Botanical Sciences 97 (4): 609-622. 2019
DOI: 10.17129/botsci.2200

Received: February 1, 2019, accepted: July 23, 2019
On line first: December 17, 2019

Ecology/Ecología

Nodule-forming *Sinorhizobium* and arbuscular mycorrhizal fungi (AMF) improve the growth of *Acacia farnesiana* (Fabaceae): an alternative for the reforestation of the Cerro de la Estrella, Mexico

Sinorhizobium formadores de nódulos y hongos micorrízicos arbusculares (HMA) mejoran el crecimiento de *Acacia farnesiana* (Fabaceae): una alternativa para la reforestación del Cerro de la Estrella, México

Selene Gómez-Acata[1], Enriqueta Amora-Lazcano[1], En Tao Wang[1], Flor N. Rivera-Orduña[1], Juan Carlos Cancino-Díaz[1], Juan Antonio Cruz-Maya[2], and Janet Jan-Roblero[1]*

[1] Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico.
[2] Unidad Profesional Interdisciplinaria en Ingeniería y Tecnologías Avanzadas, Instituto Politécnico Nacional, Mexico City, Mexico.

* Corresponding author: jjan_r@yahoo.com.mx

Abstract

Background: Cerro de la Estrella (CE) is a natural reserve in Mexico City that suffers from afforestation, and its restoration with *Acacia farnesiana* is being considered.
Question: Will the nodule-forming rhizobia and arbuscular mycorrhizal fungi (AMF) associated with the CE soil support *A. farnesiana* growth?
Study species: *Acacia farnesiana* (L.) Willd. (Fabaceae).
Methods: Mycorrhizal fungi, nodule-forming rhizobia and physicochemical characteristics of the CE soil were studied to determine if they are suitable for improving the growth of *Acacia farnesiana*.
Results: Four different families of AMF were found which generated 13 % mycorrhization with *A. farnesiana*. However, *A. farnesiana* from CE did not nodulate, suggesting the lack of native rhizobia. The CE soil has low fertility. Nodules of *A. farnesiana* were obtained from the soil in Ticuman, Morelos, and 66 rhizobia were isolated from them. *Rhizobium* isolates were individually added to *A. farnesiana* grown in the CE soil. Five of the 66 isolates yielded significant differences in shoot dry weight, shoot height, number of nodules, nodulation time and nitrogenase activity compared with the *Sinorhizobium americanum* CFNEI 156 control strain ($p < 0.05$). Three isolates were named as *S. americanum* ENCBTM1, ENCBTM31 and ENCBTM43, and last two as *Sinorhizobium* sp. ENCBTM34 and ENCBTM45.
Conclusions: CE soil had low fertility and lacked specific rhizobia for *A. farnesiana*. The individual addition of *S. americanum* (ENCBTM1, ENCBTM31 or ENCBTM43) or *Sinorhizobium* sp. (ENCBTM34 or ENCBTM45) improved the growth of *A. farnesiana*.
Keywords: *Acacia farnesiana*, mycorrhiza, rhizobia, symbiosis.

Resumen

Antecedentes: El Cerro de la Estrella (CE) es una reserva natural deforestada en la Ciudad de México cuya restauración con *Acacia farnesiana* es considerada.
Pregunta: ¿Los rizobios nodulantes y los hongos micorrízicos arbusculares (HMA) del CE apoyarán el crecimiento de *A. farnesiana*?
Especies de estudio: *Acacia farnesiana* (L.) Willd. (Fabaceae).
Métodos: Se estudiaron los HMA, los rizobios nodulantes y las características fisicoquímicas del suelo del CE para determinar si mejoran el crecimiento de *A. farnesiana*.
Resultados: Cuatro familias de HMA generan 13 % de micorrización con *A. farnesiana*. La planta no noduló creciendo en suelo del CE sugiriendo la falta de rizobios nativos. El suelo del CE tiene una baja fertilidad. Nódulos de *A. farnesiana* se obtuvieron de Ticuman, Morelos y 66 rizobios se aislaron de ellos, los cuales se analizaron individualmente en *A. farnesiana* empleando suelo del CE y solo cinco produjeron diferencias significativas en el peso seco, altura del brote, el número de nódulos, el tiempo de nodulación y la actividad de la nitrogenasa en comparación con la cepa control *Sinorhizobium americanum* CFNEI 156 ($p < 0.05$). Tres aislados fueron identificados como *S. americanum* ENCBTM1, ENCBTM31 y ENCBTM43, y dos como *Sinorhizobium* sp. ENCBTM34 y ENCBTM45.
Conclusiones: El suelo CE tiene baja fertilidad y carece de rizobios específicos para *A. farnesiana*. La adición individual de *S. americanum* (ENCBTM1, ENCBTM31 o ENCBTM43) o *Sinorhizobium* sp. (ENCBTM34 o ENCBTM45) mejoró el crecimiento de *A. farnesiana*.
Palabras clave: *Acacia farnesiana*, micorrízas, rizobios, simbiosis.

This is an open access article distributed under the terms of the Creative Commons Attribution License CCBY-NC (4.0) international.
https://creativecommons.org/licences/by-nc/4.0/

Cerro de la Estrella (CE) is a zone located in the Transverse Neovolcanic Axis within Mexico City. Since 1991, CE has been designated a natural reserve. However, due to expansion of the city, environmental pollution and displacement of the urban regions, the forest has been degraded, and the natural environments have gradually been destroyed. Some species of trees, such as *Eucalyptus* L'Hér. and *Cupressus lusitanica* Mill., have been introduced into CE but their introduction was excessive or inadequate and resulted in the displacement of some native endemic species, such as *Acacia farnesiana* (L.) Willd., known locally as huisache dating to the pre-Hispanic period (Montero-García 2002). Efforts to replant *A. farnesiana* to recover the natural ecosystem in CE have been proposed with the support of the National Institute of Anthropology and History of Mexico.

A. farnesiana is a leguminous tree widely distributed around the world, native to the Neotropics and planted across Mexico (Barrientos-Ramírez *et al*. 2012). It is used as forage, medicine and a source of fuel, glue, and tannins (Monroy-Ata *et al.* 2007). This multipurpose tree is used to revegetate degraded ecosystems because of its ability to form symbiotic associations with rhizobia and mycorrhizal fungi. These bacterial and fungal symbionts are known to be key components of natural ecosystems, since they help to govern the cycles of major plant nutrients and sustain the vegetation cover in natural habitats (Lauriano-Barajas & Vega-Frutis 2018). Rhizobia are soil Gram-negative bacteria that are able to form nodules on the roots or stems of legumes. Inside the nodules, these bacteria can fix nitrogen from the atmosphere to form ammonia, which can be assimilated by the leguminous plants (Lei *et al*. 2008). Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that belong to the phylum Glomeromycota. They form symbioses with various terrestrial plant species. The symbioses are biotrophic and normally mutualistic with the long-term compatible interactions based largely on bidirectional nutrient transfer and disease tolerance. The soil fungi provide the plants with phosphorous and other immobile nutrients, and in exchange, the plants supply carbohydrates to them. In addition, these fungi can protect plants against pathogens and drought (Camargo-Ricalde 2017, Monroy-Ata *et al.* 2007). Particularly, for the Mexican *Acacia* species, a new species of *Sinorhizobium* isolated from *Acacia acatlensis* Benth. was reported from the Sierra de Huautla in Morelos, Mexico. This new species was named *S. americanum* and the type strain is CNFN156, which is able to nodulate *A. acatlensis* and *A. farnesiana* (Toledo *et al*. 2003).

