8	3.8 ± 0.88	-	+
	A	*Chitinophaga japonensis* BIP-9	7.43 ± 1.38	-	+
BIPS	**F**	*Pseudomonas mohnii* BIPS-10	6.05 ± 0.42	3.68 ± 1.29	+
(n=3)	**F**	*Pseudomonas corrugata* BIPS-11	6.25 ± 0.99	3.42 ± 0.65	+
	A	*Burkholderia graminis* BIPS-12	2.86 ± 0.52	1.33 ± 0.49	+

F = Forest soil, A = Agricultural soil, I = Indol acetic acid producer, S = siderophores producer, P = solubilizer of phosphate (PO_4^{-3}), (+) positive; (-) negative. Data taken from Flores-Núñez *et al*. 2018.

Subsequently, bacterial growth was evaluated by CFU and a concentration of 10^7-10^8 CFU mL^{-1} was inoculated to each seedling.

The bacteria that had significant results in the different growth parameters of the sorghum seedlings in the hydroponics bioassay were selected to make mixtures among them and tested in the low-nutrient soil bioassay (as mentioned below). Multi-bacterial inoculum was prepared from pure cultures, following the previously described procedure. For this, 5 mL of each bacterium was taken and mixed.

Hydroponic nutrient solution (HNS). The synthetic hydroponic nutrient solution (HNS) was prepared from a macronutrient stock solution that contained: 1 mM $NH_4H_2PO_4$, 5.75 mM $Ca(NO_3)_2$, 1.3 mM $CaCl_2$, 2 mM $MgSO_4 \cdot 7H_2O$, 6 mM KNO_3, 24 µM H_3BO_3, 12 nM $Na_2MoO_4 \cdot H_2O$, 0.24 µM $CuCl_2 \cdot H_2O$, 5µM $ZnCl_2$, 0.88 µM $MnCl_2 \cdot 4H_2O$ and 14.2 nM Fe-EDTA. Modifications to the HNS were made in the assays of assimilation of phosphorus, wherein the phosphorus concentration was changed as follows: a) without phosphorus, HNS-wP, $NH_4H_2PO_4$ was replaced by the addition of 0.5 mM NH_4NO_3; b) half of phosphorus as insoluble tricalcium phosphate $Ca_3(PO_4)_2$ and the other half as soluble $NH_4H_2PO_4$, HNS-mP, was composed of 0.5 mM $NH_4H_2PO_4$ (instead of 1 mM), 0.5 mM $Ca_3(PO_4)_2$ and 0.25 mM NH_4NO_3 (to compensate the nitrogen source); and c) total phosphorus as insoluble tricalcium phosphate $Ca_3(PO_4)_2$, HNS-tP, 1 mM $Ca_3(PO_4)_2$, and 0.5 mM NH_4NO_3.

Sorghum seed germination. The seeds of *Sorghum bicolor* (L.) Moench were disinfested with 3 % NaClO for 1 min and subsequently washed with sterile distilled water. The disinfected seeds were placed on a paper previously wet with sterile distilled water with a separation of 3 cm between each seed, the paper containing the seeds was rolled up and incubated at 28 ºC in darkness for 6 days.

Hydroponics bioassay. The hydroponics bioassay was done with tyndallized vermiculite as the support for the development of sorghum seedlings. Inoculation of IAA-producing bacteria was conducted by dispensing a bacterial suspension from 10^7 to 10^8 CFU mL^{-1} over the neck area of the five-day-old sorghum homogenous seedlings, which were in containers with vermiculite. Subsequently, 15 mL of HNS was added to allow the inoculum to cover the root, facilitating colonization of the rhizosphere. The bioassay was kept during four weeks under greenhouse conditions with the exposure of 12 h of light and 12 h of darkness at room temperature (25-30 ºC), watering every other day with 15 mL of HNS diluted with distilled water in a ratio of 1:4. Each treatment included a total of 6 replicates and a control group was included without the inoculation of any bacteria.

Low-nutrient soil bioassay. This was performed with a mixture of sterile sand (121 °C, 15 PSI, for 15 min) with sieved soil (< 2 mm) from Cerro de la Estrella, Mexico City (19° 20' 31" N, 99° 05' 22" W, altitude 2,460 m); the sieved soil was sterilized by tyndallization by 3 cycles of flowing steam for 45 min, with 24 h of aeration at room temperature in each cycle. The sand and soil were mixed in a ratio of 2:5 volume/volume. The soil is considered as a low-nutrient soil due to the organic matter (4.46 %), nitrogen content (0.13 %) and soluble phosphorus (1.7 % mg Kg^{-1}) as determined previously (Gómez-Acata *et al*. 2019). Two control groups without bacterial inoculation were included, one was irrigated only with water and the other watered with HNS.

