

## *Trichoderma* spp. and *Bacillus* spp. as growth promoters in maize (*Zea mays* L.)

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**Abstract.** Microbes that are beneficial to plants are used to enhance the crop growth, yield and are alternatives to chemical fertilizers. *Trichoderma* and *Bacillus* are the predominant plant growth-promoting fungi and bacteria. The objective of this study was select, characterize, and evaluate isolates of *Trichoderma* spp. and *Bacillus* spp. native from the northern region of Sinaloa, Mexico, and assess their effect on growth promotion in maize (*Zea mays* L.). In greenhouse conditions, four *Trichoderma* isolates and twenty *Bacillus* isolates, as well as two controls, were tested in a completely randomized design with three replicates. We selected the two best strains of *Trichoderma* and *Bacillus*: TB = *Trichoderma asperellum*, TF = *Trichoderma virens*, B14 = *Bacillus cereus* sensu lato and B17 = *Bacillus cereus*, which were evaluated in the field in a completely randomized blocks in factorial arrangement design with three replicates applying different rates of nitrogen fertilizer (0, 150 kg N/ha, and 300 kg N/ha). Treatments 5 (B17 = *B. cereus*) and 11 (TF = *T. virens*) both fertilized with 150 kg N/ha showed similar yields and they did not reveal significant differences from the treatments fertilized with 300 kg N/ha. This indicated that treatment 5 (B17 = *B. cereus* with 150 kg N/ha) and treatment 11 (TF = *T. virens* with 150 kg N/ha) were efficient as growth promoters, by not showing significant differences in root volume and dry weight of foliage. The results indicated a reduction of 50% in the rate of nitrogen to fertilizer required for maize (*Zea mays* L.) crops. These microorganisms *Trichoderma* and *Bacillus* could be an alternative to reduce the use of chemical fertilizers in maize.

**Keywords:** *Trichoderma*; *Bacillus*; Native strains; Biofertilizers.

## INTRODUCTION

In Mexico, the most important crop according to its cultivated surface and the role played in the diet is maize (*Zea mays* L.). Sinaloa is the state with the greatest yields per surface unit, obtaining in average 10.5 t/ha; however, this grain crop requires the highest amount of fertilizers, mainly nitrogen to obtain these yields (SIAP-SAGARPA, 2015).

In the last years, mineral fertilizers have been used indiscriminately and have led to serious imbalances in agroecosystems by contamination of the soil, water, air, and foods, being able to cause degradation of soil and resistance to pests, even risking human health (Armenta-Bojórquez et al., 2012; Pulido et al., 2003).

For this reason, it is of great interest to reduce synthetic fertilization and to recover the microflora of the soil by means of strategies that allow improvements related to agricultural productivity in a nonpolluting way (Pulido et al., 2003).

A biological inoculant is a product elaborated based on live microorganisms, fungi and/or bacteria, which, when being applied to the seeds, surfaces of the plants or the soil, colonize the rhizosphere or the interior of the plant and promote growth, increase availability of nutrients, improve the vegetal health in the host plant, and increase or maintain yield with a reduced dose or even without chemical fertilizers (Sánchez-Yañez, 2006; Reyes et al., 2008).

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The native microorganisms of a region for the production of inoculants as vegetal growth promoters have greater possibilities for their adaptation and multiplication in the soil, whereas the non-native microorganisms can have difficulties to adapt to edaphic and climatic changes. (Hungria et al., 2010; Aguirre et al., 2009; Rives et al., 2007; Bacilio-Jiménez et al., 2003). *Trichoderma* and *Bacillus* are fungi and bacteria considered very powerful effective agents of biological control against a great variety of pathogens of different crops of commercial interest, using different control mechanisms, like antibiosis, lysis, mycoparasitism (López-Valenzuela et al., 2015; De la Garza, 1996). Studies have been made to verify that they induce and increase the growth response of the plants (Figueiredo & Martínez, 2008), by means of enzymes like auxins, cytokinins, gibberellins or by solubilizing phosphates, manganese (Chen et al., 2006; Altomare et al., 1999). The ability of *Trichoderma* spp. as opportunistic fungi is well known, characterized by their fast growth, the capacity to assimilate an ample range of substrates, and the production of a variety of microbial compounds, stimulating the rate of growth and vegetal development. *Trichoderma* spp. act like biostimulants in herbaceous species, such as lettuce (*Lactuca sativa* L.), maize (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), zapallo (*Cucurbita maxima* L.), petunia (*Petunia hybrida* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annum* L.), potato (*Solanum tuberosum* L.) among others (Candelero et al., 2015; Cano, 2011). Studies made in maize indicate that the roots colonized by *Trichoderma* spp. require 40% less nitrogen fertilizer in comparison to non-colonized roots, reducing fertilizers' application costs and improving the environment (Páez, 2006; Harman, 2006). On the other hand, Guigón & González (2004) found in greenhouse conditions that all the treatments that included strains of *Trichoderma* spp., native of the south of Chihuahua, Mexico, exerted a good control on *Phytophthora capsici* and presented the best growth response for pepper (*Capsicum annum* L.) plants in comparison to the control and the four non-native strains. Similarly, García et al. (2012) evaluated, in greenhouse conditions, inoculating potato (*Solanum tuberosum* L.) plants with three strains of *Trichoderma asperellum*; they obtained an increase in the growth variables, photosynthesis, and production of tubercles. The effects of *Bacillus* spp. bacteria as growth promoters has been studied also, obtaining an increase in the emergence, vigor, dry weight of seedling, roots volume, and an increase from 25 to 30% in the production of crops of commercial interest, like potato (*Solanum tuberosum* L.), radish (*Raphanus sativus* L.), tomato (*Solanum lycopersicum* L.), wheat (*Triticum aestivum* L.), soy (*Glycine max* L.), and pepper (*Capsicum annum* L.) (Altamirano et al., 2002; Hernández-Castillo et al., 2014). Armenta-Bojórquez et al. (2015) evaluated isolates of native *Rhizobium* spp. and *Bacillus* spp. as biological fertilizers in the greenhouse and in the field on bean (*Phaseolus vulgaris* L.) crops; they demonstrated that with the combination of *Rhizobium* and *Bacillus* they obtained the best yield, higher