Restoration of the native vegetation and the regeneration of functioning ecosystems is now a major conservation focus in several locations of the world where poor management practices have resulted in serious land degradation problems. The revegetation of CE with *A. farnesiana* is an attractive proposal to reestablish a functional and stable ecosystem. However, to achieve the revegetation of CE with *A. farnesiana,* it is important to study the contribution of the nodule-forming rhizobia and AMF associated to CE soil and the physicochemical characteristics of this soil. Therefore, the present work was focused on performing these analyses.

Materials and methods

Physicochemical analysis of the soil. Three soil samples were collected randomly around 1 m^2 at Cerro de la Estrella, Mexico City (19° 20' 31" N, 99° 05' 22" W, altitude 2,460 m), air dried and sieved for physicochemical characterization, including soil texture (Bouyoucos hydrometer method), water holding capacity (gravimetric method) (Blažka & Fischer 2014), pH (using a glass electrode, 1:2 soil: distilled water suspension) and electrical conductivity (1:2 soil: distilled water suspension) (Rhoades *et al.* 1989). Organic carbon content (C) was determined by oxidation with $K_2Cr_2O_7$ and titration of excessive dichromate with $(NH_4)_2FeSO_4$. Cation exchange capacity was measured by extracting soil with a solution of calcium chloride, followed by cation exchange of Ba^{2+} by Mg^{2+} in the extract. The Mg^{2+} was then titrated with a solution of EDTA. In addition, the content of nitrogen (Kjeldahl method), phosphorus (Olsen method), arsenic and lead (atomic absorption spectrometry) were determined.

Strain isolation and growth conditions. Strains of rhizobia were isolated from the nodules of *Acacia farnesiana* (L.) Willd. grown in pots filled with soil from Ticuman, Morelos, Mexico. The rhizobia were isolated as described by Vincent (1970) using yeast extract mannitol (YEM) media. The strains were purified by several subcultures on YEM plates at 28 °C. All the rhizobia were maintained in YEM and stored in 20 % glycerol at -80 °C. The control strains *Sinorhizobium americanum* CFNEI 156 isolated from *Acacia acatlensis* Benth. and *S. americanum* CFNE 54 from *Acacia macilenta* Rose, were provided by Dr. E. Martinez from Universidad Nacional Autónoma de Mexico (UNAM). These strains were used as positive and comparative controls against the rhizobia isolates obtained in this work.

Plant infection tests. All rhizobial isolates (66 strains) were tested for their ability to nodulate *A. farnesiana*. The seeds of *A. farnesiana* were manually scarified and superficially disinfected with ethanol at 95 % for 30 sec, followed by 3 % (v/v) calcium hypochlorite for 5 min and rinsed with sterile water (Vincent 1970). The seeds were germinated for 48 h on sterile sand at 28 °C. The seedlings were placed aseptically in powder pouches (Somasegaran & Hoben 1994) and supplemented with a nitrogen-free plant nutrient solution (Vincent 1970). Each pouch was inoculated with 0.1 mL of rhizobial suspension (10^7 CFU/seedling) seven days after planting. *S. americanum* CFNEI 156 and *S. americanum* CFNEI 54 were included as positive controls. Uninoculated plants and plants supplied with nitrogen [6 mM KNO_3, 1 mM $NH_4H_2PO_4$, and 5.7 mM $Ca(NO_3)_2$] were used as negative controls. Plants were grown under greenhouse conditions for 40 days. The shoot height and shoot dry weight were measured to evaluate the plant growth efficiency, while the nodule appearance and numbers were counted. Each isolate was analyzed in triplicate and the mean values are reported. In the statistical analysis was compared each rhizobial isolate with the positive control strains.

Acetylene reduction assay. To evaluate the nitrogenase activity of the nodules, *A. farnesiana* plants inoculated with each isolate and with pool A (a combination of isolates with high effectiveness) and pool B (a combination of isolates and reference strains) were grown for ten weeks in pots filled with moisturized vermiculite and N-free plant nutrient solution. The roots containing nodules were cut off after harvesting and placed in perfectly sealed vials. Subsequently, 0.6 mL of air was extracted from each vial, replaced with the same volume of acetylene and incubated for one hour at room temperature. The content of acetylene and ethylene (product of acetylene reduction) was determined using a Varian 3,300 (Walnut Creek, CA, USA) gas chromatograph with a flame ionization detector. Roots without nodules served as blank controls. Roots with nodules from *S. americanum* CFNEI 54 and nodules from *S. americanum* CFNEI 156 were also included as positive controls. The nitrogenase activity was expressed as nmol ethylene h^{-1} $plant^{-1}$. Each isolate was analyzed in triplicate and the nitrogenase activity of the isolates was compared with the positive controls.

Analysis of AMF. The rhizosphere soil samples of the plants located in CE were collected to analyze the AMF spores. The spores were extracted from 50 g of soil, separated by wet sieving, decanting (Genderman & Nicolson 1963) and sucrose centrifugation. Healthy spores were counted, and the abundance of the AMF in the soil was expressed as the number of spores/gram of dry soil. Each spore type was mounted in PVLG (polyvinyl-lacto-glycerol) and PVLG/Melzer's reagent (Brundrett *et al.* 1996). To identify AMF taxonomically at the genus level, different ontogenetic and morphological criteria of the spores were examined, such as the presence/absence and type of hyphae, constitution of the spore wall (number of layers and ornamentation), presence of a germinal wall, presence of germinal shields and a histological response of any of the layers of the wall to Melzer's reagent. The databases of the International Collection of Mycorrhizal Fungi (INVAM 2019) and the International Bank of Glomeromycota (IBG 2016) were consulted to identify the isolates at the species level.

To obtain AMF that could be adapted to colonize *Acacia farnesiana* using the spores obtained, *A. farnesiana* were grown in pots filled with soil samples of CE under greenhouse conditions. The plants were harvested to assess the degree of mycorrhizal infection after three months. The roots were washed in water, cleared and stained as described by Phillips & Hayman (1970). The AMF colonization was estimated using the slide method (Giovannetti & Mosse 1980). The presence of vesicles, arbuscules or typical mycelia within the roots was considered to be colonization, and the results were expressed as a percentage.

Statistical analysis. The results were analyzed in SPSS 15.0 for Windows. The data were subjected to an ANOVA analysis at $p < 0.05$ and a Dunnett's test with $p < 0.05$ was used to compare the results of the isolates with the positive controls.

DNA extraction. PY broth cultures of selected isolates were boiled with 0.1 % Tween 20 for 10 min and immediately cooled on ice to induce lysis. This suspension was used as the PCR template.

