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Effect of plant growth promoting rhizobacteria (PGPR) on the growth of chili habanero pepper (*Capsicum chinense* Jacq.)

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ABSTRACT

Objective: To evaluate the effect of plant growth promoting rhizobacteria (PGPR) on the growth and production of habanero pepper (*Capsicum chinense* Jacq.).

Design/Methodology/Approach: Twelve strains of PGPR were evaluated in habanero pepper seeds of the orange variety. The species of PGPR were *Rhizobium leguminosarum*: (CP Méx 46), *Pseudomonas* spp: (*P. fluorescens*, C2, A7, A9, A9m, Avm); *Azospirillum*, (Sp7, Sp 59, UAP 40, UAP154), plus a control treatment, giving a total of 13 treatments. The study variables were seedling emergence (SE), plant height (PH), white fruit incidence (WFI), virotic plants (VP), days to flowering (DF) and fresh fruit yield (FFY). The experimental design was random blocks with four repetitions.

Results: An effect on the growth of habanero pepper from PGPRs was found in all the variables studied. Seedling emergence and their height was favored by strains Sp9 (84.16%) and A7 (73.44). The number of white flies decreased with the inoculation of CP Méx 46, while the incidence of virosis decreased in plants inoculated with SP9 (32.00%). The highest yield of fresh fruit was found in plants with the strain AVM with 16636 kg ha^{-1}.

Findings/Conclusions: The effect of inoculation with PGPR is in function of the strain used and the study variable, growth stage and development stage of the habanero pepper plant.

Keywords: Inoculation, *Rhizobium*, *Pseudomonas* spp, *Azospirillum*.

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INTRODUCTION

Habanero pepper (*Capsicum chinense* Jacq.) is one of the most important crops of the Yucatan Peninsula in Mexico. The average surface cultivated annually is 700 ha; however, it has increased in various states in Mexico to cover the national and international demand. An ecologically acceptable alternative to increase the crop's yield is the inoculation with growth promoting microorganisms, called biostimulants or biofertilizers (Compant *et al.*, 2010).

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One of these microorganisms is plant growth promoting rhizobacteria (PGPR). Rhizobacteria exert beneficial effects on the plants through direct and indirect mechanisms, or a combination of both (Parray *et al*., 2016). Direct mechanisms happen when the bacteria synthesize metabolites that ease or increase the availability of nutritional elements, required for their metabolism and nutritional process (Gómez-Luna *et al*., 2012). Among the direct mechanisms, the following stand out: nitrogen fixation (N); synthesis of plant hormones, vitamins and enzymes, solubilization of phosphorus (P) (Esquivel-Cote *et al*., 2013), while the indirect mechanisms are characterized by the PGPR causing the decrease or elimination of plant pathogen microorganisms, whether through the production of antimicrobial substances or antibiotics, lithic enzymes or a combination of these; from competition of nutrients or space in the ecological niche (Esquivel-Cote *et al*., 2013). According to Constantino *et al*. (2008), the vegetative growth of plants and the fruit yield of *Capsicum chinense* were higher when inoculated with *A. brasilense*, *Azotobacter chroococum* and *Rhizophagus* spp. than in plants without inoculation. For their part, Reyes-Ramírez *et al*. (2014), in their evaluations with three strains of rhizobacteria in habanero pepper plants treated with *Pseudomonas* found greater height of the plant, stalk diameter and total dry biomass than the control plants 120 (ddt).

They also found that the yield was higher (899.84 g per plant) and the fruits had greater length, diameter and weight, concluding that the inoculation when transplanting with *Pseudomonas* spp. to habanero pepper plants increased their growth and the size of the fruit. In collections and evaluations of rhizobacteria strains evaluated in poblano chili pepper plants (*Capsicum annuum*), an increase of 20% was found in the weight of the aerial part of the plant, concluding that the rhizobacteria strains can be used to increase the quality of poblano chili seedlings, which could guarantee a better establishment and health of these in the field (González-Mancilla *et al*., 2017). Complementary studies in tomato and pepper point to the use of rhizobacteria of the genus *Bacillus* spp. which increased the percentage of germination of pepper seeds in 7% and the biomass from 16 to 37% (Luna-Martínez *et al*., 2013). In this context, the present study evaluated the effect of different experimental PGPR strains on the growth of habanero pepper (*Capsicum chinense* Jacq.).