In the case of the phosphate-solubilizing bacteria, the test was carried out in a similar way to the inoculation of the bacteria described above, with the following modifications: a) HNS without phosphorus (HNS-wP); b) HNS with the half of phosphorus as insoluble tricalcium phosphate and half of the remaining phosphorus as $NH_4H_2PO_4$ (HNS-mP) and c) HNS with total of phosphorus as insoluble tricalcium phosphate (HNS-tP).

The bioassay was maintained for a duration of four weeks under greenhouse conditions with the exposure of 12 h of light and 12 h of darkness at room temperature (25-30 ºC), irrigating every seven days with the respective modified HNS and every other day with distilled water. Each treatment included six replicates.

Growth parameters of the sorghum seedlings. For both bioassays (hydroponics and low-nutrient soil), the seedlings growth parameters were determined. The height of the shoot was determined from the neck to the tip of the apical tissue. The stem diameter was determined at the height of the neck of the plant with a vernier caliper. The shoot and the root of the plant were cut, and both were placed in an oven at a temperature of 70 °C, for three days. After this time, the dry weight of the shoot and the root was determined.

Statistical analysis. Firstly, the results obtained from the bioassays were determined if they comply with the normality and homoscedasticity tests, to apply a parametric test. In all experiments, the results behaved as parametric data (Table S1). The parametric test used for the statistical analysis was a one-way ANOVA by Dunnett's statistical test with a significance level of 0.05. All statistical tests were performed with the GraphPad PRISM® program ver. 8.0.1.

Results

Evaluation of non-solubilizing phosphate (PO_4^{-3}) bacteria on sorghum seedlings in hydroponics bioassays. It was observed that there is a significant difference in plant growth in the presence of bacteria with respect to the control. Regarding the stem diameter of sorghum, the most noticeable result was that obtained with *Bacillus simplex* BIS-4 (Table 1; BIS group; Figure 1A), which produced an 18 % increase compared to the control group (sorghum without bacterial inoculum; $P < 0.05$). Regarding the height of shoot, it was observed that in four of the five bacteria, there was an increase that ranged from 7 to 15 % with respect to the control group ($P < 0.05$), except for *Bacillus drentensis* BIS-5 (Figure 1B). Regarding the effect on the biomass of the shoot, all the bacterial strains increased from 28 to 55 % with respect to the control group ($P < 0.05$; Figure 1C). In addition, a significant increase in the dry weight of the root was also observed with the treatments of the strains *Pseudomonas monhii* BI-2, *Bacillus subtilis* BI-3 and *B. simplex* BIS-4, of 76, 59 and 42 % compared to control group ($P < 0.05$; Figure 1D), respectively.

In general, it is observed that all the strains of the BI group had a positive effect on most of the growth parameters

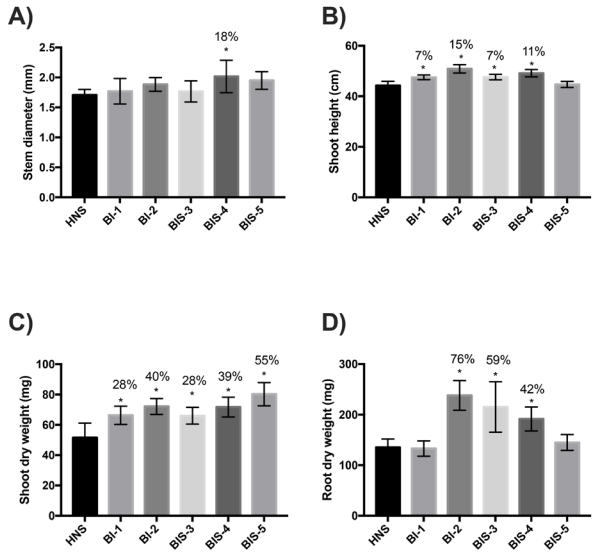

Figure 1. Effect of inoculation of IAA-producing strains (groups BI and BIS) on sorghum seedlings under hydroponic conditions. A) diameter of the stem, B) height of the shoot, C) shoot dry weight, and D) root dry weight. Error bars represent the standard deviation. *Treatments that show a significant difference with respect to the control (HNS) using the Dunnett's test ($P < 0.05$) (n = 6).