16S rRNA, atpD, recA and glnII gene amplification. The primers used for 16S rRNA gene amplification were fD1 (5´-AGAGTTTGATCCTGGCTCAG-3´) and rD1 (5´-AAGCTTAAGGTGATCCAGCC-3´). The *atpD* fragment was amplified using *atpD255F* (5'- GCT SGG CCG CAT CMT SAA CGT C -3') and *atpD*782R (5'- GCC GAC ACT TCM GAA CCN GCC TG -3'). A partial *recA* gene was amplified using *recA41F* (5'- TTC GGC AAG GGM TCG RTS ATG -3') and *recA640R* (5'- ACA TSA CRC CGA TCT TCA TGC -3'). Finally, a partial *glnII* gene was obtained with *glnII12F* (5'- YAA GCT CGA GTA CAT YTG GCT -3') and *glnII*689R (5'- TGC ATG CCS GAG CCG TTC CA -3') (Vinuesa *et al.* 2005). The amplifications were conducted in 50 µL reaction volumes (template DNA, 1x reaction buffer, 2 mM $MgCl_2$, 1 U Taq polymerase, 200 µM dNTP's and 15 pmol of each primer). The PCR cycles were 95 °C for 3.5 min, followed by 35 cycles of 94 °C for 70 sec, alignment at 56 °C for 40 sec, 72 °C for 2 min with a final extension step at 72 °C for 6 min 10 sec. The alignment temperature for the amplification of *recA* and *glnII* was changed to 58 °C for 1 min. The amplified fragments were purified using QIAquick and sequenced at the UNAM-FES-Iztacala UBIPRO, Biochemistry Molecular Laboratory.

Sequence analysis of the 16S rRNA gene and multilocus sequence analysis (MLSA). The taxonomic identification of the bacteria was based on the level of similarity with reference sequences from the GenBank and phylogenetic relationship analysis. The sequences acquired were compared with those in the GenBank database using the program BLAST (Camacho et al. 2009). The *16S rRNA, recA, atpD* and *glnII* gene sequences were aligned independently using CLUSTAL X software (Larkin *et al*. 2007). The sequences of the *recA, atpD* and *glnII* genes were concatenated manually. Phylogenetic analyses of both data were performed by maximum likelihood using PhyML (Guindon *et al.* 2010). The jModelTest 3.06 software was used to select appropriate models of sequence evolution using the AIC (Akaike information criterion) (Darriba *et al.* 2012). The statistical confidence of the nodes was estimated by bootstrapping using 1,000 replications, and *Ensifer garamanticus* was used as an outgroup. Similarities among the sequences were calculated using the MatGAT v.2.01 software (Campanella *et al.* 2003).

Results

Microbiological and physicochemical characterization of the CE soil. Microbiological and physicochemical characteristics of the CE soil were studied to determine its quality and the viability for reforestation with *Acacia farnesiana* (L.) Willd. In regard to the physicochemical characteristics only those indicators that determined the degree of fertility of the CE soil were evaluated in three soil samples from the same area

without comparison with another soil. Based on the criteria of Aprile & Lorandi (2012), the CE soil presents low fertility due to the low value of electrical conductivity which in turn suggests a low sodium concentration. In addition, the high values of pH and cation exchange capacity are indicative of a high concentration of cations while the elements of nitrogen and phosphorous are low (Table 1). The low soil fertility of CE could be a consequence of its parent material. In addition, the high amount of arsenic found based on the NOM-147-SEMARNAT/SSA1-2004 (SEMARNAT 2007) could have been due to its volcanic origin. This result suggests that the physicochemical properties are influencing the quality and fertility of the soil. Besides, the low amount of nitrogen in the CE soil could have an important effect on *A. farnesiana* growth, based on this idea, nodule-forming and nitrogen-fixing rhizobia were studied in order to improve the plant growth.

The AMF and nodule-forming rhizobia associated with the CE soil were studied to determine the microbiological characteristics of the soil. The presence of different spores was observed from CE soil samples (Figure 1A). The quantification of the rhizospheric soil spores was between 10 to 40 spores per 10 g of dry soil, indicating a quality criterion between low and medium (Tovar-Franco 2006). Native colonization assays using *A. farnesiana* plants grown in the CE soil showed approximately 13 % of mycorrhization, and the stained roots of the *A. farnesiana* plants showed the presence of characteristic structures of AMF (arbuscules, vesicles and cenocitic hyphae; Figure 1B and C), which indicates that the fungi present in the CE soil are infective. Subsequently, the spores were identified based on their morphological characteristics, and ten AMF morphotypes belonging to four different families (Appendix 1) included: *Acaulospora laevis* (Figure 2A), *Acaulospora spinose* (Figure 2B), *Gigaspora decipiens* (Figure 2C), *Racocetra gregaria* (Figure 2D), *Scutellospora pellucida* (Figure 2E), *Septoglomus constrictum* (Figure 2F), *Funneliformis mosseae* (Figure 2G), *Glomus* sp. (Figure 2H), *Glomus aurantium* and *Paraglomus occultum*.

Table 1. Physicochemical characteristics from Cerro de la Estrella Soil.

Characteristics	Cerro Estrella	NOM-SEMARNAT
Soil texture	Sandy loam	
Organic matter (%)	4.46±0.8	Low*
Water holding capacity (%)	33±9	
Moisture (%)	16±5	
pH	8.1±0.6	Moderately alkaline*
Electrical conductivity (mS cm^{-1})	0.06±0.02	Low *
Cation exchange capacity (meq100g^{-1})	13.30±1.5	High**
Nitrogen (%)	0.13±0.1	Low *
Phosphorous (mg Kg^{-1})	1.7±0.2	Low *
Arsenic (mg Kg^{-1})	5310±311.5	High**
Lead (mg Kg^{-1})	32±3.5	Normal*

* Observations based on NOM-021-SEMARNAT 2000 (SEMARNAT 2002), which establishes the specifications of fertility, salinity and soil classification, study, sampling and analysis. ** Observation based on NOM-147-SEMARNAT / SSA1-2004 (SEMARNAT 2007), which establishes the criteria to determine the remediation concentrations of soils contaminated by arsenic, barium, beryllium, cadmium, hexavalent chromium, mercury, nickel, silver, lead, selenium, thallium and / or vanadium.

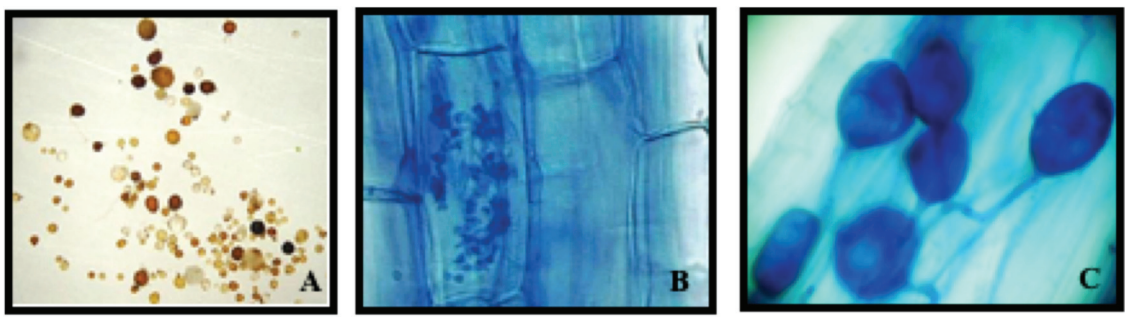

Figure 1. Structures of the AMF present in the CE soil and the *A. farnesiana* root. (A) CE soil spores; (B) cenocitic hyphae and arbuscules inside the cortical cells of the *A. farnesiana* root; (C) vesicles and cenocitic hyphae.