MATERIALS AND METHODS

The experiment was conducted in Chiná, Campeche, Mexico (19° 45' 18" N and 90° 26' 23" W). The orange variety of habanero chili pepper was used. Twelve experimental strains of plant growth promoting rhizobacteria (PGPR) were evaluated: CP Méx 46 (*Rhizobium leguminosarum*), *P. fluorescens*, C2 (*P. fluorescens*), *P. putida*, A7 (*Pseudomonas* sp.), A9 (*Pseudomonas* sp.), A9m (*Pseudomonas* sp.), Avm (*Pseudomonas* sp.), Sp7 (*Azospirillum brasilensis*), Sp 59 (*Azospirillum brasilensis*), UAP 40 (*Azospirillum brasilensis*), and UAP154 (*Azospirillum brasilensis*).

The chili seeds were inoculated with bacteria suspensions of each strain evaluated with a bacterial suspension volume of 2.5 mL applied to each lot of seeds (10^9 cells per milliliter of suspension). Once the habanero pepper seeds were inoculated, they were sown manually in polystyrene trays with 200 cavities, depositing one seed per cavity at a depth of one centimeter.

The substrate used was Canadian peat moss™. Ten days after sowing (DAS) the emergence of the seedlings began, and the trays were placed in seed banks of a rustic greenhouse. The irrigation was applied according to the needs of the plant, and irrigating once daily was enough. Derosa® 1+Previcur® at a dose of 1.0 mL L^{-1} were applied, respectively. Raizal® 0.5 g L^{-1} of water was applied in later irrigation to promote the rooting of the seedling. Fertilization was carried out by the leaf using the formula 20-30-10 (N-P-K), giving on average two applications per week at a rate of 2 g L^{-1} of water.

Transplant was conducted at 60 DAS. The distances between plants were 50 cm and 1.5 m between furrows. In the field, habanero pepper plants were subjected to the agronomic management in fertigation recommended by Soria *et al.* (2002). The experimental unit consisted of three furrows 10 m long and 1.5 m wide, considering as a useful plot the central furrow constituted by 21 plants. The study variables were the seedling emergence (SE), which was done through visual counting of the sowing substrate expressed in percentage at 10 DAS; plant height (PH) prior to transplant, days to flowering, plants with virosis (PV) and total fresh fruit yield (FFY). A completely random block experimental design with four repetitions was used. The treatments were the 12 strains of rhizobacteria inoculated on habanero chili seeds and a control treatment without inoculation. The data obtained were subjected to a variance analysis and Tukey's means comparison test ($P \leq 0.05$), through the statistical analysis software SAS (Statistical Analysis System Institute).

RESULTS AND DISCUSSION

Plant emergence

The effect of the rhizobacteria on the emergence of habanero chili seedlings was variable. The two best treatments were the non-inoculation of seeds, with 84.36 plants emerged at 10 DAS, and the effect of strain Sp 59 (*Azospirillum* spp.) with 84.16 plants (Table 1). This effect is barely within the percentage of emerged plants necessary for the commercial production of habanero pepper plants in trays with 80%; therefore, the impact of PGPRs was not clear for the conditions of study. Lagunas *et al.* (2001) reported that the inoculation with *Bacillus firmus* increased their germination in 6.0%, effect of similar magnitude to that found in this assay. Similar results have shown that inoculation with *Bacillus* spp. in poblano chili (*Capsicum annuum*) promoted increments in germination of 7% (Jalili *et al.*, 2009). This improvement in germination can be explained by the reduction of the levels of ethylene in the seed and the increase of indole-3-acetic acid, stimulating the cell division and an increase in germination.