production of biomass, number of nodules per plant, and dry weight of nodules, surpassing the control. In addition, they did not find significant differences in fertilization, which suggests that the combination of *Rhizobium* and *Bacillus* can replace synthetic fertilization in bean (*Phaseolus vulgaris* L.). Sánchez-Lopez et al. (2012), when inoculating bacterial promoters of vegetal growth in tomato (*Solanum lycopersicum* L.), under greenhouse conditions, found that the bacteria increased significantly the height of the plant, dry weight of the plant, and fruits yield. Similarly, Sánchez et al. (2006) showed that inoculating endophytic bacteria *Burkholderia* sp. and the enteric *Klebsiella oxitogena* of teosinte (*Zea mays* sp. mexicana) into maize (*Zea mays* L.) seeds in the field, colonized roots, stems, leaves, and promoted growth reducing 50% the amount of urea recommended for the region of northern Michoacán and southern Guanajuato, Mexico, which is equivalent to diminishing from 280 kg N/ha to 140 kg. Tests made with diverse bacterial promoters of vegetal growth as *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, and *Streptomyces* (Loredo et al., 2004; Martínez, et al., 2010; Beracohea, 2011), in different soils, climatic regions, and crops of agronomic importance, demonstrated a 5% to 30% yield increase, as well as a diminution from 25% to 50% in the amount of the used chemical fertilizer (Hernández-Escareño et al., 2015; García et al., 2012; Adesemoye et al., 2009). Assays performed in maize demonstrated up to 70% increase in yields (Nezarat & Gholami, 2009) and a 50% reduction in the chemical fertilizer dose (Aguado & Moreno, 2008; García et al., 2007); however, contradictory results have also been reported in which the expected positive response was not attained, probably because microorganisms did not adapt to the soil conditions that were quite different from those of their origin, or they did not compete successfully with the native biota or were unable to survive the unfavorable conditions, (Díaz et al., 2001; Loredo et al., 2004; García et al., 2007).

Biofertilization with microorganisms (*Trichoderma* and *Bacillus*) is a method used in different crops in different parts of the world, the use of non-native strains may present difficulties with their persistence in soil, due to their need to adapt to new environments. Therefore, the selection of native isolates that are adapted to the conditions of the soil and climate of the area where they will be used is of utmost importance.

For this reason, this research was aimed at selecting isolates of *Trichoderma* and *Bacillus* native to the North region of Sinaloa and to estimate their effects as growth promoters in maize crops.

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## MATERIALS AND METHODS

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### Microorganisms

In this study, three native isolates of *Trichoderma* spp. and 20 isolates of *Bacillus* spp. were used, obtained from the rhizosphere of plants established in gardens, orchards,

and agricultural fields of the North of Sinaloa, Mexico, in the 2013-2014 agricultural cycle. The isolates belong to a collection of microorganisms kept in the Department of Soil, Plant and Water of the School of Agriculture of the Valle del Fuerte. In addition, a stock of non-native *Trichoderma* spp. isolated from the roots of a walnut crop in the Lagunera region, Torreón, Coahuila, Mexico, was evaluated.

Isolates of *Trichoderma* spp. were obtained by the technique reported by Papavizas & Davey (1959) and isolates of *Bacillus* spp. were obtained by the method used by Ohba & Aizawa (1986). The geographic data of the sampling sites and the origin of the isolates are reported in Table 1.

### Preparation of inocula

#### Inocula with *Trichoderma* spp.

The *Trichoderma* spp. inoculum was prepared in solid medium, following the technique of Lewis & Papavizas (1984). Inoculated flasks were incubated during 7 days at  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$  until growth and sporulation. Once *Trichoderma* had sporulated completely in the flask, the inoculum produced in wheat straw (*Triticum aestivum* L.) (solid-inoculum) was subjected to a series of continuous washings with sterile distilled water until collecting the largest amount of spores contained in the straw. Later, the inoculum was placed in liquid form in containers previously sterilized and kept in refrigeration at  $4^{\circ}\text{C}$  until its use. The concentration of the inoculum, determined by the spores counting technique, using a Neubauer chamber, and by dilution of the inoculate, was gauged to the desired concentration (spores/mL) for its application.

#### Inocula with *Bacillus* spp.

Isolates of *Bacillus* spp. were reactivated in LB medium and grown at  $28^{\circ}\text{C}$  during 24 h. One milliliter of the culture was transferred to 50 mL of LB broth and incubated for 12 h at  $28^{\circ}\text{C}$  and 250 rpm in an orbital shaker (Lab-Line, model 2345, Cat. No1542, USA). Finally, 5 mL of the previous culture was transferred into an Erlenmeyer flask with 250 mL of LB broth and incubated during 12 h at  $28^{\circ}\text{C}$  and 250 rpm. The concentration of the bacterial cultures (CFU/ mL) was assessed by the optical density (OD) and confirmed by the serial dilution.

### Greenhouse trial

#### Selection of *Trichoderma* and *Bacillus* isolates as growth promoters

Inoculation with *Trichoderma* spp. was made impregnating the seed with 3 mL of inoculum at a concentration of  $1 \times 10^7$  spores/mL. With *Bacillus* spp., the seed was impregnated with 2 mL of inoculum at a concentration of  $1 \times 10^6$  CFU/mL. Maize seeds of the ASGROW Caribú® hybrid were used. As substrate, a mixture of sifted soil and sand and peat-moss (2/1/1) was used after making a soil analysis, which showed a neutral pH (7.1) and electrical conductivity without salt problems (0.08 ds/m).

Two experiments were carried out: one to evaluate *Trichoderma* and the other to evaluate *Bacillus*. In the first, four *Trichoderma* spp. isolates were tested and in the second 20 isolates of *Bacillus* spp. Two controls were used for comparison of results: positive control (without inoculating and with fertilizer equivalent to 300 kg N/ha) and negative

