PGPR inoculation improves growth, nutrient uptake and physiological parameters of Capsicum chinense plants

La inoculación con PGPR mejora el crecimiento, la absorción de nutrientes y parámetros fisiológicos en plantas de Capsicum chinense

Castillo-Aguilar C de la C1, JJ Zúñiga-Aguilar2, AA Guzmán-Antonio2, R Garruña3

Abstract. The Habanero pepper (Capsicum chinense) is intensively cultivated in the Yucatan peninsula, México. Because of adverse environmental conditions, it required seeding in germination trays, from which six-week-old seedlings were transplanted to the soil. Adequate nursing and fertilization programmes were made to improve health and vigour before seedlings transplanting. During seed germination, we investigated the effects of inoculation with four plant growth-promoting rhizobacteria (PGPR) on growth, nutrient uptake and gas exchange of 8-week-old Capsicum chinense plants. Inoculation was made with Pseudomonas sp. -P61-, Pseudomonas sp. -A46-, Bacillus pumilus -R44-, and Paenibacillus polymyxa -BSP1.1-. The BSP1.1 strain produced the highest increase in plant height (22.52 cm), leaf area (99 cm²), fresh weight of roots (0.337 g) and shoots (2.83 g), and dry weights of roots (0.05 g) and shoots (0.43 g). Nitrogen uptake was similar (P>0.05) among treatments. However, seedlings inoculated with BSP1.1 had a higher accumulation than controls when they were exposed to P and K (50 and 40%, respectively). Likewise, seedlings inoculated with the BSP1.1 strain improved both photosynthesis (2 μmol/m²/s) and water use efficiency (7.3 μmol CO₂/mmol H₂O), and decreased transpiration rate (0.27 mmol/m²/s). Thus, the inoculation with the BSP1.1 strain was the best option to enhance growth and vigour of Capsicum chinense plants.

Keywords: Habanero pepper; Plant growth-promoting rhizobacteria; Leaf area; Biomass; Bioprotection.

Resumen. El chile habanero (Capsicum chinense) es cultivado extensivamente en la península de Yucatán México. Debido a los factores ambientales adversos, la siembra de las semillas se debió efectuar en bandejas germinadoras. Cuando las plántulas tuvieron seis semanas de edad fueron trasplantadas al suelo. Se efectuaron programas adecuados de semillero y fertilización para incrementar la salud y el vigor de las plántulas antes del trasplante. Se evaluó el efecto de la inoculación de cuatro cepas de rizobacterias promotoras del crecimiento vegetal (Pseudomonas sp. -P61-, Pseudomonas sp. -A46-, Bacillus pumilus -R44-, y Paenibacillus polymyxa -BSP1.1-) sobre el crecimiento, la absorción de nutrientes y el intercambio de gases en plantas de 8 semanas de edad de Capsicum chinense. La cepa BSP1.1 promovió el mayor incremento en altura de planta (22.52 cm), área foliar (99 cm²), peso fresco de raíz (0.337 g) y de la parte aérea (2.83 g), y peso seco de raíz (0.05 g) y de la parte aérea (0.43 g). No hubo diferencias significativas entre tratamientos en la absorción de N. Sin embargo, las plántulas inoculadas con BSP1.1 tuvieron una mayor acumulación de P y K que los controles (50 y 40%, respectivamente). Además, las plántulas inoculadas con la cepa BSP1.1 mejoraron la tasa fotosintética (2 μmol/m²/s) y el uso eficiente de agua (7,3 μmol CO₂/mmol H₂O), y disminuyeron la tasa de transpiración (0.27 mmol/m²/s). Así, la inoculación con la cepa BSP1.1 fue la mejor opción para aumentar el crecimiento y el vigor de las plantas de Capsicum chinense.