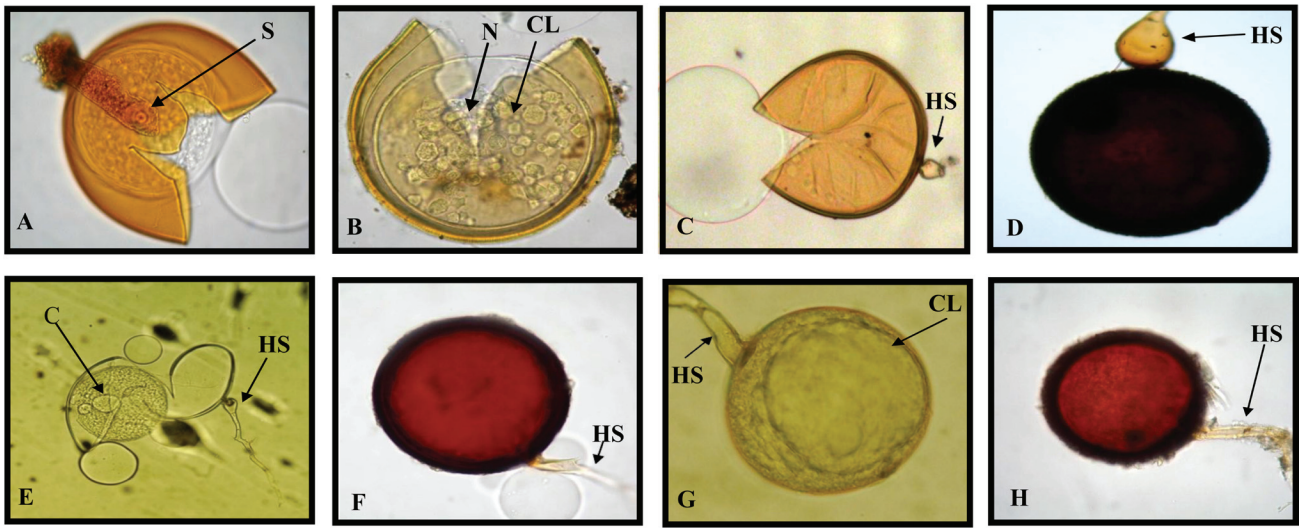

Figure 2. Identification of the AMF spores present in the CE soil. (A) *Acaulospora laevis*; (B) *A. spinosa*; (C) *Gigaspora decipiens*; (D) *Racocetra gregaria*; (E) *Scutellospora pellucida*; (F) *Septoglomus constrictum*; (G) *Funneliformis mosseae*, and (H) *Glomus* sp. S: scar. HS: support hypha, CL: lipid content, N: nuclei.

These results showed that there are different species of mycorrhizal fungi in the soil of CE that are capable of colonization.

Nodule formation experiments were conducted to isolate and identify the rhizobia. The plants of *A. farnesiana* obtained from CE lacked of nodules. Thus, nodules were sought in other plant species of the same region, and *Acacia schaffneri* Thell. showed the presence of nodules when they were collected in the field (Figure 3A). The four rhizobial isolates CE1, CE2, CE3 and CE4 were isolated from them. Subsequently, nodulation experiments of the CE1, CE2, CE3 and CE4 isolates with *A. farnesiana* were conducted in the laboratory using the soil from CE, including plants of *A. farnesiana* growing in soil only (as a negative control). After independent treatment with each isolate of rhizobia (CE1, CE2, CE3 or CE4), *A. farnesiana* did not form nodules (Figure 3B). *A. farnesiana* with the addition of the control strains *Sinorhizobium americanum* CFNEI 156 (control 1) and *S. americanum* CFNE 54 (control 2) formed nodules (Figure 3C). The results indicated that the CE soil lacked of specific rhizobia capable of nodulating *A. farnesiana*. It was also found that *S. americanum* CFNEI 156 and *S. americanum* CFNEI 54 are able to nodulate *A. farnesiana*.

Isolation of beneficial rhizobia and Acacia farnesiana assay. As a result of the previous experiments, the next strategy was to use soil from Ticuman, Morelos, which is an area where *A. farnesiana* grows naturally and is close to Mexico City. Therefore, the soil should contain rhizobia specific for *A. farnesiana*, whose plants growing in Ticuman soil formed nodules in the roots after eight weeks of growth. Sixty-six isolates of rhizobia were obtained from these conditions. Subsequently, the 66 isolates were tested with *A. farnesiana* in the CE soil. Only 36 isolates formed nodules in the root

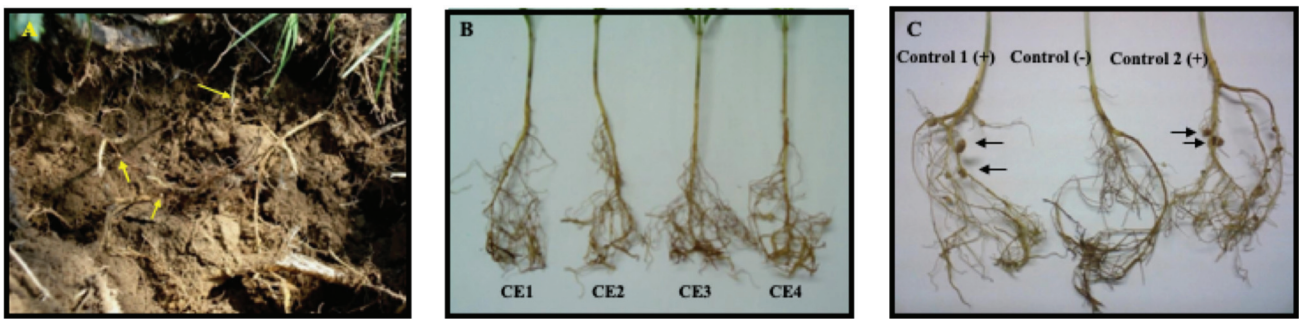

Figure 3. Nodulation tests of *A. farnesiana* (A) Nodules of *A. schaffneri* collected at the field of CE. (B) *A. farnesiana* inoculated with strains CE1, CE2, CE3 and CE4. (C) *A. farnesiana* inoculated with the reference strains *S. americanum* CFNE156 (control 1) and *S. americanum* CFNE54 (control 2), control (-) corresponds to *A. farnesiana* plants without the addition of bacterial inoculum.

and each one of them was compared with the positive controls. When the other parameters of plant growth were determined, including shoot dry weight (Figure 4A), shoot height (Figure 4B), the number of nodules (Figure 4C) and the time at which nodule sprout (Figure 4D), only the five isolates ENCBTM1, ENCBTM31, ENCBTM34, ENCBTM43 and ENCBTM45 presented a significant difference (Table 3) in all these parameters compared with the negative control, which consisted of *A. farnesiana* plants growing in soil without any additions, and the positive control (*A. farnesiana* added with *S. americanum* CFNEI 156). Figure 4 shows a representative of the 36 isolates.