**Table 1.** Geographic and descriptive information of native isolates of the northern region of Sinaloa, Mexico.

| ISOLATES           | HOST PLANT  | LATITUDE      | LENGTH         | ALTITUDE<br>(m.a.s.l) |
|--------------------|-------------|---------------|----------------|-----------------------|
| <i>Trichoderma</i> |             |               |                |                       |
| TB                 | Bougambilia | 25°49'15,6" N | 109°02'06,9" O | 8                     |
| TF                 | Ficus       | 25°34'25,0" N | 108°27'54,2" O | 16                    |
| TM                 | Mango       | 25°35'14,0" N | 108°31'49,8" O | 4                     |
| <i>Bacillus</i>    |             |               |                |                       |
| B1                 | Obelisk     | 25°32'46,1"N  | 108°28'55,3"O  | 24                    |
| B6                 | Jatropha    | 25°32'48,2"N  | 108°29'04,3"O  | 16                    |
| B7                 | Mango       | 25°35'14,0"N  | 108°31'49,8"O  | 4                     |
| B8                 | Mango       | 25°35'17,8"N  | 108°31'53,2"O  | 5                     |
| B12                | Sorghum     | 25°55'44,7"N  | 109°16'45,8"O  | 13                    |
| B13                | Sorghum     | 25°55'40,0"N  | 109°16'51,7"O  | 9                     |
| B14                | Mango       | 25°55'28,8"N  | 109°17'13,0"O  | 10                    |
| B15                | Maize       | 25°56'13,0"N  | 109°17'14,5"O  | 9                     |
| B17                | Maize       | 25°54'37,3"N  | 109°15'46,3"O  | 14                    |
| B18                | Maize       | 25°56'57,6"N  | 109°08'37,7"O  | 15                    |
| B20                | Sorghum     | 25°56'26,7"N  | 109°07'59,4"O  | 20                    |
| B21                | Maize       | 25°49'33,7"N  | 109°01'23,4"O  | 14                    |
| B22                | Cane        | 25°50'05,2"N  | 109°01'01,9"O  | 16                    |
| B23                | Mango       | 25°55'42,7"N  | 109°05'33,8"O  | 13                    |
| B27                | Avocado     | 25°45'18,6"N  | 108°49'27,4"O  | 20                    |
| B31                | Lemon       | 25°45'18,1"N  | 108°49'26,5"O  | 25                    |
| B32                | Cane        | 25°47'52,7"N  | 108°55'45,7"O  | 14                    |
| B33                | Orange      | 25°42'56,0"N  | 108°58'28,0"O  | 8                     |
| B40                | Sorghum     | 25°49'17,3"N  | 108°57'50,0"O  | 27                    |
| B42                | Maize       | 25°45'32,8"N  | 108°58'35,1"O  | 43                    |

control (without inoculating and without fertilizer) in both experiments. Four replicates were made in a randomized complete blocks design in each case.

The irrigations were applied based on the water needs presented by the plants during their growth and for the fertilization applying 300 kg N/ha (0.358 g urea) to each flowerpot in replicate, corresponding to the positive control, which was the only fertilization treatment.

### Measured variables

Measurements of the variables were made at 30 days after sowing (dds) the four plants, which corresponded to each treatment during development of the crop. Height of plant (cm) was measured with a measuring tape (reading error: 0.05 cm), considering as height the length from the neck to the last bud. Diameter of the stem (cm) was measured with a Vernier Caliper (530 Series-Standard Model) considering as key point the neck of the plant. Volume of the root (mL), the root of the plant was introduced in a container with water to soften the substrate surrounding it, and then washed with running water. A test tube was gauged with liquid to 100 mL, the root was introduced, and the volume was measured by displacement of the liquid. Dry weight of the foliage (g), the foliage of the plants was washed and placed individually in brown paper bags previously labeled. These bags were introduced in a drying furnace with air recirculation to 70 °C during 48 h; afterwards, bags were weighed with a digital balance.

The results were subjected to variance analysis and the Duncan test ( $p \leq 0.05$ ) to compare averages using the SAS software (2004).

### Molecular identification

The two best isolates of *Trichoderma* (TB and TF) and the two best isolates of *Bacillus* (B14 and B17) selected in the greenhouse were identified at the molecular level in the Laboratory of Molecular Ecology of the Rhizosphere of the IPN-CIIDIR-Sinaloa (see López-Valenzuela et al., 2015).

### Field trial

#### Evaluation of strains of *Trichoderma* and *Bacillus* as growth promoters

This research was undertaken during the agricultural cycle, autumn-winter 2014-2015, in the municipality of Ahome, Sinaloa, Mexico, in an agricultural lot located at coordinates: Latitude: 25°56' 57,6" North, Longitude: 109°08' 37,7" West, at a height of 15 m above sea level (m.a.s.l.). The soil was of average texture, without salt problems, with low organic matter content (1%) and a suitable amount of the necessary phosphorus for maize (18 ppm) and with high potassium content (0.92 Cmol/kg). The soil was prepared, according to the methods used by the agriculturists from the northern region of Sinaloa, Mexico. A randomized complete blocks design with three replicates was used. Fifteen treatments were established, which included the two best

native strains of *Trichoderma* and of *Bacillus* determined in the greenhouse. *Bacillus* treatments were the following: T1, T2, and T3 = strain B14 with 0, 150 kg N/ha, and 300 kg N/ha, respectively; T4, T5, and T6 = strain B17 with 0, 150, and 300 kg N/ha. *Trichoderma* treatments were the following: T7, T8, and T9 = strain TB with 0, 150 kg N/ha, and 300 kg N/ha; T10, T11, and T12 = strain TF with 0, 150 kg N/ha, and 300 kg N/ha; and three controls: T13 = commercial *Bacillus* with 300 kg N/ha, T14 = commercial *Trichoderma* with 300 kg N/ha, and T15 = without inoculating and with 300 kg N/ha. The area of each experimental unit consisted of four furrows, each one with 0.75 m × 10 m. Each experimental lot consisted of 30 m<sup>2</sup> of total area. The inoculation with *Trichoderma* spp. was made impregnating the seed with 3 mL of the inoculum at a concentration of  $1 \times 10^7$  spores/mL. For *Bacillus* spp. the seed was submerged in 2 mL of bacterial solution at a concentration of  $1 \times 10^6$  CFU/mL, shaken for 1 min and left to rest 5 min before seeding. In both conditions, the inoculum was mixed with the carboxymethyl-cellulose adherent at 0.25 L/ha. Hybrid Caribú® seeds from ASGROW were used. Eight seeds per linear meter were deposited in furrows with lengths of 10 m, and 75 cm of separation between furrows. An approximate seeding amount of 106,700 plants per hectare was reached. Fertilization was made prior to seeding in a single application of 300 kg and 150 kg of nitrogen per hectare, depending on the treatment to be evaluated, using urea at 46% as nitrogen source, based on the results from the soil analysis performed before seeding. Application of phosphorus and potassium was not necessary because the analysis reported acceptable amounts for maize. A pre-sowing irrigation was applied; the first auxiliary watering was applied at 58 dds, the second at 79 dds, and the third (last) at 109 dds; irrigation was applied allowing for sufficient water to cover the hydric necessities of the crop through a gravimetric irrigation system.