Plant height

The plant height of habanero chili at the time of transplant presented more evident effects regarding the rhizobacterial inoculation, which is why the inoculated plants presented a higher response to inoculation and therefore a greater plant height than those not inoculated (Table 1). The results showed that the strains with greatest effect were a A7 (*Azospirillum*) and Avm (*Pseudomonas*) with plants of 23.44 and 20.8 cm height, superior to those found at treatment without inoculation (14.02 cm).

Table 1. Seedling germination and plant height at plant transplant of *Capsicum chinense* Jacq. inoculated with PGPR.

Treatment (strain)	Species	Germination	Heigth (cm)
Cp Mex 46	(*Rizobium leguminozarum* by *phaseoli*)	79.34 d	16.95 def
P. fluorescens	*P. fluorescens*	72.34 b	14.38 ab
C2	(*P. fluorescens*)	78.30 d	18.33 f
P. putida	(*Pseudomonas putida*)	76.14 c	15.69 bcd
A7	(*Pseudomonas* sp. cepa 7)	74.06 b	23.44 i
A9	(*Pseudomonas* sp. cepa 9)	74.14 b	14.28 ab
A9m	(*Pseudomonas* sp. cepa A9m)	81.34 e	17.12 ef
Avm	(*Pseudomonas* sp. cepa Avm)	74.06 b	20.8 h
Sp 7	*Azospirrillum brasilensis* cepa Sp7	76.47 c	17.33 ef
Sp 59	*Azospirrillum brasilensis* cepa Sp59	84.16 f	15.05 abc
UAP 40	*Azospirrillum brasilensis* cepa UAP40	79.06 d	16.02 cde
UAP 154	*Azospirrillum brasilensis* cepa Sp154	81.32 e	15.43 abc
Not inoculated		84.36 f	14.02 a
DMS		1.16	1.39

Treatments with the same letter are statistically equal (Tukey α=0.05).

This increase in the growth can be translated into more vigorous plants, better nourished than those that use less time to obtain the adequate height of transplant (12 cm). On average, the plants inoculated with strains of rhizobacteria presented a height at harvest of 22.07 compared to the 14.02 cm obtained in plants without inoculating, which implies less time in the production of plants without devaluing their quality. These results can be compared to those obtained by Castillo *et al.*, (2017) in their evaluations with different strains of experimental rhizobacteria in the production of habanero chili plants, who found that inoculation with rhizobacteria in habanero chili with rhizobacteria strains BSP1.1, R44 and P61 promoted a significant increase in the height of plants in tray (39, 35 and 25%, respectively), compared to the control plants. It is important to consider that colonization of rhizobacteria requires time and happens only when there is compatibility between the microorganisms and the intrinsic factors of the plant, such as root exudates (Khalid *et al.*, 2004, Trivedi *et al.*, 2012). In addition, it is necessary to consider the existence of other physiological mechanisms promoted by PGPRs such as better water absorption and efficient use of mineral elements (Egamberdiyeva, 2007), production of indole-3-acetic acid (IAA), which promotes root or vegetative development (García *et al.*, 2010), with the consequent reduction of ethylene in addition to a better use of the nutrients in the growth substrate (Husen *et al.*, 2011).

Incidence of white fly

The plants inoculated with the strains CP Mex 46, *P. fluorescens* and C2 (*Pseudomonas*) presented lower incidence of white fly (Table 2), showing on average 17, 16.5 and 17 insects per trap, respectively. The variation of the results found can be attributed to the effects of each strain of rhizobacteria in the study and the rhizosphere interaction in the cultivation site, as well as the physical and chemical conditions of the soil. With relation to this variable, authors such as Chiquito (2002) in jalapeño pepper and tomato crops, found that plants inoculated with rhizobacteria presented low incidence of white fly, attributing that to the presence of siderophores, hydrogen cyanide, and salicylate in plants. The low incidence of white fly can also be attributed to the suppression of substances that attract the white fly in inoculated plants (Zendher *et al.*, (1997). According to Martínez and Carrillo (1990) the genera *Pseudomonas*, *Rhizobium* and *Azotobacter* are the ones that activate most the synthesis of siderophores.