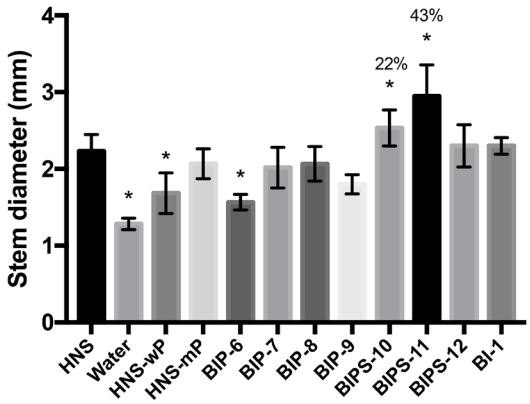

Figure 2. Effect of inoculation of phosphate solubilizing bacteria (BIP and BIPS groups) on sorghum seedlings under 50 % phosphate condition. Diameter of the stem in a bioassay with 50 % of tricalcium phosphate using vermiculite as support. Error bars represent the standard deviation. * Treatments showing significant difference with respect to the control of sorghum without bacterial inoculum and with HNS including 50 % tricalcium phosphate (HNS-mP). The Dunnett test ($P < 0.05$) (n = 6) was utilized.

of the sorghum seedlings, with a better performance of the *Pseudomonas monhii* BI-2 strain over the *Pseudomonas* sp. BI-1 strain. In the BIS group, only the *B. simplex* BIS-4 strain exerted a positive effect. Taking into consideration these results, all bacteria from groups BI and BIS were selected for the following low-nutrients soil bioassay.

Effect of phosphate (PO_4^{-3})-solubilizing bacteria on sorghum under hydroponic bioassay. The groups of bacteria that produce IAA and solubilize phosphate, BIP and BIPS groups (Table 1), were tested for their ability to solubilize phosphate, for this purpose two bioassays of availability of phosphate were included, one corresponds to applying half of the phosphorus (mP) as tricalcium phosphate (insoluble phosphate) and the remaining as diacid ammonium phosphate (soluble phosphate) (Figure 2), and in the other bioassay to adding only insoluble tricalcium phosphate (tP; Figure 3). The following controls were included, without the inoculation of any bacteria with HNS, HNS without phosphorus (HNS-wP), water only, and HNS with 50 % tricalcium phosphate (HNS-mP). In addition, a treatment with the inoculation of a non-solubilizing phosphate bacterium (*Pseudomonas* sp. BI-1) with HNS-mP was included.

In the bioassay with 50 % insoluble tricalcium phosphate, the controls corresponding to the growth of sorghum in the presence of HNS and 50 % insoluble phosphate (HNS-mP) did not show a significant difference between them; however, in the sorghum controls growing without phosphate (water or HNS-wP), there was a significant difference in all the growth parameters compared to the control in the presence of HNS, indicating the importance of phosphate for the growth of sorghum.

Regarding to the inoculated strains in the bioassy with 50 % insoluble tricalcium phosphate, *Pseudomonas mohnii* BIPS-10 and *Pseudomonas corrugata* BIPS-11 improve the stem diameter of the plant with an increment of 22 and 43 %, respectively, as compared to the HNS-mP control, without bacterial addition ($P < 0.05$; Figure 2), the *Pseudomonas* sp. BI-1 strain did not improve this parameter because it does not solubilize phosphate (control). Regarding the other three parameters no significant differences were observed (Figure S1).

In the bioassay with 100 % of insoluble tricalcium phosphate, the controls behaved similarly to the previous bioassay with 50 % of insoluble tricalcium phosphate. In the treatments with the inoculated strains, significant increases

were observed in the shoot dry weight (Figure 3A) and the root dry weight (Figure 3B) for *P. mohnii* BIPS-10 and *P. corrugata* BIPS-11 strains, with respect to the HNS-tP control without bacterial addition ($P < 0.05$); in contrast to the *Pseudomonas* sp. BI-1 strain (control) in which the percentage was lower. With the other two parameters no significant differences were obtained (Figure S2). From these results, *P. mohnii* BIPS-10 and *P. corrugata* BIPS-11 strains are the best options, since they have greater ability to solubilize phosphate in both conditions tested.

Effect of PGPB on sorghum seedlings using a low-nutrients soil bioassay. Based on the results obtained in the hydroponic conditions, the strains selected from the BI and BIS groups (Table 1) and the *P. monhii* BIPS-10 and *P. corrugata* BIPS-11 strains provided the best results. The next challenge was to test these selected strains in a low-nutrients soil bioassay. The bacteria were tested alone and in a multi-strains inoculum M1 (*P. monhii* BI-2 + *B. simplex* BIS-4 + *P. monhii* BIPS-10) and M2 (*P. monhii* BI-2 + *B. simplex* BIS-4 + *P. corrugata* BIPS-11).