Palabras clave: Chile habanero; Bacterias promotoras de crecimiento vegetal; Área foliar; Biomasa; Bioprotección.
INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) is a term applied to a group of soil bacteria associated with the rhizosphere and the root surface of many plant species. The association of PGPR with the root system can stimulate plant growth and defence responses against pathogens (Vessey, 2003). There are various mechanisms explaining how PGPR favours the growth of plants. For example, through mostly the (1) biological fixation of nitrogen (Bashan et al., 2014); (2) regulation of the synthesis of plant growth-regulators, which can promote root growth, enhancing the uptake of water and minerals from the soil; (3) decrease in ethylene content of developing or stressed plants, inducing the elongation of the root system (Ahemad & Kibret, 2014); (4) the solubilization of soil-insoluble compounds, such as calcium di-and tri-phosphates and other minerals (Marschner, 2007); (5) growth inhibition of both pathogenic and non-pathogenic microorganisms, which may compete for nutrients and antibiosis (Caballero-Mellado, 2006); and (6) biosynthesis of siderophores, which can solubilize and chelate iron from the soil (Marschner, 2007).

PGPR have been applied to commercially important crops, and their growth-promoting capacity has been evaluated (Fernández-Herrera et al., 2007). Reyes-Ramírez et al. (2014) found that the inoculation of Capsicum chinense seedlings with Pseudomonas sp. increased plant height, stem diameter and dry weight, as well as yield and size of the fruits.

Landrace genotypes of the Capsicum chinense have been traditionally cultivated in the Yucatán Peninsula under adverse environmental conditions (Garruña-Hernández et al., 2014a). It has contributed to generating one of the most pungent peppers worldwide (Bosland & Votava, 2000; Canto-Flick et al., 2008). However, adverse conditions constraint seed germination and seedling establishment, and seeds must be previously sown in pots under greenhouse conditions. The loss of seed viability and the establishment of vigorous seedlings in the field are among the main challenges in these cultivars (Garruña-Hernández et al., 2014b). The performance of plants late in their productivity period is highly dependent on the first stages of development. Thereafter, the establishment of efficient nursery and fertilization protocols is necessary to improve both the health and vigour of seedlings during the first stages of development. The use of PGPR has been considered as an alternative to fortify seedlings before transplantation (Canto-Martín et al., 2004). As a result, the aim of the current study was to evaluate the capacity of four plant growth-promoting rhizobacteria for promoting increases in both growth and vigour of Capsicum chinense seedlings, after adding them during seed germination and in the seedling nursery.

MATERIALS AND METHODS

**Biological materials and culture conditions.** Four bacterial strains isolated from the potato rhizosphere grown in the Valley of Toluca (P61, Pseudomonas tolaasii; R44, Bacillus pumilus) or on an andisol soil from the Valley of Allende (BSP1.1, Paenibacillus polymyxa; A46, Pseudomonas tolaasii), Mexico, were tested in this study. The research was conducted under greenhouse conditions at the Yucatan Scientific Research Center (CICY), Mexico. Seeds of the orange (“Akil”) variety of Capsicum chinense were sown in polystyrene trays with sterilized sphagnum peat moss (Premier Mexico, Mexico). After sowing, the seeds were inoculated with a broth culture of the respective PGPR isolates (P61, A46, R44, or BSP1.1), containing 10^7 colony-forming units of bacteria (10^7 CFU/mL broth). The control seeds were soaked with distilled water. Two weeks after sowing, the inoculated seedlings were dipped again for five min into broth cultures of the respective PGPR strain (10^7 CFU/mL broth). Control seedlings without bacterial inoculation were treated accordingly. The fertilization programme began 25 days after seed sowing, by submerging the trays twice a week in a solution with 18:30:15 (N:P:K). Measurement of growth and physiological parameters was performed eight weeks after seed sowing.

**Growth parameters.** Eight weeks after sowing, plant diameter and height were measured using a digital Vernier scale, and leaf area was determined using an area metre (LiCor LI-3100, Nebraska, USA). Fresh (FW) and dry (DW) weight were measured for individual organs: leaves and stem (shoot), and roots were separated and weighed. Thereafter, they were placed in a forced-air oven at 70 °C until obtaining a constant mass (approximately 72 h).