### Measured variables

The variables were the root volume, dry weight of the foliage, and grain yield. The root volume and the dry weight were measured at 134 dds during the stage of physiological maturity, taking nine plants from the usable area of each treatment in three replicates, under the procedure described previously in the greenhouse trial.

The harvest was manual at 185 dds. To calculate the yield of the grain by hectare, humidity was adjusted to 14% in each treatment and repetition in the usable area. The size of the usable area consisted of two central furrows of 8 m in length × 1.50 m width, eliminating 1 m on each end, obtaining a harvested area of 12.0 m<sup>2</sup>.

**Table 2.** Average of variables evaluated in the maize crop inoculated with isolates of *Trichoderma* spp. as promoters of growth under greenhouse conditions.

| ISOLATES         | PLANT HEIGHT<br>(cm) |   | STEM DIAMETER<br>(cm) |   | ROOT VOLUME<br>(mL) |    | FOLIAGE, DRY WEIGHT<br>(g) |     |
|------------------|----------------------|---|-----------------------|---|---------------------|----|----------------------------|-----|
| TB               | 51.80                | b | 0.67                  | b | 2.47                | b  | 2.20                       | ab  |
| TM               | 48.70                | c | 0.53                  | d | 2.00                | c  | 1.87                       | c   |
| TF               | 52.67                | b | 0.69                  | b | 2.62                | ab | 2.13                       | abc |
| TN               | 48.13                | c | 0.60                  | c | 2.17                | c  | 1.97                       | bc  |
| Positive control | 57.64                | a | 0.74                  | a | 2.83                | a  | 2.36                       | a   |
| Negative control | 35.35                | d | 0.43                  | d | 1.50                | d  | 1.46                       | d   |
| <i>F</i>         | 96.46                |   | 79.56                 |   | 36.80               |    | 11.84                      |     |
| <i>p</i>         | <0.0001              |   | <0.0001               |   | <0.0001             |    | 0.0003                     |     |

Equal letters are not different (Duncan,  $p \leq 0.05$ ).

*F* = Fisher's test

*p* = statistical probability

**Table 3.** Average of variables evaluated in the maize crop inoculated with isolates of *Bacillus* spp. as promoters of growth under greenhouse conditions.

| ISOLATES         | PLANT HEIGHT<br>(cm) |     | STEM DIAMETER<br>(cm) |    | ROOT VOLUME<br>(mL) |   | FOLIAGE, DRY WEIGHT<br>(g) |   |
|------------------|----------------------|-----|-----------------------|----|---------------------|---|----------------------------|---|
| B1               | 39.87                | i   | 0.52                  | fg | 2.00                | f | 1.56                       | k |
| B6               | 51.43                | de  | 0.52                  | fg | 1.66                | h | 1.60                       | j |
| B7               | 48.16                | g   | 0.59                  | de | 2.00                | f | 2.13                       | d |
| B8               | 53.53                | bc  | 0.49                  | gh | 2.00                | f | 1.66                       | j |
| B12              | 48.55                | g   | 0.65                  | c  | 1.66                | h | 1.80                       | f |
| B13              | 50.66                | ef  | 0.63                  | cd | 1.50                | i | 1.76                       | g |
| B14              | 54.56                | b   | 0.70                  | b  | 2.66                | b | 2.36                       | a |
| B15              | 49.32                | fg  | 0.59                  | de | 2.50                | c | 2.30                       | b |
| B17              | 54.49                | b   | 0.69                  | b  | 2.83                | a | 2.33                       | b |
| B18              | 53.75                | bc  | 0.49                  | gh | 1.83                | g | 1.76                       | g |
| B20              | 52.64                | cd  | 0.70                  | b  | 2.33                | d | 2.23                       | c |
| B21              | 43.23                | h   | 0.50                  | gh | 2.16                | e | 1.63                       | i |
| B22              | 50.65                | ef  | 0.60                  | de | 2.33                | d | 2.16                       | d |
| B23              | 51.07                | def | 0.64                  | c  | 2.00                | f | 2.00                       | e |
| B27              | 50.86                | def | 0.57                  | e  | 2.00                | f | 2.00                       | e |
| B31              | 53.85                | bc  | 0.56                  | ef | 1.83                | g | 2.13                       | d |
| B32              | 41.77                | h   | 0.49                  | gh | 1.83                | g | 1.70                       | h |
| B33              | 35.55                | i   | 0.41                  | i  | 1.50                | i | 1.33                       | n |
| B40              | 36.90                | i   | 0.40                  | i  | 1.50                | i | 1.53                       | l |
| B42              | 49.40                | fg  | 0.47                  | h  | 1.83                | g | 1.76                       | g |
| Positive control | 57.64                | a   | 0.74                  | a  | 2.83                | a | 2.36                       | a |
| Negative control | 35.35                | i   | 0.43                  | i  | 1.50                | i | 1.46                       | m |
| <i>F</i>         | 137.6                |     | 49.8                  |    | 473.2               |   | 852.0                      |   |
| <i>p</i>         | 0.0001               |     | 0.0001                |    | 0.0001              |   | 0.0001                     |   |

Equal letters are not different (Duncan,  $p \leq 0.05$ ).

*F* = Fisher's test

*p* = statistical probability

## RESULTS

### Greenhouse trial

#### Selection of *Trichoderma* and *Bacillus* isolates as growth promoters

##### Selection of *Trichoderma* spp.

All the measured variables differed significantly among isolates (Table 2). Isolated native TB and TF showed the highest values in plant height, diameter of the stem, and dry weight of foliage, without significant difference among them. In root volume, the highest value was shown by the TF isolate, followed by TB, presenting significant differences among them. In all the measured variables, the negative control significantly showed the lowest values ( $p \leq 0.05$ ; Table 2).

##### Selection of *Bacillus* spp.

Significant differences in height of the plant and diameter of the stem were found; native isolates B14 and B17 showed the highest values, whereas B1, B33, and B40 showed the lowest values (Table 3). In root volume, the isolate B17 showed the highest value, followed by the isolate B14. In dry weight of foliage, the isolate B14 showed the highest value, followed by isolate B15 and B17 without significant difference between them, but showing significant difference with the rest of the isolates. In all the measured variables, the negative control significantly showed the lowest values ( $p \leq 0.05$ ; Table 3).