Number of virotic plants

The number of virotic plants from the effect of inoculation with rhizobacteria varied significantly (Table 2). On average, the percentage of virotic plants was lower in plants with PGPR (58.88%) compared to the plants not inoculated (62.25%). The results showed that the strains *Sp9 and Avm* presented lower percentage of virotic plants, 32% and 34%, respectively, significantly lower incidence than the control treatment (62.25%).

These percentages could be favored by the intense precipitations of the prior agricultural cycle, which abated drastically the populations of white fly in the field, giving as a result a reduction of the transmission of virosis. It should be considered that the resistance induced is associated with the capacity of PGPRs to promote the growth of plants and protect them against the attack from pathogens. The effect of repelling the white fly or keeping the plant from becoming a host can be attributed to the induced resistance provoked by a large variety of microorganisms such as *Pseudomonas fluorescens* and *Pseudomonas putida*,

Table 2. Incidence of white fly (*Bemisia tabasi*), virotic plants, and day to flowering *Capsicum chinense* Jacq. plants inoculated with PGPR.

Treatment (strain)	Species	Whitefly incidence (PL)	Virotic plants (%)	Flowering (days)
Cp Mex 46	(*Rizobium leguminozarum* by *phaseoli*)	17.00 a	54.67 d	62.27 d
P. fluorescens	*P. fluorescens*	16.75 a	68.25 h	58.24 a
C2	(*P. fluorescens*)	17.25 a	60.67 df	63.24 e
P. putida	(*Pseudomonas putida*)	19.75 ab	64.05 g	62.72 de
A7	(*Pseudomonas* sp. cepa 7)	30.50 b	60.67 df	58.70 ab
A9	(*Pseudomonas* sp. cepa 9)	24.00 b	61.37 f	58.72 ab
A9m	(*Pseudomonas* sp. cepa A9m)	38.50 c	69.00 h	59.27 b
Avm	(*Pseudomonas* sp. cepa Avm)	29.00 b	34.62 b	58.11 a
Sp 7	*Azospirrillum brasilensis* cepa Sp7	19.50 ab	58.57 f	58.76 ab
Sp 59	*Azospirrillum brasilensis* cepa Sp59	21.00 ab	32.00 a	59.24b
UAP 40	*Azospirrillum brasilensis* cepa UAP40	34.75 b	40.35 c	58.29 a
UAP 154	*Azospirrillum brasilensis* cepa Sp154	38.50 c	62.25 fg	58.24 a
Not inoculated		38.50 c	89.30 i	61.05 c
DMS		3.51	2.35	0.85

Treatments with the same letter are statistically equal (Tukey α=0.05).

species used in this study, which could produce various metabolites as a defense system, among which the following stand out: AS, lipopolysaccharides (LPS), siderophores, cyclic lipopeptides, 2,4-diacetylphloroglucinol, homoserine lactones, and volatile compounds such as acetoin and 2,3-butanediol (Molina-Romero *et al.*, 2015). Also, it is important to consider that the plants have genes that codify to generate chemical defense mechanisms against the attack of pathogenic organisms, whether by decreasing or impeding such an attack. This biological phenomenon has been called systemic resistance (Gómez and Reis, 2011).

Days to flowering

The effect of inoculation with PGPR on 50% of the flowering of habanero chili plants was variable, and the following strains were found to be outstanding: Avm (*Pseudomonas* sp.) with 58.11 days, followed by UAP 154Y *P. fluorescens* with 58.24 days for both, less time than that found with the control treatment of 61.05 days.