The treatments with *Pseudomonas* sp. BI-1 and *P. mohnii* BIPS-10 strains increased significantly ($P < 0.05$) the stem diameter by 32 % compared to the control (sorghum without bacterial inoculum and irrigated only with water, Figure 4A). In contrast, eight of the nine treatments significantly improved the shoot height ($P < 0.05$) from 23 to 48 % in relation to the control (Figure 4B), being the strain *Pseudomonas* sp. BI-1 the one that achieved the highest percentage (48 %). Finally, seven of the nine treatments increased the biomass of the shoot dry weight significantly ($P < 0.05$), from 61 to 140 % compared to the control, highlighting the results obtained with *Pseudomonas* sp. BI-1 (140 %) and *P. mohnii* BIPS-10 (123 %) strains, with respect to the control (Figure 4C), a similar result was observed in the dry weight of the root of *Pseudomonas* sp. BI-1 (79 %) and *P. mohnii* BIPS-10 (67 %), with respect to the control (Figure 4D). Although most strains presented increases in sorghum growth, *Pseudomonas* sp. BI-1 presented the highest percentages in the four parameters tested. For example, 32, 48, 140 and 79 % in stem diameter, height and dry weight of the shoot and dry weight of the root, respectively, followed by the *P. mohnii* BIPS-10 strain. The other bacterial strains also improved the growth of sorghum, but not in the four parameters analyzed. For this reason, they are of less interest.

Discussion

PGPB promotes plant growth and development through a variety of mechanisms. The exact mechanism by which PGPB stimulates plant growth is not clearly understood, although various mechanisms such as the production of phytohormones, the suppression of harmful organisms, the phosphate solubilization and the improvement in absorption of mineral nutrients are involved in promoting plant growth (Santos *et al.* 2019). This work was focused on determining the effect of PGPB for promoting plant growth in sorghum.

First, we analyzed the bacteria that only produced IAA in the hydroponic bioassay using vermiculite as support. It was observed that most of the tested strains increased different growth parameters of sorghum. These parameters are established as indicators of the PGP, since an increase in the shoot height of the plant is highly relevant for the access to sunlight to carry out photosynthesis (Mahanty *et al*. 2017), making it more competitive by minimizing its permanence in greenhouse conditions, and therefore the cost associated with it. Furthermore, the increase in the height of shoot of the sorghum has the effect of a higher productivity of the grain (Grover *et al*. 2013). The same occurs with the increase in root biomass, which represents a benefit to the plant, since it increases the absorption surface for the uptake of nutrients and water, in addition to improving its stability since it has a better anchorage to the ground (Santos *et al*. 2019). It was observed that the majority of the tested strains increased different growth indicators of sorghum, and the increase in sorghum root biomass generated by the studied strains could be attributable to the IAA production, since the IAA production has been reported to promote root proliferation (Khalid *et al*. 2004, Keswani *et al.* 2020).

Regarding the effect of phosphate solubilizing bacteria on the growth of sorghum seedlings using an HNS with 50 % tricalcium phosphate, the results were unexpected. In the study reported by Flores-Núñez *et al.* 2018, it was exhibited that *Burkholderia phytofirmans* BIP-7, *Phylobacterium* sp. BIP-8, *Chitinophaga japonensis* BIP-9, *Pseudomonas*

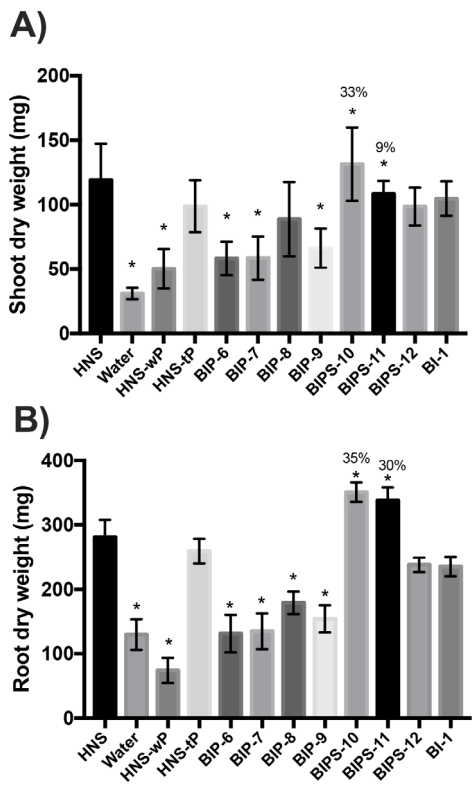

Figure 3. Effect of inoculation of phosphate solubilizing bacteria (BIP and BIPS groups) on sorghum seedlings under 100 % phosphate condition. A) shoot dry weight, and B) root dry weight in a bioassay with 100 % of tricalcium phosphate using vermiculite as support. Error bars represent the standard deviation. * Treatments showing significant difference with respect to the control of sorghum without bacterial inoculum and with HNS including 100 % tricalcium phosphate (HNS-tP). The Dunnett test ($P < 0.05$) (n = 6) was utilized.

corrugata BIPS-11 and *Burkholderia graminis* BIPS-12 solubilized tricalcium phosphate *in vitro*. However, under the experimental conditions tested here, these same strains did not improve the growth of sorghum seedlings. This result was unexpected since there is evidence that phosphate-solubilizing bacteria increase the yields of different crops in both, at greenhouse level and in an experimental field (Chuang *et al*. 2007, Valverde *et al*. 2007). The probable causes that led to this result could be that these strains inhibit the activity of solubilizing phosphate in the presence of the sorghum plant, another possibility is that the activity of solubilizing phosphate by these strains is low or the amount of inoculum used was not adequate to meet the demand of available phosphate for sorghum. More experiments are required to answer these questions.