Alternatively, nitrogenase activity and the shoot dry weight and height of *A. farnesiana* inoculated with the isolates previously selected were measured when they were grown in vermiculite. The ENCBTM1 had a significative nitrogenase activity compared with *S. americanum* CFNEI 156 and *S. americanum* CFNEI 54 (Tables 2 and 3). The rest of the isolates produced amounts of nitrogenase similar to those of the reference strains (positive controls). In addition, we studied pool A that consisted of five rhizobia and pool B that consisted of the components of pool A plus the control strains. Pool B did not present significant nitrogenase activity with the control strains, and the pool A result was similar to the obtained with the ENCBTM1 isolate and present significant difference with the control strains (Tables 2 and 3). The shoot dry weight following treatment with the isolates ENCBTM1, ENCBTM31, ENCBTM34, ENCBTM43, ENCBTM45 and the pool A were significant superior compared to the control strain *S. americanum* CFNEI 156 (Tables 2 and 3) but not with the control strain *S. americanum* CFNEI 54 ($p > 0.05$). However, there was no significant difference in shoot height between the strains isolated and both control strains.

Identification of the rhizobia by analysis of the 16S rRNA gene and multilocus sequence analysis (MLSA). The 16S rRNA gene phylogenetic analysis (Appendix 2 and 3) showed that the five strains ENCBTM1, ENCBTM31, ENCBTM34, ENCBTM43, and ENCBTM45 were grouped with different sequences of the genus *Sinorhizobium*. The clustering analysis based on the 16S rRNA gene did not define clear positions for the five strains. The strains ENCBTM31 and ENCBTM43 showed high similarity of 99.9 % with *S. americanum* (NR_025251), *Sinorhizobium fredii* (Y260145) and *Sinorhizobium xinjiangense* (AM181732), while the strains ENCBTM34 and ENCBTM45 showed 99.4 and 99.6 % with *Sinorhizobium saheli* (NR_026096), respectively. The remaining strain (ENCBTM1) showed a similarity of 99.4 % with *Ensifer adhaerens* (EU928872).

The tree built with the concatenated genes resulted in two groups (A-B) with a bootstrap support of > 80 % (Figure 5). The group B was comprised of two subclades (BI-BII). The three strains ENCBTM1, ENCBTM31, and ENCBTM43 grouped directly with *S. americanum* (clade BI) with a boot-

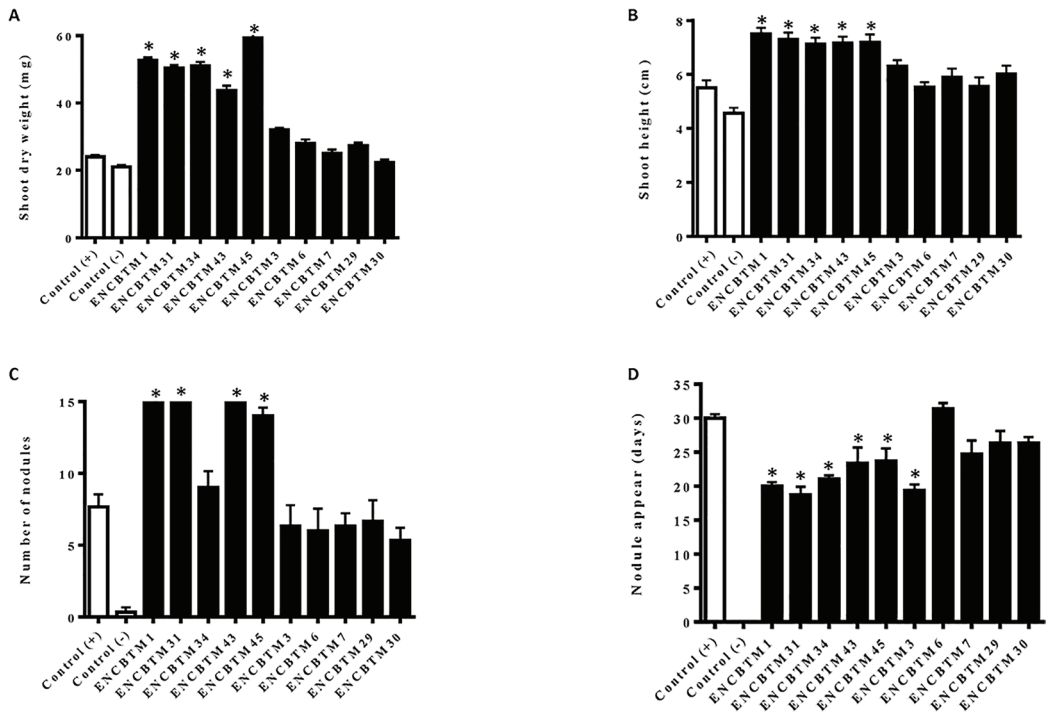

Figure 4. Effectiveness of the isolates in *A. farnesiana* in the selection tests on powder pouches. A black bar showed the selected isolates based on Dunnett's test. (A) Shoot dry weight. (B) Shoot height. (C) Total number of nodules and (D) Nodule sprouts (days). All data were determined 60 days post-inoculation. ANOVA, Dunnett's test, * $p < 0.05$ compared to the positive control.

Table 2. Nitrogenase activity evaluated in isolates selected.

Strain	Nitrogenase activity (nmol etilene h^{-1} $plant^{-1}$)	Shoot dry weight (mg)	Shoot Height (cm)
ENCBTM1	24.74 ± 2*	120.92 ± 3*	12.55 ± 0.9
ENCBTM31	11.87 ± 1.5	121.08 ± 2*	13.43 ± 1
ENCBTM34	11.66 ± 3	120.35 ± 2*	13.30 ± 0.6
ENCBTM43	12.46 ± 2	145.08 ± 4*	13.62 ± 0.8
ENCBTM45	12.40 ± 1.8	140.05 ± 3*	13.51 ± 0.4
S. americanum CFNEI 156	9.72 ± 3	112.00 ± 2	11.87 ± 1.1
S. americanum CFNEI 54	15.23 ± 2	146.80 ± 4	15.92 ± 0.7
Pool A	20.65 ± 3*	154.25 ± 3*	13.25 ± 0.8
Pool B	11.26 ± 2	111.43 ± 2	11.30 ± 0.6
Control 1 (without N)	0.00	82.30 ± 3	9.20 ± 1
Control 2 (0.05% N)	0.00	123.52 ± 2	11.80 ± 0.9

Pool A: ENCBTM1, ENCBTM31, ENCBTM34, ENCBTM43 and ENCBTM45; Pool B: ENCBTM1, ENCBTM31, ENCBTM34, ENCBTM43, ENCBTM45, and *S. americanum* CFNEI 156 and 54. *significant difference compared to the control strain *S. americanum* CFNEI 156 and *S. americanum* CFNEI 54.