**Determination of nutrient content in plants.** All glassware was washed three times with concentrated nitric acid, and rinsed with MilliQ deionized water (18.2 MΩ) three times each. Five mL of 70% nitric acid ACS (JT Baker, Phillipsburg, USA) were added to wash Teflon-covered receptacles. Then, the receptacles were heated in a microwave oven (CEM, MARS-Xpress) for 10 min at 200 °C. After washing, the receptacles were rinsed thoroughly with MilliQ deionized water (18.2 MΩ) as described above. The protocol reported by Bhandari & Amarasingharden (2000) was followed for digestion and analysis of nutrients. In brief, Capsicum chinense plants were harvested eight weeks after sowing and rinsed thoroughly with tap water. Then, whole plants were dried at 70 °C for three days and ground to fine dust in an electric mill. For nutrient analysis, 0.5 g samples of milled plants tissues were mixed with 9 mL of nitric acid (TraceUltra Sigma) and 1 mL of hydrogen peroxide (TraceUltra Sigma), and digestion proceeded for 15 min at 200 °C. After digestion, the receptacles remained inside the microwave oven until they reached
Effect of PGPR inoculation in *Capsicum chinense*

None of the PGPR treatments promoted significant differences in plant height (39, 35 and 25%, respectively) with respect to the control plants, while the relative humidity (RH) was ca. 75% and the average daily temperature was 27 °C. Those parameters were measured using data loggers (HOBO H08–004–02; Onset Computer Corp., Bourne, MA, USA) and Quantum sensors (LI–190SB; LI–COR, Lincoln, NE, USA) placed on the top of and inside the greenhouse. Gas exchange analyses were conducted inside a greenhouse under relevant growth conditions by a non-invasive method, using a portable infrared gas analyser system (IRGA; LICOR, LI–6400, Nebraska, USA). At 12:00 h, 30 fully expanded young leaves from each treatment were placed in the gas-exchange leaf chamber of the IRGA LI–6400 (ten plants per treatment were exclusively for gas exchange analyses). Photosynthesis \((A_o)\) and transpiration \((E)\) were estimated under the greenhouse conditions described above. Water use efficiency was subsequently calculated \((A_o/E)\).

Experimental design. The determination of growth parameters, including height, stem diameter, leaf area, and biomass (dry and fresh weight) of root and shoot (stem and leaves) was performed using samples from 20 plants. Experimental data were collected from five independent treatments in a randomized complete block design with three replicates. One-way analysis of variance (ANOVA) was calculated for every variable with the Statistical analysis system 9.1.3 (SAS Institute Inc., Cary, NC, USA). After the F tests were significant \((P<0.05)\), multiple comparisons of means were made using the Tukey test, with a significance level of 5%.

**RESULTS**

Growth parameters. Inoculation of *Capsicum chinense* seeds with the BSP1.1, R44 and P61 strains promoted a statistically significant increase in plant height (39, 35 and 25%, respectively) with respect to the control plants, while the result with the A46 strain was statistically similar to the control plants (Table 1). The leaf area of plants inoculated with the BSP1.1 and R44 strains was significantly increased by 28 and 21% with respect to the control plants, respectively (Table 1). None of the PGPR treatments promoted significant differences in the stem diameter of plants (Table 1).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>Control</td>
<td>0.083 b</td>
<td>1.62 b</td>
</tr>
<tr>
<td>P61</td>
<td>0.138 b</td>
<td>2.43 a</td>
</tr>
<tr>
<td>A46</td>
<td>0.094 b</td>
<td>2.16 ab</td>
</tr>
<tr>
<td>R44</td>
<td>0.141 b</td>
<td>2.62 a</td>
</tr>
<tr>
<td>BSP1.1</td>
<td>0.337 a</td>
<td>2.83 a</td>
</tr>
<tr>
<td>LSD</td>
<td>0.084</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Data are means. LSD = Least Significant Difference. Different letters in the same column represent statistically significant differences \((Tukey \alpha = 0.05)\).