The evaluation of the effect of PGPB on sorghum seedlings using a low-nutrient soil as support yielded significant increases in stem diameter, height and biomass of shoot, and biomass of root. These increases are attractive since it demonstrates that the plant-microorganism interaction allowed a better utilization of the resources provided by the soil, allowing the plant to grow more effectively.

It was observed that there was a better growth of sorghum when a single bacterial inoculum was used instead of a multi-strains inoculum. This could be explained by the antagonistic activity of some metabolite or by a simple competition for the space on the root surface among the members of the inoculum. However, it would be necessary to carry out the compatibility experiments that confirm the above. The biochemical activity of the bacteria being in consortium is complex and perhaps our bacterial mixtures were not adequate because of their similar PGP activities, such as *P. mohnii* BI-2, *B. simplex* BIS-4 and *P. mohnii* BIPS-10, with PGP activities that overlap, since they all produce IAA and two of them produce siderophores. It is possible that a multi-strains inoculum with different

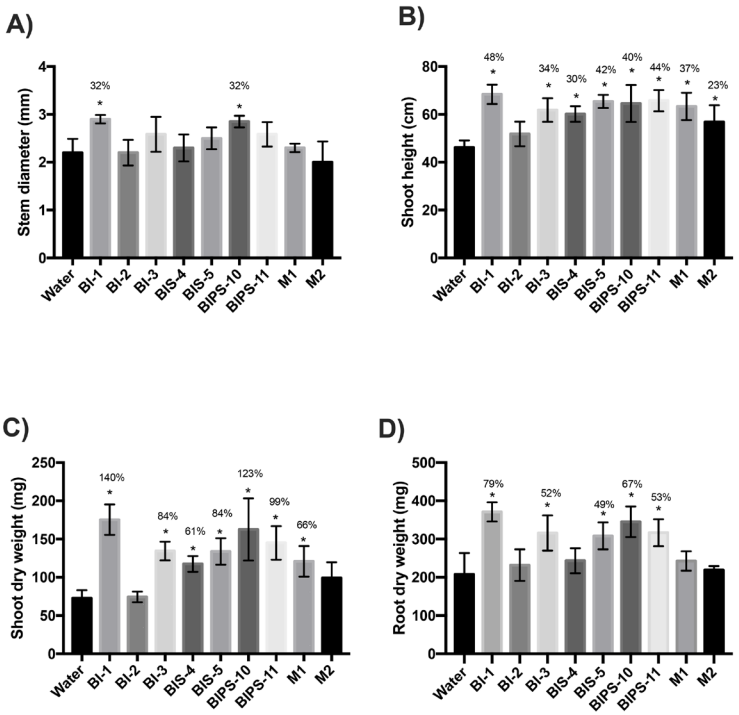

Figure 4. Effect of inoculation of selected PGPB on sorghum seedlings using a low-nutrient soil bioassay. A) diameter of the stem, B) height of the shoot, C) shoot dry weight and D) root dry weight. *Treatments that show significant difference with respect to the control irrigated with water. The Dunnett test ($P < 0.05$) (n = 6) was utilized.

PGP activities produce a synergistic effect, such as *Pseudomonas* sp. TR15 (ACC deaminase producer) and *Bacillus aerophilus* TR15c (siderophore producer) which have a synergistic effect promoting growth of *Helianthus annuus* L. (Kumar *et al.* 2021). In fact, our results are similar to those obtained in another study (Abdel-Salam *et al*. 2015), in which inoculated sorghum seedlings with *Azotobacter chroococcum, Azospirillum brasilense, Bacillus megaterium* and *Bacillus circulans* individually and in different bacterial mixtures, obtaining increases of 63-81 % with single strains rather than bacterial mixtures. Similarly, the individual inoculation of *Luteibacter* sp. XiU1292, *Pseudomonas vranovencis* XiU1297 or *Acinetobacter iwoffii* XiU12138 in sorghum seedlings, improved the growth parameters evaluated 4 weeks after inoculation (Mull *et al*. 2017). *Luteibacter* sp. XiU1292 produces IAA, siderophores and solubilizes phosphate, similarly to the *P. mohnii* BIPS-10 strain reported in this work. It has also been reported that the inoculation of *Bacillus* spp. in sorghum seedlings generated better growth, in terms of shoot length and root biomass, with an average increase of 7 to 9 % compared to non-inoculated plants (Grover *et al*. 2013). The strains used in that study are IAA producers, solubilize phosphate and do not produce siderophores, which are the same PGP activities of our strains. In contrast, soybeans co-inoculated with *Bradyrhizobium japonicum* and *A. brasilense* produced a better result compared to the individual-strain inoculum (Hungria *et al*. 2013). Furthermore, the same result was obtained with the co-inoculation of beans with *Rhizobium tropici* and *A. brasilense*. This suggests that the synergistic effects depend on the bacterial species involved.