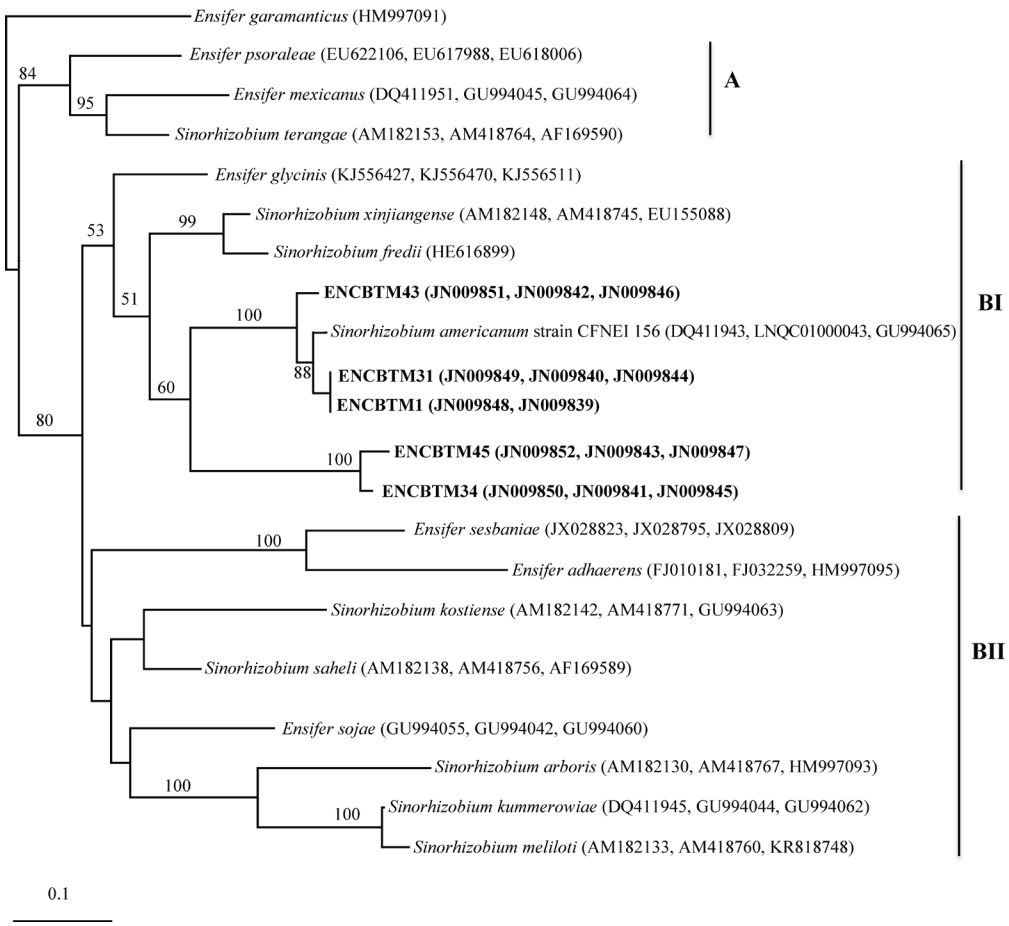

Figure 5. Maximum-likelihood phylogenetic tree based on the concatenated *recA* (446 bp), *atpD* (474 pb) and *glnII* (603 pb) gene sequences. The analysis was performed using the nucleotide substitution model GTR + G with −InL = -7974,11 and Gamma shape parameter = 0.193. Bootstrap values > 50% (based on 1000 replications) are shown at each node.

Table 3. Statistical data of ANOVA and the Dunnett's test.

	Shoot dry weight[1]			Shoot height[1]			Number of nodules[1]			Nodule appear[1]			Nitrogenase activity[2]			Shoot dry weight[2]			Shoot Height[2]		
Strain	Mean Diff.	*q*	*p* < 0.05	Men Diff.	*q*	*p* < 0.05	Mean Diff.	*q*	*p* < 0.05	Mean Diff.	*q*	*p* < 0.05	Mean Diff.	*q*	*p* < 0.05	Mean Diff.	*q*	*p* < 0.05	Mean Diff.	*q*	*p* < 0.05
ENCBTM1	-28.67	21.42	Yes	-2.0	5.557	Yes	-3.333	2.303	No	10	5.282	Yes	-14.99	11.29	Yes	-8.8	8.04	Yes	1.583	1.626	No
ENCBTM31	-26.33	19.68	Yes	-1.8	5.0	Yes	-4.333	2.994	Yes	11.33	5.986	Yes	-1.950	1.468	No	-9.37	8.56	Yes	0.667	0.684	No
ENCBTM34	-27.0	20.17	Yes	-1.633	4.538	Yes	-1.333	0.9213	No	5.0	2.641	No	-1.927	1.45	No	-8.533	7.79	Yes	0.8	0.821	No
ENCBTM43	-19.67	14.69	Yes	-1.667	4.631	Yes	-4.0	2.764	No	6.667	3.521	Yes	-2.76	2.07	No	-34.97	31.95	Yes	0.633	0.65	No
ENCBTM45	-35.33	26.4	Yes	-1.7	4.723	Yes	-2.333	1.612	No	6.33	3.345	Yes	-2.76	2.07	No	-28.33	25.89	Yes	0.7	0.719	No
Pool A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-10.84	8.16	Yes	-42.32	38.67	Yes	0.783	0.804	No
Pool B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-1.627	1.225	No	0.5667	0.517	No	2.767	2.842	No
ANOVA data	$F = 217.4, p < 0.0001$			$F = 13.61, p < 0.0001$			$F = 10.39, p < 0.0001$			$F = 36.21, p < 0.0001$			$F = 60.89, p < 0.0001$			$F = 706.0, p < 0.0001$			$F = 5.91, p < 0.0004$		

[1]Assays carried out with CE soil; results presented in Figure 4.
[2]Assays carried out with vermiculite; results presented in the Table 2.
Mean Diff. It means difference of the means between the control (*S. americanum* CFNEI 156) and the isolates.
ND means not determined.

strap support of 100 %. The sequence similarities of these strains with *S. americanum* type strain were 92.1 to 100 % *(recA),* 99.8 to 100 % (*atpD*) and 97.2 to 99.8 % (*glnII*). While two strains ENCBTM34 and ENCBTM45 are associated in an independent subgroup, but related with the clade of *S. americanum.* These strains showed a lower degree of similarity of < 93.1 % with *S. americanum* type strain suggesting that could be a new species of *Sinorhizobium*.