Based on the values obtained for the variables measured in the greenhouse, height of plant, diameter of the stem, root volume, and dry weight of the foliage, we selected the two best native isolates of *Trichoderma* (TB and TF) and of *Bacillus* (B14 and B17), aimed at proving their effect on growth promotion in the maize crop under field conditions.

### Molecular identification

The molecular analysis of the selected *Trichoderma* and *Bacillus* isolates in the greenhouse revealed that the sequences compared in the Gene Bank database indicated that the isolate TB had a 99% identity with *Trichoderma asperellum* and the isolate TF presented 100% of similarity with *Trichoderma virens*. The isolate B14 presented high percentage of similarity (99-100%) with several species of the *Bacillus* genus, although it could not be identified to the species level; it was possibly associated with the *Bacillus* group *cereus* sensu lato, whereas the isolate B17 presented a high percentage of similarity (99%) with *Bacillus cereus*.

### Field trial

#### Evaluation of *Trichoderma* and *Bacillus* strains as growth promoters

Significant differences in the field-evaluated variables were found (Table 4).

**Table 4.** Average of variables evaluated in the maize crop with strains of *Trichoderma* and *Bacillus* in combination with nitrogen fertilization as promoters of growth under conditions of field.

| TREATMENT | DESCRIPTION OF TREATMENTS |  | ROOT VOLUME (mL) | FOLIAGE, DRY WEIGHT (g) |
|-----------|---------------------------|--|------------------|-------------------------|
| 1         | <i>Bacillus</i> (B14)     | <i>B. cereus</i> sensu lato without nitrogen (N) | 30.00 e          | 85.80 f                 |
| 2         |                           | <i>B. cereus</i> sensu lato with 150 kg N/ha     | 76.67 ab         | 128.42 abc              |
| 3         |                           | <i>B. cereus</i> sensu lato with 300 kg N/ha     | 70.00 abcd       | 119.00 bcde             |
| 4         | <i>Bacillus</i> (B17)     | <i>B. cereus</i> without (N)                     | 50.00 cde        | 109.13 de               |
| 5         |                           | <i>B. cereus</i> with 150 kg N/ha                | 70.00 abcd       | 136.86 a                |
| 6         |                           | <i>B. cereus</i> with 300 kg N/ha                | 53.33 bcd        | 119.51 bcd              |
| 7         | <i>Trichoderma</i> (TB)   | <i>T. asperellum</i> without (N)                 | 46.67 de         | 104.90 e                |
| 8         |                           | <i>T. asperellum</i> with 150 kg N/ha            | 70.00 abcd       | 117.66 bcde             |
| 9         |                           | <i>T. asperellum</i> with 300 kg N/ha            | 63.33 abcd       | 112.93 bcd              |
| 10        | <i>Trichoderma</i> (TF)   | <i>T. virens</i> without (N)                     | 50.00 cde        | 108.10 de               |
| 11        |                           | <i>T. virens</i> with 150 kg N/ha                | 86.67 a          | 136.77 a                |
| 12        |                           | <i>T. virens</i> with 300 kg N/ha                | 60.00 bcd        | 118.17 bcde             |
| 13        | Positives controls        | Comercial <i>Bacillus</i> with 300 kg N/ha       | 66.67 abcd       | 135.63 a                |
| 14        |                           | Comercial <i>Trichoderma</i> with 300 kg N/ha    | 73.33 abc        | 113.37 de               |
| 15        |                           | Without strain with 300 kg N/ha                  | 76.67 ab         | 130.26 ab               |
|           | <i>F</i>                  |  | 4.09             | 12.66                   |
|           | <i>p</i>                  |  | <0.0001          | <0.0001                 |

Equal letters are not different (Duncan,  $p \leq 0.05$ ).

*F* = Fisher's

*p* = statistical probability

### Root volume

Treatment 11 yielded the highest value, showing a significant difference with treatments 1, 4, 6, 7, 10, and 12. Treatment 1 presented the lowest significant value in root volume ( $p \leq 0.05$ ; Table 4).

### Dry weight of the foliage

Treatments 5 and 11 showed the highest value of dry weight, without presenting significant differences neither between them, nor with treatment 2 and controls, but there was a significant difference with the rest of treatments. Treatment 1 showed significantly the lowest value in dry weight of foliage ( $p \leq 0.05$ ; Table 4). This indicates that treatment 5 and treatment 11 were efficient as growth promoters, by not showing significant differences in root volume and dry weight of foliage.

### Grain yield (t/ha)

Significant differences were revealed in the grain yield among treatments. Treatment 11 showed the highest value, presenting significant difference with treatments 7, 1, and 4, and these last two presented the lowest yield ( $p \leq 0.05$ ; Table 5). These results indicate that the inoculation with native *Trichoderma virens* spp. strains (treatment 11) was more effective in promoting plant growth with only 50% of the nitrogen dose as compared to the controls.

## DISCUSSION

### Selection of *Trichoderma* isolates in the greenhouse

Isolates of the native *Trichoderma* (TB and TF) promoted growth, which was reflected in a greater height of plant, diameter of the stem, root volume, and dry weight of foliage when compared to maize foliage treated with non-native strains, which indicates that the inoculated maize plants with these native microorganisms presented greater efficiency, because they were better adapted to the environmental conditions of the region. The selected isolated native strains did not present significant difference with the treatment fertilized only with 300 kg N/ha (positive control) and showed greater yield than the treatment without inoculation and fertilizer (negative control). This agrees with that reported by Guigón & González (2004) on growth promotion of pepper plants (*Capsicum annuum* L.) in greenhouse conditions. These authors observed that all the treatments that included strains of *Trichoderma* spp., native to the south of Chihuahua, Mexico, had the best effects as compared to the controls and the four non-native strains. Similarly, García et al. (2012) in greenhouse conditions inoculated potatoes (*Solanum tuberosum* L.) with three strains of *Trichoderma asperellum* and obtained increases in all the physiological variables of growth, photosynthesis, and production of tubercles. González et al. (1999) and Cupull et al. (2003)

**Table 5.** Average of maize yield (t/ha) inoculated with strains of *Trichoderma* and *Bacillus* in combination with nitrogen fertilization under field conditions.