Gas exchange analysis. Eight-week-old plants inoculated at seed sowing with BSP1.1 had 36, 54, 32 and 66% higher photosynthesis than plants inoculated with P61, A46, and R44 and the non-inoculated (control) plants, respectively.
(Fig. 1A). This was when photosynthetic photon flux density was at its maximum (at noon). In contrast, the non-inoculated (control) plants had a transpiration rate that was 41, 50, 14 and 58% higher than plants inoculated with P61, A46, R44 and BSP1.1, respectively (Fig. 1B). Plants inoculated with BSP1.1 had a water use efficiency 55, 62, 67 and 86% higher than that on plants inoculated with P61, A46, and R44 and the controls, respectively (Fig. 1C).

**Nutrient uptake.** Plants inoculated with BSP1.1 had a P uptake 40 and 50% higher than that on plants inoculated with A46 and the controls, respectively (Table 3). Likewise, plants inoculated with BSP1.1 had a K uptake 40% higher than that on controls (Table 3). There were no significant differences between treatments in N uptake (Table 3).

Table 3. Nutrient uptake (N:P:K) of Capsicum chinense plants inoculated with PGPR.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N (mM)</th>
<th>P (mM)</th>
<th>K (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>907.9</td>
<td>2172.1</td>
<td>121.7</td>
</tr>
<tr>
<td>P61</td>
<td>1456.7</td>
<td>2736.5</td>
<td>171.3</td>
</tr>
<tr>
<td>A46</td>
<td>1247.2</td>
<td>2611.8</td>
<td>159.8</td>
</tr>
<tr>
<td>R44</td>
<td>1711.0</td>
<td>3468.8</td>
<td>179.9</td>
</tr>
<tr>
<td>BSP1.1</td>
<td>2250.0</td>
<td>4352.8</td>
<td>203.3</td>
</tr>
<tr>
<td>LSD</td>
<td>2828.4</td>
<td>1738.7</td>
<td>46.2</td>
</tr>
</tbody>
</table>

Data are means. LSD = Least Significant Difference. Different letters in the same column represent statistically significant differences (Tukey $\alpha = 0.05$).