Discussion

Few native AMF spores were found (10 to 40 spores per 10 g of dry soil) in the CE soil, indicating that they are in a level between low and medium (Tovar-Franco 2006). The abundance of the AMF spores present in the soil may be influenced by the different seasonal patterns of fungal sporulation, which vary depending on each species, and by the synchronization of the flowering of the plant with the sporulation of some AMF. Despite the low amount of AMF spores in the CE soil, the AMF colonization test in the roots of *Acacia farnesiana* (L.) Willd. harvested from the pots with CE soil samples showed 13 % mycorrhization, and this colonization was observed by the presence of cenocitic hyphae and vesicles in the roots of *A. farnesiana* (Figure 1B, 1C). This result suggests that the AMF of the CE soil, although at low quantity, are able to colonize the roots of *A. farnesiana* and could be contributing to the growth of this plant. The AMF from the CE soil were identified as *Acaulospora laevis, Acaulospora spinosa, Gigaspora decipiens, Racocetra gregaria, Scutellospora pellucida, Septoglomus constrictum, Funneliformis mosseae, Glomus aurantium* and *Paraglomus occultum*. These fungi have been shown to be mycorrhizal and have a beneficial relationship with plants. Thus, all the AMF isolated in this study, with the exception of *G. aurantium*, have been reported in natural ecosystems or in agroecosystems in Mexico (Montaño *et al*. 2012). Notably, this is the first report of *G. aurantium* in soils in Mexico. In particular, *A. laevis, A. spinosa, G. decipiens, S. pellucida, S constrictum, F. mosseae* and *P. occultum* have been found in extensive agricultural systems in Mexico in which *A. spinosa, R. gregaria* and *S. constrictum* were isolated from soil of fruit orchards; finally, *A. spinosa* and *S. constrictum* had been found in agroforestry systems in Mexico (Montaño *et al*. 2012). Alternatively, *A. farnesiana* has been primarily associated with AMF, such as *Acaulospora foveata, Gigaspora albida, Glomus fasciculatum, Glomus geosporum* and *Sclerocystis sinuosa* (Udaiyan *et al*. 1996). In addition, the AMF do not have a specific host, and they have a high ability to colonize the root of various plants. The results of the AMF identified in this study show that they can establish mycorrhizal association with *A. farnesiana* determined at microscopic level and could suggest the improvement of the plant growth. Thus, the AMF identified in this study could be considered as potential contributors to the growth of *A. farnesiana* when this plant will be introduced into the CE. On the other hand, it is reported that a combination of AMF and rhizobia enhance nutrition and plant growth in legumes (Meghvansi *et al.* 2008). The above is supported because

AMF increases the uptake of nutrients specially zinc, phosphorus and nitrogen as well as increasing the crop production in legumes (Camargo-Ricalde 2017). Whereas that, the association of rhizobia with the root of plants form symbiotic root nodules and within the cell of these nodule, nitrogen-fixing rhizobia provide the host plant with fixed nitrogen. The rhizobium-legume symbiosis has a crucial impact on the global nitrogen cycle, besides an important supply of nitrogen for the soil and the improvement of the crops (Oldroyd & Dixon 2014). With this same concern, an assay combining the AMF and rhizobia was conducted in the soil samples of CE, formation of nodules in their roots and colonization by native AMF was observed after ten weeks (data not shown) suggesting that *A. farnesiana* could establish association with AMF and rhizobia.

In contrast to the AMF, the rhizobia are more specific to the host they colonize (Kazmierczak *et al.* 2017). However, no rhizobia specific for *A. farnesiana* were found in the CE soil. When rhizobia isolated from another soil (Ticuman, Morelos), were introduced, they were capable of forming nodules and presented nitrogenase activity similar to that of the *Sinorhizobium americanum* CFNEI 156 strain in *A. farnesiana*, showing a symbiotic association between these exo-rhizobia and the plant. To explain the lack of specific rhizobia of *A. farnesiana* in the CE soil, the physicochemical characteristics of the soil were determined and only the macronutrients that estimate the degree of fertility of a soil were evaluated. It has been reported that the values of nitrogen, phosphorus and electrical conductivity are indicators of the fertility of a soil (Aprile & Lorandi 2012, Delsouz-Khaki *et al.* 2017). Considering the values of the macronutrients, the CE soil was classified as a soil of low fertility because of its low amount of nitrogen and phosphate, and its low value of electrical conductivity. The low fertility of the CE soil could be partially explained by the lack of specific rhizobia for *A. farnesiana*, therefore it was necessary to use exogenous microorganisms that enhance plant nutrition, such as rhizobia, to ensure the establishment of *A. farnesiana*. The soil micronutrients from the CE could also have a role in the establishment or permanence of specific rhizobia for the plant. In this work, we did not determine them, however, we think that the CE soil has the requirements for the permanence of rhizobia since it was possible to isolate nodule-forming rhizobia (CE1, CE2, CE3 and CE4) from *Acacia schaffneri* Thell. which inhabits the same region (Figure 3A), but which are not specific to *A. farnesiana*. In addition, in the soil samples arsenic was found at high levels; it has been documented that plants growing on As-contaminated soils tend to be mycorrhized (Meharg & Hartley-Whitaker 2002). Therefore, we suggest that the AMF could be highly tolerant to As and improve the plant nutrition, since it has been described that mycorrhizal plants resist high concentrations of arsenic (Xia *et al.* 2007, Xu *et al.* 2008). Also, it was reported that rhizobia have resistance genes for arsenic when they grew in soils contaminated with this element (Sá-Pereira *et al.* 2007, Mandal *et al.* 2008); in this manner, we suggest that the absence of specific rhizobia for *A. farnesiana* in the CE soil is not caused by the high concentration of As. Another explanation of the lack of specific rhizobia for *A. farnesiana* is the absence of the plant in the CE since the colonial period. Thrall *et al.* (2005) suggested that the survival of rhizobia in the soil is usually dependent on the presence of their hosts. In fact, native rhizobia are often undetectable in soils where native legumes have been removed. Therefore, in disturbed or degraded areas where revegetation is crucial, seeded legumes may require inoculation with effective strains of rhizobia to maximize their establishment and growth.

Due to the deficiency of specific rhizobia of *A. farnesiana* in the CE soil, the isolation of rhizobia capable of forming nodules and being infective was conducted. Five nodule-forming rhizobia out of 66 isolates efficiently improved the growth of *A. farnesiana* in CE soil and the high nitrogenase activities obtained by them, indicated that these nodule-forming rhizobia were adapted to CE soil conditions, since they improved the growth of the plant. In addition, the adaptation of the 5 nodule-forming rhizobia in the CE soil indicates that this soil has the macro- and micronutrient requirements necessary for the biological function of the rhizobia.

The identification of these five isolates from the analysis of different genes showed that the isolates were closely related to *S. americanum* into the same subclade B1 (Figure 5). The ENCBTM1 and ENCBTM31 isolates are closely related to *S. americanum* CFNEI 156 (Toledo *et al*. 2003) and although ENCBTM43 isolate is in the same subgroup of type strain CFNEI 156, it is not so closely related to it. We suggest that the ENCBTM1, ENCBTM31 and ENCBTM43 isolates are biovariants of *S. americanum* CFNEI 156. The isolates ENCBTM34 and ENCBTM45 were in a separate subgroup into the subclade BI and due to low similarity values obtained in the phylogenetic reconstruction of the concatenated tree, we proposed that this could be a new species of *Sinorhizobium*. However, it is necessary to carry out tests from a polyphasic taxonomic approach as phenotypic, genotypic and chemotaxonomic assays (biochemical characteristics, Box-PCR fingerprinting, fatty acid profile, % GC and DNA–DNA hybridization), for making the correct assignation of species of these isolates.

In this work, we evidenced that *S. americanum* CFNI 156 nodulates *A. farnesiana,* which is consistent with was previously reported by Toledo *et al*. (2003), indicating an association with this species of *Acacia*. However, the strains ENCBTM1, ENCBTM31 and ENCBTM43, were significantly different from *S. americanum* CFNI 156 with respect to the improvement of growth of *A. farnesiana*, thus, our isolates were better than the CFNI 156 strain. In addition, the strains ENCBTM34 and ENCBTM45 identified as *Sinorhizobium* sp. also improved the growth of this plant under the conditions used in this work. To our knowledge, this is the first report on the improvement of *A. farnesiana* growth associated with these rhizobium species. In addition, it is pointed out that other different species of *S. americanum* are capable of nodulating *A. farnesiana*. Different rhizobia isolated from nodules of *Acacia* from diverse geographical origins have been described (Romdhane *et al*. 2006, Diouf *et al*. 2007, Rodríguez-Echeverría *et al*. 2007, Fall *et al*. 2008). In addition, in Mexico it has been reported that *S.*

americanum is native from root nodules of *Acacia acatlensis* Benth. (Toledo *et al*. 2003) and *Ensifer mexicanus* from *Acacia angustissima* (Mill.) Kuntze (Lloret *et al*. 2007).