| TREATMENT | DESCRIPTION OF TREATMENTS  |  | YIELD<br>(t/ha) |
|-----------|----------------------------|--|-----------------|
| 1         | <i>Bacillus</i><br>(B14)   | <i>B. cereus</i> sensu lato without nitrogen (N) | 7.56 c          |
| 2         |                            | <i>B. cereus</i> sensu lato with 150 kg N/ha     | 9.43 abc        |
| 3         |                            | <i>B. cereus</i> sensu lato with 300 kg N/ha     | 8.76 abc        |
| 4         | <i>Bacillus</i><br>(B17)   | <i>B. cereus</i> without (N)                     | 7.68 c          |
| 5         |                            | <i>B. cereus</i> with 150 kg N/ha                | 9.97 ab         |
| 6         |                            | <i>B. cereus</i> with 300 kg N/ha                | 9.38 abc        |
| 7         | <i>Trichoderma</i><br>(TB) | <i>T. asperellum</i> without (N)                 | 8.07 bc         |
| 8         |                            | <i>T. asperellum</i> with 150 kg N/ha            | 8.39 abc        |
| 9         |                            | <i>T. asperellum</i> with 300 kg N/ha            | 9.07 abc        |
| 10        | <i>Trichoderma</i><br>(TF) | <i>T. virens</i> without (N)                     | 8.40 abc        |
| 11        |                            | <i>T. virens</i> with 150 kg N/ha                | 10.38 a         |
| 12        |                            | <i>T. virens</i> with 300 kg N/ha                | 9.01 abc        |
| 13        | Positives<br>controls      | Comercial <i>Bacillus</i> with 300 kg N/ha       | 10.36 a         |
| 14        |                            | Comercial <i>Trichoderma</i> with 300 kg N/ha    | 9.00 abc        |
| 15        |                            | Without strain with 300 kg N/ha                  | 9.73 ab         |
| <i>F</i>  |                            |  | 2.21            |
| <i>p</i>  |                            |  | 0.036           |

Equal letters are not different (Duncan,  $p \leq 0.05$ ).

*F* = Fisher's test

*p* = statistical probability

demonstrated that *Trichoderma harzianum* and *Trichoderma koningii* increased the dry weight of the tobacco root (*Nicotiana tabacum* L.) and influenced the vegetative growth of potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.). The previous results, to a great extent, are attributed to *Trichoderma* spp. role in the promotion of vegetal growth, through a series of mechanisms implemented to increase the radicle's growth and development of the plants. These mechanisms include the competition for space and nutrients, tolerance to stress, organic acid presence that retains cations and acidifies the rhizosphere, which solubilizes nutrients for their absorption by the plants; in addition, these mechanisms are attributed to the release of chelating or siderophore structures that favors ion accumulation, such as Ca, Fe<sub>2</sub>O<sub>3</sub>, MnO<sub>2</sub>, Cu, and Zn, and it is through these mechanisms that nutrients are usually better assimilated by the plants (Valencia, 2005; Altomare et al., 1999; Kleifeld & Chet, 1992).

#### Selection of *Bacillus* isolates in greenhouse conditions

Native *Bacillus* (B14 and B17) isolates promoted growth, inducing greater volume of roots and dry weight of foliage in the maize plants. The selected native isolates showed the same values as the non-inoculated but fertilized plants (positive control) and greater values than the plants without inoculating and without fertilizer (negative control). The previous agrees with the report by Sánchez-López et al. (2012), who inoculated bacteria, like *Enterobacter* spp., *Pseudomonas* spp. and *Bacillus* spp., into tomato (*Solanum lycopersicum* L.) plants under greenhouse conditions and demonstrated that the bacteria increased significantly the length of the plant, dry weight of the plant, and yield of fruits. Mena & Olalde (2007) found that *Bacillus subtilis* increases the greenhouse production of tomato (*Solanum lycopersicum* L.) in 25%.

The results of the present study indicate that the bacterial strains of *Bacillus* spp. promote plants growth, attributed to a great extent to their ability to produce phytohormones and to solubilize phosphorus from the soil. This produces a remarkable increase in the growth of roots and foliage of the treated plants (Castro & Revillas, 2005; Bashan & Of-Bashan, 2002; Egamberdiyeva & Höflich, 2004).

#### Field evaluation of strains

Treatments 5 (B17 = *Bacillus cereus*) and 11 (TF = *Trichoderma virens*), both fertilized with 150 kg N/ha, showed the same effectiveness in growth promotion as treatments and controls fertilized with 300 kg N/ha. These *B.* and native *T. virens* isolates showed to be potential biofertilizers by yielding the highest values in root volume and dry weight of foliage, reducing 50% the dose of nitrogen fertilizer required by the maize crop in field conditions.

These results can be due to the fact that these microorganisms synthesize phytohormones that increase the volume of roots, which favors the plant by increasing the space of soil exploration to obtain greater amounts of nutrients and water.

The results obtained in this study agree with Sánchez et al. (2006), who in a greenhouse test found that when inoculating the maize seeds with endophytic bacteria promoters of growth, *Burkholderia* sp. and the enteric *Klebsiella oxitogena*, of teosinte (*Zea mays* sp. *mexicana*), they individually increased in average 30% the dry weight of the foliage of maize as compared to that obtained with control 1 (without inoculating and fertilized with 140 kg N/ha = 50% of the recommended rate). The response of this teosinte maize (*Zea mays* sp. *mexicana*), in terms of dry weight of the foliage, was similar statistically ( $p \leq 0.05$ ) to the dry weight of the foliage of the plants corresponding to control 2 (without inoculating and fertilized with 280 kg N/ha = 100% of the recommended rate).

#### Grain yield in t/ha

The inoculation with the native *Trichoderma virens* strain (treatment 11) exerted the same effectiveness on the yield of grain as compared to the positive control (treatment 15). The effectiveness of the isolated native *Trichoderma virens* as a promoter of vegetal growth in maize was demonstrated by reducing to 50% the dose of nitrogen fertilization. The previous agrees with Sanchez et al. (2006), who reduced to 50% the rate of nitrogen fertilization in the maize crop in the field inoculating endophytic bacteria of teosinte maize (*Zea mays* sp. *mexicana*) *Burkholderia* sp. and the enteric *Klebsiella oxitogena*. Other studies made by Harman (2006), in maize crops, indicate that the roots colonized by *Trichoderma* spp. require 40% less of nitrogen fertilizer in relation to the roots that are not colonized. This agrees with Páez (2006) on the inoculation with *Trichoderma* spp., which reduced the rate of nitrogen required by maize plants and thereby the costs, obtaining an appreciable improvement of the environment. Other studies conducted with diverse bacterial promoters of vegetal growth, in different soils, climates and crops of agronomic importance, demonstrated 5% to 30% of increase in the yield, as well as a diminution from 25% to 50% of the dose of chemical fertilizers, like nitrogen and phosphorus (Hernandez-Escareño et al., 2015; García et al., 2012; Adesemoye et al., 2009; Aguado & Moreno, 2008). The results obtained with *Trichoderma virens* in this research can be ascribed to the participation of this fungus in the biotransformation of cellulose, acceleration of cellular reproduction, mineralization of nitrogen, and the solubilization of the phosphorus present in the soil, but also due to the increase in roots volume, fostering their space for exploration and thereby obtaining more nutrients and water. All these biological processes favor the growth of plants (Cupull et al., 2003).