The difference of 2.94 days between AVM and the control seems small, although in three days the prices of the fruit of the habanero chili can vary drastically. The results found could be influenced by the management of the crop, fertilization, water availability, temperature, presence of diseases, or some pest insect, conditions that could induce the reproductive stage earlier. With relation to this, Molina-Romero *et al.* (2015) established that PGPRs have the capacity to produce growth promoting substances (auxins; gibberellins; and cytokinins), and with this to promote the floral induction, floral differentiation, and floral development, processes that entail metabolic changes in the plant, such as gibberellin production (Yuri *et al.*, 2002).

Fresh fruit yield

The habanero chili pepper yield was favored in different magnitude from rhizobacterial inoculation. The best treatment found was inoculation with the strain Avm (*Pseudomonas* spp.) with average yield of 16636.30 kg ha^{-1}, compared to the treatment without inoculating with a yield of 12554.80 kg ha^{-1} with a difference of 4081.50 kg ha^{-1} (Figure 1).

Recent studies with habanero chili in greenhouse conditions inoculated with different strains of rhizobacteria showed that *Pseudomonas* spp. induced a significant difference in fruit yield compared to the control treatment of 899.84 g per planta. Rini and Sulochiana (2006) documented the significantly higher growth and yield of the *C. annuum* plants inoculated with *Pseudomonas fluorescens* compared to the control without inoculating. For their part, Constantino *et al.* (2008) observed a higher growth and yield in the *Capsicum chinense* plants treated with *Azospirillum brasilense* and a consortium of *Rhizophagus* spp. compared to plants not inoculated.

Authors such as Reyes-Ramírez *et al.* (2014) found an increase in habanero chili in the fruit yield and number of fruits ($p \leq 0.05$) from inoculation with *Pseudomonas fluorescens*. This effect on the fresh fruit yield of the chili has possibly been observed in different plant species such as tomato from inoculation with *Bacillus* spp., *Aeromonas* spp. and *Pseudomonas lini*, plus an effect of the rhizobacteria-substrate interaction.

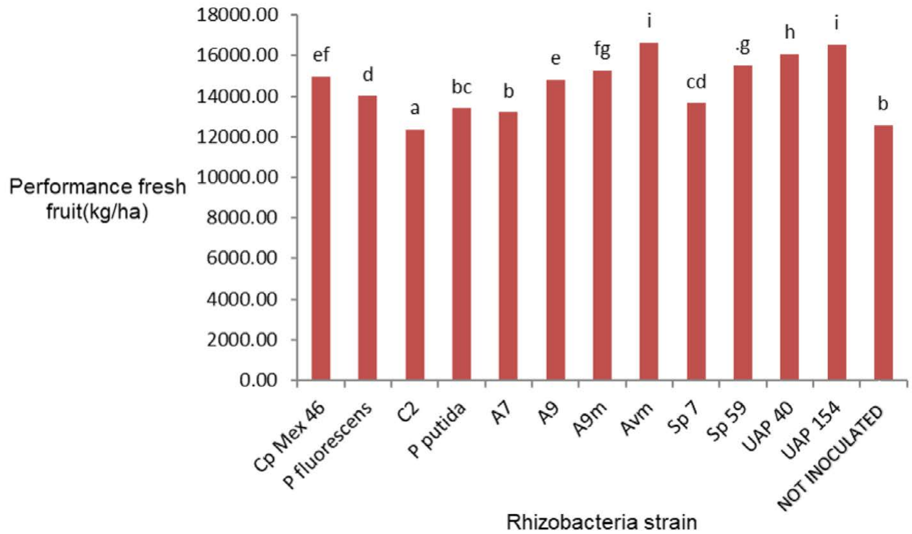

Figure 1. Yield by treatment of fresh fruit of habanero pepper. Treatments with the same letter are statistically the same (Tukey α=0,05). DMS=392.04

CONCLUSIONS

The results in this study indicated that there is a promotion of the growth of the habanero chili plant from the effect of the inoculation with rhizobacteria with variation due to the strain and the variable or the state of development of the plant. It is concluded that the *Pseudomonas* genus is potentially usable in the production of habanero chili seedling and fruit.

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