**DISCUSSION**

The efficiency of rhizobacteria to promote larger plants depends on the strain used (Khalid et al., 2004). In the current study, the strain of *Paenibacillus polymyxa* (BSP1.1) had the best effects, enhancing plant height, leaf area and biomass (in both roots and shoots), and promoting plant quality. According to Lee et al. (2012), *Paenibacillus polymyxa* is a group of PGPR, very similar to other rhizobacteria, that act as beneficial microbes improving the microbiome of plant roots. Many mechanisms are involved in efficient rhizobacteria-plant interaction. PGPR can also induce the production of enzymes, better absorption of water, and efficient use of mineral elements provided by fertilization (Egamberdiyeva, 2007). Thus, Timmusk et al. (1999) showed that *P. polymyxa* could metabolize cytokinins. In this way, plants and plant-associated micro-organisms have been found to contain over 30 growth-promoting compounds of the cytokinin group (Nicander et
al., 1993; Timmusk et al., 1999). In the current study, it was evident that the BSP1.1 (P. polymyxa) strain improved the quality traits of Capsicum chinense in comparison to untreated controls. In this way, the taller plants with more root biomass (BSP1.1 treatment) likely produced phytohormones that are considered to enhance root growth and surface area (i.e., bigger roots and more lateral roots and root hairs), leading to an increased plant nutrition (Richardson et al., 2009). On this last point, the BSP1.1 strain improved nutrient uptake (P and K). In this way, according to Lal & Tabacchiioni (2009), P. polymyxa (formerly Bacillus polymyxa) is a non-pathogenic and endospore-forming bacillus with a wide range of properties, including nutrient fixation, plant growth promotion and soil phosphorus solubilization (Shi et al., 2009). Supporting our results, Guemouri-Athmani et al. (2000) demonstrated the nitrogen fixing ability of P. polymyxa (in 14 out of the 23 strains tested). However, it has not been demonstrated that plant growth promotion by P. polymyxa is primarily correlated with its nitrogen-fixing ability (Lal & Tabacchiioni, 2009). The BSP1.1 strain tested in the current study probably had nitrogen fixing ability. In addition, the BSP1.1 strain increased the phosphorus-fixing ability. The use of phosphate-solubilizing bacteria as inoculants increases P uptake by plants (Chen et al., 2006), and P. polymyxa has been reported as a P solubilizer (De Freitas et al., 1997). It is generally accepted that the predominant mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms (Rodriguez & Fraga, 1999). On the other hand, P. polymyxa is not reported to be a potassium-solubilizing bacteria. However, Han & Lee (2006) showed that inoculation with P. polymyxa increased the availability of P and K in the soil, the uptake of N, P and K by the roots, and the growth of peppers and cucumbers. According to Schachtman & Schroeder (1994), potassium is crucial for plant nutrition and growth. In the current study, the larger plants, suggests that plant-PGPR interaction improves P and K uptake in Capsicum chinense plants.

Based on the gas exchange results, inoculation with PGPR had a positive effect on the photosynthetic rate, leading to an increased growth of Capsicum chinense plants. In a previous work with beans, inoculation with different strains of Rhizobium and Pseudomonas improved the seedling physiology (Ahmad et al., 2013). Similarly, previous studies have shown that inoculation with PGPR increases 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Nadeem et al., 2009), which might have helped a greater soil water uptake by plants (Hamdia et al., 1998). Moreover, the improvement may be due to the higher dry mass of the roots. Li & Xu (2014) showed that in plants inoculated with PGPR, indole-3-acetic acid (IAA) increases. In the current study, it was observed that water use efficiency (WUE) increased due to inoculation with BSP1.1. This result indicates that the BSP1.1 strain promoted a better investment in water consumption by plants. In this way, Timmusk & Wagner (1999) showed with a gnotobiotic system (with quantification of mRNA levels) that P. polymyxa isolates confer resistance to drought stress in Arabidopsis thaliana. Thus, a decrease in transpiration rate and an increase in photosynthesis (CO₂ assimilation rate) enhanced growth parameters (fresh mass, dry mass, leaf area and height), and subsequently improved water use efficiency of Capsicum chinense plants inoculated with P. polymyxa. The results obtained in the current study demonstrated that growth, nutrient uptake and photosynthesis activity were stimulated in Capsicum chinense plants after seeds were inoculated with PGPR during sowing. However, the effects were dependent on the used strain. Thus, of the four strains used in the current study (P61: Pseudomonas tolaasii; A46: Pseudomonas tolaasii; R44: Bacillus pumilus, BSP1.1: Paenibacillus polymyxa), the strain of P. polymyxa (BSP1.1) was better than the rest. Inoculation with P. polymyxa particularly improved the photosynthetic activity and water use efficiency of C. chinense plants.

These results suggest that inoculation with the BSP1.1 (P. polymyxa) strain could reduce the use of fertilizers and water. In addition, this research prompted the development of further experiments in the field to test productivity and fruit quality traits in seedlings inoculated with P. polymyxa.

**ACKNOWLEDGEMENTS**

This work was made with the support of the Plant Biochemistry and Molecular Biology Unit, of the Yucatan Scientific Research Center (CICY), Mexico. We thank the area of soil microbiology of the Colegio de Postgraduados at Campus Montecillo, who provided the rhizobacteria strains tested in this study.

**REFERENCES**