#### CONCLUSION

This study concluded that isolates of native *Trichoderma* spp. and *Bacillus* spp. had better response as promoters of vegetal growth in contrast to the non-native strains, both



in greenhouse and field conditions. *Trichoderma* spp. and *Bacillus* spp. in field conditions reduced 50% the dose of nitrogen fertilization used for the maize crop. The use of these native microorganisms as biofertilizers can be an alternative with ample future in sustainable agriculture. They reduce the nitrogen chemical fertilizer use, the production costs, and the contamination of soil and seas by the excessive use of nitrogen fertilizers.

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## REFERENCES

- Adesemoye, A., H. Torbert & J. Kloepper (2009). Plant growth promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microbial Ecology* 58: 921-929.
- Aguado, A. & B. Moreno (2008). Potencial de bioinoculantes microbianos como una alternativa para reducir costos de fertilización en maíz de temporal. In: Memorias Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, INIFAP/SAGARPA. Guanajuato, Mexico.
- Aguirre Medina JF, MB. Irizar Garza, A. Durán Prado, OA. Grajeda Cabrera, MA. Peña del Río, C. Loredó Ostil & A. Gutierrez Baeza (2009). Los Biofertilizantes microbianos: alternativa para la agricultura en México. *Inifap* 5: 1-86.
- Altamirano D., W. Kent & T. Eric (2002). Microbial communities and their interactions in soil and rhizosphere ecosystems. *Annual Review of Microbiology* 56: 211-236.
- Altomare, C., W.A. Norvell, T. Bjorkman & G.E. Harman (1999). Solubilization of phosphates and micronutrients by the plant growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Applied Environmental Microbiology* 65: 2926-2933.
- Armenta Bojórquez, A.D., C. Cervantes Medina, J.A. Galaviz Lara, J.R. Camacho Báez, M. Mundo Ocampo & C. García Gutiérrez (2012). Impacto de la fertilización nitrogenada en agua para consumo humano en el municipio de Guasave, Sinaloa, México. *Ra Ximhai* 8(3): 11-16.
- Armenta Bojórquez, A.D., H.R. Roblero Ramírez, J.R. Camacho Báez, M. Mundo Ocampo M, C. García Gutiérrez & A. Armenta Medina (2015). Organic versus synthetic fertilisation of beans (*Phaseolus vulgaris* L.) in Mexico. *Experimental Agriculture* 52(1): 154-162.
- Bacilio Jiménez, M., S. Aguilar Flores, E. Ventura Zapata, E. Pérez Campos, E. Bouquelet & E. Zenteno (2003). Chemical characterization of root exudates from rice (*Oryza sativa*) and their effects on the chemotactic response of endophytic bacteria. *Plant and Soil* 249: 271-277.
- Bashan, Y. & L.E. De-Bashan (2002). Reduction of bacterial speck (*Pseudomonas syringae* pv. *tomato*) of tomato by combined treatments of plant growth-promoting bacterium *Azospirillum brasilense*, streptomycin sulfate and chemo-thermal seed treatment. *European Journal of Plant Pathology* 108: 821-829.
- Beracochea, M. (2011). Respuesta de variedades comerciales de maíz (*Zea mays* L.) a la inoculación con bacterias endófitas-diazótrofas nativas. (Tesis de Licenciatura). Universidad de la Republica, Montevideo, Uruguay.
- Cano, M.A. (2011). Interacción de microorganismos benéficos en plantas: Micorrizas, *Trichoderma* spp. y *Pseudomonas* spp. *Actualidad & Divulgación Científica* 14: 15-31.
- Candelero D.J, A.J. Cristóbal, R.A. Reyes, S.J.M. Tun, A.M.M. Gamboa & S.E. Ruíz (2015). *Trichoderma* spp. promotoras del crecimiento en plántulas de *Capsicum chinense* Jacq. y antagonicas contra *Meloidogyne incognita*. *Phyton* 84: 113-119.
- Castro, A.M. & C.A. Revillas (2005). Biorregulación de *Rhizoctonia solani* en germinadores de café. Boletín CENICAFÉ. Avance técnico No. 336.
- Chen, Y.P., P.D. Rekha, A.B. Arun, F.T. Shen, W. Lai & C.C. Young (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology* 34: 33-41.
- Cupull, S.R., R.C.M. Andréu, N.C. Pérez, P.Y. Delgado & M.C. Cupull (2003). Efecto de *Trichoderma viride* como estimulante de la germinación, el desarrollo de posturas de cafetos y el control de *Rhizoctonia solani* Kuhn. *Centro Agrícola* 30 (1): 21-25.
- De la Garza, J.L. (1996). Fitopatología General. Universidad Autónoma Nuevo León. Facultad de Agronomía. Marín Nuevo León. pp. 515.
- Díaz, P., R. Ferrera, J. Almaraz & G. Alcantar (2001). Inoculación de bacterias promotoras del crecimiento en lechuga. *Terra* 19: 327-335.
- Egamberdiyeva, D. & G. Höflich (2004). Effect of plant growth-promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region of Uzbekistan. *Journal en Arid Enviroments* 56: 293-301.
- Figueiredo, M.V.B. & C.R. Martinez (2008). Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). *Microbiology Biotechnology* 24: 1187-93.
- García Crespo, R.G., M.A. Arcia Montesuma, M.R. Pérez Tortolero & R.F. Riera Tona (2012). Efecto de *Trichoderma* sobre el desarrollo de papa y el biocontrol de *Rhizoctonia* bajo tres tiempos de inicio de aplicación. *Agronomía Tropical* 62(1-4): 77-95.
- García, J., V. Moreno, I. Rodríguez, A. Mendoza & N. Mayek (2007). Efecto de cepas de *Azospirillum brasilense* en el crecimiento y rendimiento de grano de maíz. *Revista Fitotecnica Mexicana* 30(3): 305-310.