Pseudomonas sp. BI-1, which only produces IAA as the only PGP activity, was one of the strains that yielded the best growth promotion results for sorghum. In this context, it has been shown that the inoculation of sorghum with *A. brasilense* strain SM with IAA production as the only PGP activity, produced increase of shoot height, and shoot dry weight (10-weeks), shoot height, and shoot dry weight (16-weeks) (Kochar & Srivastava 2011). This suggests that in the case of sorghum, the association with a bacterium that produces IAA is enough to improve its growth.

In view that *P. mohnii* BIPS-10 (IAA producer, phosphate solubilizer and siderophore producer) improved sor-

ghum growth below the *Pseudomonas* sp. B1, that only produces IAA, this could suggest that a bacterium with multiple PGP activities does not guarantee that it is better compared to a bacterium with mono-PGP activity. In the same context, Kumar *et al.* (2012), mentioned that the possession of multiple PGP activities in an isolate does not imply that its utilization as inoculum for the plant will yield better results. In fact, these authors showed that the strain *Pseudomonas* sp. P28 (IAA producer, siderophore, HCN, protease, phosphate solubilizer) did not increase the growth of sorghum seedlings with respect to the control, unlike the strain *Pseudomonas* sp. P38 (with a single PGP activity, P-solubilization), which increased the root volume and root dry weights of sorghum in 10 %, compared to the control. There are other works that describe the same behavior, but in other bacterial genera (Mareque *et al*. 2015, da Silva *et al*. 2018).

Apparently, in sorghum, the bacterial IAA is essential to improve its growth, or it may be possible that the bacterial species promote the improvement of this parameter. Strains of *Pseudomonas* genus inoculated to plants of *Phaseolus vulgaris* (beans), *Zea mays* (corn), *Solanum tuberosum* (potato) and *Lycopersicum esculentum* (tomato), under greenhouse conditions has promoted plant growth (Santillana-Villanueva 2006). In another study, *Solanum lycopersicum* L. (tomato), *Abelmoschus esculentus* (okra), and *Amaranthus* sp. (African spinach) were inoculated with the cultures of *P. aeruginosa* and *B. subtilis,* and observed increases in the different growth parameters of these plants (Adesemoye *et al*. 2008). Nonetheless, the above shows that the genus *Pseudomonas* are rhizobacteria that improve the growth parameters of various plants, including sorghum.

Furthermore, it has been shown that the most surprising results from the inoculation of PGPB are presented when utilizing soils with nutritional deficiencies, poor irrigation and adverse weather conditions (Grover *et al*. 2013, Inostroza *et al*. 2017). In our case, the soil that was utilized contained serious nutritional deficiencies in organic matter, nitrogen and soluble phosphorus, which were previously determined (Gómez-Acata *et al*. 2019). Therefore, with the results obtained, we corroborate the importance of the inoculation with *Pseudomonas* sp. BI-1 and/or *P. mohnii* BIPS-10 to improve sorghum growth under adverse conditions. It is worth mentioning that the strains utilized in this work were obtained from the same soil area that was used in the bioassays. The approach of selecting native strains has been implemented in other works, such as Koskey *et al*. (2017) which used indigenous strains with the ability to fix nitrogen in low fertility soils in Kenya in bean crops, obtaining an increase in grain yield of 30 % compared to the commercial inoculum for beans, Biofix (unreported strains).

In summary, sorghum growth has been scarcely studied in soils with nutritional stress; thus, the results of this work show that strains of *Pseudomonas* genus, with the activity of IAA production, is sufficient to improve the sorghum growth in a low-nutrient soil. This fact is attractive because it shows an alternative for the improvement of the sorghum crop by the utilization of PGPB like *Pseudomonas*. In addition, it also offers the possibility that *Pseudomonas* sp. BI-1 and *P. mohnii* BIPS-10, both producing IAA and isolated from the crop soil, could be considered as potential biofertilizers for sorghum.