- González, S.C.H., L.L. Rodríguez, C. Arjona, A. Puertas & M. Fonseca (1999). Efecto de la aplicación de *Trichoderma harzianum* sobre la composición cuantitativa de bacterias, hongos y actinomicetos de la rizósfera de Solanáceas y su influencia en el crecimiento vegetativo. *Investigación Agraria Producción y Protección Vegetales* 14: 297-306.
- Guigón, C. & P.A. González (2004). Selección de cepas nativas de *Trichoderma* spp. con actividad antagonista sobre *Phytophthora capsici* Leonian y promotoras de crecimiento en el cultivo de chile (*Capsicum annuum* L.). *Revista Mexicana de Fitopatología* 22(1): 117-124.
- Harman, G (2006). Descripción de mecanismos y aplicaciones de *Trichoderma* spp. *Phytopathology* 96: 190-194.
- Hernández Castillo, F.D., R.H. Lira Saldivar, G. Gallegos Morales, M. Hernández Suárez & S. Solis Gaona (2014). Biocontrol de la marchitez del chile con tres especies de *Bacillus* y su efecto en el crecimiento y rendimiento. *Phyton* 83: 49-55.
- Hernández Escareño, J.J., P.G. Morales, R. Farías Rodríguez & J.M. Sánchez Yáñez (2015). Inoculación de *Burkholderia cepacia* y *Gluconacetobacter diazotrophicus* sobre la fenología y biomasa de *Triticum aestivum* var. Nana F2007 a 50% de fertilizante nitrogenado. *Scientia Agropecuaria* 6 (1): 7-16.
- Hungria, M., C. Rubens, E. Souza & F. Pedrosa (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yield of maize and wheat in Brazil. *Plant and Soil* 331: 413-425.
- Kleifeld, O. & I. Chet (1992). *Trichoderma harzianum* interactions with plants y effect on growth response. *Plant and Soil* 144: 267-272.
- Lewis, J.A. & G.C. Papavizas (1984). A new approach to stimulate population proliferation of *Trichoderma* species and other potential biocontrol fungi introduced into natural soils. *Phytopathology* 74: 1240-1244.
- López Valenzuela, B.E., A.D. Armenta Bojorquez, S. Hernández Verdugo, M.A. Apodaca Sánchez, J.A. Samaniego Gaxiola, K.Y. Leyva Madrigal & A. Ortiz Valdez (2015). Selección *in vitro* e identificación de aislados de *Trichoderma* spp. y *Bacillus* spp. nativos para el control de *Phymatotrichopsis omnivora*. *ITEA* 111(4): 310-325.
- Loredo, C., L. López & D. Espinosa (2004). Bacterias promotoras del crecimiento vegetal asociadas con gramíneas. *Terra Latinoamericana* 22(2): 225-239.
- Martínez, O., M. Jorquera, D. Crowley, G. Gajardo & M. Mora (2010). Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal Soil Science Plant Nutrition* 10(3): 293-319.
- Mena Violante, G.H. & V. Olalde Portugal (2007). Alteration of tomato fruit quality by root inoculation with plant growth-promoting rhizobacteria (PGPR): *Bacillus subtilis* BEB-13bs. *Scientia Horticulturae* 113: 103-106.
- Nezarat, S. & A. Gholami (2009). Screening plant growth promoting rhizobacteria for improving seed germination, seedling growth and yield of maize. *Pakistan Journal of Biological Sciences* 12(1): 26-32.
- Ohba, M. & K. Aizawa (1986). Insect toxicity of *Bacillus thuringiensis* isolated from soil in Japan. *Journal Invertebrate Pathology* 47(1): 12-20.
- Páez, O. (2006). Uso agrícola de *Trichoderma*. <http://www.soilfertility.com/Trichoderma/espagnol/index.shtml>.
- Papavizas, G.C. & Ch.B. Davey (1959). Evaluation of various media and antimicrobial agents for isolation of soil fungi. *Soil Science* 88: 112-117.
- Pulido, L.E., N. Medina & A. Cabrera (2003). Biofertilización con rizobacterias y hongos micorrízicos arbusculares en la producción de posturas de tomate (*Lycopersicon esculentum* mill.) y cebolla (*Allium cepa* l.). i. crecimiento vegetativo. *Redalyc* 24(1): 15-24.
- Reyes, I., L. Alvarez, H. El Ayoubi & A. Valery (2008). Selección y Evaluación de Rizobacterias Promotoras del Crecimiento en Pimentón y Maíz. *Bioagro* 20(1): 37-48.
- Rives, N., Y. Acebo & A. Hernández (2007). Bacterias promotoras del crecimiento vegetal en el cultivo del arroz (*Oryza sativa* l.). Perspectivas de su uso en Cuba. *Redalyc* 28(2): 29-38.
- Sánchez López, D.B., R.M. Gómez Vargas, M.F. Garrido Rubiano & R.R. Bonilla Buitrago (2012). Inoculación con bacterias promotoras de crecimiento vegetal en tomate bajo condiciones de invernadero. *Revista Mexicana de Ciencias Agrícolas* 3(1): 1401-1415.
- Sánchez Yáñez, J.M. (2006). Bacterias promotoras de crecimiento vegetal. <http://www.monografias.com/trabajos33/crecimiento-vegetal/crecimiento-vegetal.shtml>.
- Sánchez, J.M., M.J. García & R. Farias (2006). *Burkholderia*, endófita del teocintle (*Zea mays* sp. mexicana) promotora de crecimiento de maíz (*Zea mays* L.). <http://www.monografias.com/trabajos33/burkholderia/burkholderia.shtml>.
- SAS (2004). Statistical Analysis System version 9.1. SAS Institute Inc., Cary, NC, USA.
- SIAP/SAGARPA (2015). Servicio de Información Agroalimentaria y Pesquera/ Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. Base de Datos Estadísticos de Producción. <http://www.siap.gob.mx/avance-de-siembras-y-cosechas-por-estado>.
- Valencia, H., J. Sánchez & N. Valero (2005). Producción de ácido indolacético por microorganismos solubilizadores de fosfatos presentes en la rizósfera de *Espeletia grandiflora* y *Calamagrostis effusa* del Páramo el Granizo. En: Bonilla MA (Ed): Estrategias adaptativas de plantas de páramo y del bosque andino en la cordillera oriental de Colombia. *Unibiblos*. Bogotá, Colombia, pp. 177-193.