Acknowledgments

The financial support for this investigation was provided by the Instituto Politécnico Nacional (IPN), Mexico, grants SIP20201785, SIP20211556 and SIP20210893. The authors JACM and JJR appreciate the COFAA and EDI, IPN fellowships, and the support from the SNI CONACyT.

Supplementary material

Supplemental data for this article can be accessed here: https://doi.org/10.17129/botsci.2841.

Literature Cited

Abdel-Salam A, Elhosainy O, Wissam Z, Abdel-Salam M, Hashem I. 2015. Npk biofertilizers on sorghum (*Sorghum bicolor*) grown on a light clay torrifluvent soil and interactions among them. *Egypt Journal Applied Sciences* **30**: 383-400.

Adesemoye A, Obini M, Ugoji EO. 2008. Comparison of plant growth-promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. *Brazilian Journal of Microbiology* **39**: 423-426. DOI: https://doi.org/10.1590/S1517-83822008000300003

Ahemad M, Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal King Saud University Science* **26**: 1-20. DOI: https://doi.org/10.1016/j.jksus.2013.05.001

Casanova-Sáez R, Mateo-Bonmatí E, Ljung K. 2021. Auxin metabolism in plants. *Cold Spring Harbor Perspectives in Biology* **13**: a039867. DOI: https://doi.org/10.1101/cshperspect.a039867

Castagno LN, Sannazzaro AI, Gonzalez ME, Pieckenstain FL, Estrella MJ. 2021. Phosphobacteria as key actors to overcome phosphorus deficiency in plants. *Annals of Applied Biology* **178**: 256.267. DOI: https://doi.org/10.1111/aab.12673

Chuang CC, Kuo YL, Chao CC, Chao WL. 2007. Solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger*. *Biology and Fertility of Soils* **43**: 575-584. DOI: https://doi.org/10.1007/s00374-006-0140-3

da Silva JF, da Silva TR, Escobar IEC, Fraiz ACR, Dos Santos JWM, do Nascimento TR, Dos Santos JMR, Peters SJW, de Melo RF, Signor D, Fernandes-Júnior PI. 2018. Screening of plant growth promotion ability among bacteria isolated from field-grown sorghum under different managements in Brazilian drylands. *World Journal of Microbiology and Biotechnology* **34**: 186. DOI: https://doi.org/10.1007/s11274-018-2568-7

Flores-Núñez V, Amora-Lazcano E, Rodríguez-Dorantes A, Cruz-Maya J, Jan-Roblero J. 2018. Comparison of plant growth-promoting rhizobacteria (PGPR) in a pine forest and an agricultural soil. *Soil Research* **56**: 346-355. DOI: https://doi.org/10.1071/SR17227

Gómez-Acata S, Amora-Lazcano E, Wang ET, Rivera-Orduña FN, Cancino-Diaz JC, Cruz-Maya JA, Jan-Roblero J. 2019. Nodule-forming *Sinorhizobium* and arbuscular mycorrhizal fungi (AMF) improve the growth of *Acacia farnesiana* (Fabaceae): an alternative for the reforestation of the Cerro de la Estrella, Mexico. *Botanical Sciences* **97**: 609-622. DOI: https://doi.org/10.17129/botsci.2200

Grover M, Madhubala R, Ali SZ, Ydav SK, Venkateswarlu B. 2013. Influence of *Bacillus* spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions. *Journal of Basic Microbiology* **54**: 951-961. DOI: https://doi.org/10.1002/jobm.201300250

Hao H, Li Z, Leng C, Lu C, Luo H, Liu Y, Wu X, Liu Z, Shang L, Jing HC. 2021. Sorghum breeding in the genomic era: opportunities and challenges. *Theoretical and Applied Genetics*. DOI: https://doi.org/10.1007/s00122-021-03789-z

Hungria M, Nogueira MA, Araujo RS. 2013. Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. *Biology and Fertility of Soils* **49**: 791-01. DOI: https://doi.org/10.1007/s00374-012-0771-5

Inostroza NG, Barra PJ, Wick LY, Mora ML, Jorquera MA. 2017. Effect of rhizobacterial consortia from undisturbed arid- and agro-ecosystems on wheat growth under different conditions. *Letters in Applied Microbiology* **64**: 158-163. DOI: https://doi.org/10.1111/lam.12697

Keswani C, Singh SP, Cueto L, García-Estrada C, Mezaache-Aichour S, Glare TR,

Borriss R, Singh SP, Blázquez MA, Sansinenea E. 2020. Auxins of microbial origin and their use in agriculture. *Applied Microbiology and Biotechnology* **104:** 8549-8565. DOI: https://doi.org/10.1007/s00253-020-10890-8

Khalid A, Arshad M, Zahir Z. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology* **96**: 473-480. DOI: https://doi.org/10.1046/j.1365-2672.2003.02161.x

Kochar M, Srivastava S. 2011. Surface colonization by *Azospirillum brasilense* SM in the indole-3-acetic acid dependent growth improvement of sorghum. *Journal of Basic Microbiology* **52**: 123-131. DOI: https://doi.org/10.1002/jobm.201100038

Koskey G, Mburu SW, Njeru EM, Kimiti JM, Ombori O, Maingi JM. 2017. Potential of native rhizobia in enhancing nitrogen fixation and yields of climbing beans (*Phaseolus vulgaris* L.) in contrasting environments of eastern Kenya. *Frontiers in Plant Science* **8**: 443. DOI: https://doi.org/10.3389/fpls.2017.00443

Kour D, Rana KL, Kaur T, Yadav N, Yadav AN, Kumar M, Kumar V, Dhaliwal HS, Saxena AK. 2021. Biodiversity, current developments and potential biotechnological applications of phosphorus-solubilizing and -mobilizing microbes: A review. *Pedosphere* **31**: 43-75. DOI: https://doi.org/10.1016/S1002-0160(20)60057-1

Kumar GP, Kishore N, Amalraj ELD, Ahmed S, Rasul A, Desai S. 2012. Evaluation of fluorescent *Pseudomonas* spp. with single and multiple PGPR traits for plant growth promotion of sorghum in combination with AM fungi. *Plant Growth Regulation* **67**:133-140. DOI: https://doi.org/10.1007/s10725-012-9670-x

Kumar A, Tripti, Maleva M, Bruno LB, Rajkumar M. 2021. Synergistic effect of ACC deaminase producing *Pseudomonas* sp. TR15a and siderophore producing *Bacillus aerophilus* TR15c for enhanced growth and copper accumulation in *Helianthus annuus* L. *Chemosphere* **276**: 130038. DOI: https://doi.org/10.1016/j.chemosphere.2021.130038

Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A, Tribedi P. 2017. Biofertilizers: a potential approach for sustainable agriculture development. *Environmental Science and Pollution Research international* **24**: 3315-3335. DOI: https://doi.org/10.1007/s11356-016-8104-0

Majda M, Robert S. 2018. The role of auxin in cell wall expansion. *International Journal of Molecular Sciences* **19**: 951. DOI: https://doi.org/10.3390/ijms19040951

Mareque C, Taulé C, Beracochea M, Battistoni F. 2015. Isolation, characterization and plant growth promotion effects of putative bacterial endophytes associated with sweet sorghum (*Sorghum bicolor* (L) Moench). *Annals of Microbiol*ogy **65**: 1057-1067. DOI: https://doi.org/10.1007/s13213-014-0951-7

Mull JGC, de la Riva GA, Vargas-Sámano CD, Pérez-Machado G, Agüero-Chapin G. 2017. Plant growth promoting bacteria isolated from a Mexican natural ecosystem induce water stress resistance in maize and sorghum plants. *Journal of Microbial and Biochemical Technology* **9**: 209-219. DOI: https://doi.org/10.4172/1948-5948.1000367

Nasidi M, Agu R, Deeni Y, Walker G. 2016. Utilization of whole sorghum crop residues for bioethanol production. *Journal of the Institute of Brewing* **122:** 268-277. DOI: https://doi.org/10.1002/jib.324

SAGARPA [Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación]. 2017. *Planeación agrícola nacional 2017-2030*. Primera Edición. Ciudad de México, México. https://www.gob.mx/cms/uploads/attachment/file/256433/B_sico-Sorgo_Grano.pdf (accessed May 19, 2020)

Santillana-Villanueva N. 2006. Biofertilizer production using *Pseudomonas* sp. *Ecología Aplicada* **5**: 87-91.

Santos MS, Nogueira MA, Hungria M. 2019. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB Expr*ess **9**: 205. DOI: https://doi.org/10.1186/s13568-019-0932-0

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* **2**: 587. DOI: https://doi.org/10.1186/2193-1801-2-587

Valverde A, Burgos A, Fiscella T, Rivas R, Veláquez E, Rodríguez-Barrueco C, Cervantes E, Chamber M, Igual JM. 2007. Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacterium) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. *In*: Velázquez E, Rodríguez-Barrueco C, eds. *First International Meeting on Microbial Phosphate Solubilization, Developments in Plant and Soil Sciences*. Dordrecht: Springer Netherlands, pp. 43-50. DOI: https://doi.org/10.1007/978-1-4020-5765-6_5

Associate editor: Joel Flores

Author contributions: EAL performed the hydroponic bioassays; HJQG, and CIOO carried out low-nutrient soil bioassay; JACM accomplished the statistical analysis; JJR designed the experiments and wrote the manuscript.