We determined that there are no specific rhizobia in CE soil associated to *A. farnesiana*. Therefore, the systematic inoculation of strains of rhizobia capable of improving plant growth, such as *S. americanum* strain ENCBTM1, ENCBTM31 or ENCBTM43 or *Sinorhizobium* sp. strain ENCBTM34 or ENCBTM45, in nursery seedlings, is required to improve the growth of *A. farnesiana* in CE soil. In addition, these isolates were statistically better compared to the strain *S. americanum* CFNEI 156.

Acknowledgments

This work was supported by Grant no. SIP20180359 and SIP20195543 from the Instituto Politécnico Nacional. We thank Susana Xelhuantzi, Jesús Evaristo Sánchez and José Luis Alvarado of the National Institute of Anthropology and History of Mexico for inviting us to participate in the Cerro de la Estrella project and for the aid provided for obtaining the soil samples and the *Acacia farnesiana* seeds. The authors En Tao Wang, Flor N. Rivera-Orduña, Juan C. Cancino-Diaz, Juan A. Cruz-Maya and Janet Jan-Roblero acknowledge the fellowships to Comisión de Operación y Fomento de Actividades Académicas and Estímulo al Desempeño de los Investigadores, Instituto Politécnico Nacional and Sistema Nacional de Investigadores, Consejo Nacional de Ciencia y Tecnología.

Literature cited

Aprile F, Lorandi R. 2012. Evaluation of cation exchange capacity (CEC) in tropical soil four different analytical methods. *Journal of Agricultural Science*. **4**: 278-289. DOI: https://doi.org/10.5539/jas.v4n6p278

Barrientos-Ramírez L, Vargas-Radillo J, Rodríguez-Rivas A, Ochoa-Ruiz H, Navarro-Arzate XF, Zorrilla J. 2012. Evaluación de las características del fruto de huizache (*Acacia farnesiana* (L.) Willd.) para su posible uso en curtiduría o alimentación animal. *Madera y Bosques* **18**: 23-35.

Blažka P, Fischer Z. 2014. Moisture, water holding, drying and wetting in forest soils. *Open Journal of Soil Science* **4**: 174-184. DOI: https://doi.org/10.4236/ojss.2014.45021

Brundrett M, Bougher BD, Grove T, Malajczuk N. 1996. Working with Glomalean fungi. *In*: Brundrett M, Bougher BD, Grove T, Malajzuk N, eds. *Working with Mycorrhizas in Forestry and Agricultura*. Camberra, Australia: Australian Centre for International Agricultural Research, pp. 141-186. ISBN-13: 978-1863201810

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. *BMC Bioinformatics* **10**: 421. DOI: https://10.1186/1471-2105-10-421

Camargo-Ricalde S. 2017. Some biological aspects of the arbuscular mycorrhizal fungi (AMF). *Botanical Sciences* **68**: 15-32. DOI: https://doi.org/10.17129/botsci.1633

Campanella JJ, Bitincka L, Smalley J. 2003. MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences. *BMC Bioinformatics*. **4**: 29. DOI: https://doi.org/10.1186/1471-2105-4-29

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*. **9**: 772. DOI: https://doi.org/10.1038/nmeth.2109

Delsouz-Khaki B, Honarjoo N, Davatgar N, Jalalian A, Torabi-Golsefidi H. 2017. Assessment of two soil fertility indexes to evaluate paddy fields for rice cultivation. *Sustainability* **9**: 1299. DOI: https://doi.org/10.3390/su9081299

Diouf D, Samba-Mbaye R, Lesueur D, Ba AT, Dreyfus B, de Lajudie P, Neyra M. 2007. Genetic diversity of *Acacia seyal* Del. rhizobial populations indigenous to senegalese soils in relation to salinity and pH of the sampling sites. *Microbial Ecology* **54**: 553-566. DOI: https://doi.org/10.1007/s00248-007-9243-0

Fall D, Diouf D, Ourarhi M, Faye A, Abdelmounen H, Neyra M, Sylla SN, Missbah Idrissi El M. 2008. Phenotypic and genotypic characteristics of *Acacia senegal* (L.) Willd. root-nodulating bacteria isolated from soils in the dryland part of Senegal. *Letters in Applied Microbiology* **47**: 85-97. DOI: https://doi.org/10.1111/j.1472-765X.2008.02389.x

Genderman JW, Nicolson JH. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society,* **46**: 235-244. DOI: https://doi.org/10.1016/S0007-1536(63)80079-0

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular- arbuscular mycorrhizal infection in roots. *New Phytologist* **84**: 489-500. DOI: https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Guindon S, Dufayard JF, Lefort V, Anisimova M. Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59**: 307-321. DOI: https://doi.org/10.1093/sysbio/syq010

IBG. 2016. The international bank for the Glomeromycota. Leibniz-Institute for vegetables and crops. http://www.i-beg.eu (accessed August 18, 2016).

INVAM. 2019. International culture collection of (vesicular) arbuscular mycorrhizal fungi. West Virginia University. https://invam.wvu.edu/ (accessed July 29, 2019).

Kazmierczak T, Nagymihály M, Lamouche F, Barrière Q, Guefrachi I, Alunni B, Ouadghiri M, Ibijbijen J, Kondorosi É, Mergaert P, Gruber V. 2017. Specific host-responsive associations between *Medicago truncatula* accessions and *Sinorhizobium* strains. *Molecular Plant Microbe Interacttions* **30**: 399-409. DOI: https://doi.org/10.1094/MPMI-01-17-0009-R

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947-2948. DOI: https://doi.org/10.1093/bioinformatics/btm404

Lauriano-Barajas J, Vega-Frutis R. 2018. Infectivity and effectivity of commercial and native arbuscular mycorrhizal biofertilizers in seedlings of maize (Zea mays). *Botanical Sciences* **96**: 395-404. DOI: https://doi.org/10.17129/botsci.1855

Lei X, Wang ET, Chen WF, Sui XH, Chen WX. 2008. Diverse

bacteria isolated from root nodules of wild *Vicia* species grown in temperate region of China. *Archives of Microbiology* **190**: 657-671. DOI: https://doi.org/10.1007/s00203-008-0418-y

Lloret L, Ormeño-Orrillo E, Rincón R, Martínez-Romero J, Rogel-Hernández MA, Martínez-Romero E. 2007. *Ensifer mexicanus* sp. nov. a new species nodulating *Acacia angustissima* (Mill.) Kuntze in Mexico. *Systematic and Applied Microbiology* **30**: 280-290. DOI: https://doi.org/10.1016/j.syapm.2006.12.002

Mandal SM, Pati BR, Das AK, Ghosh AK. 2008. Characterization of a symbiotically effective *Rhizobium* resistant to arsenic: Isolated from the root nodules of *Vigna mungo* (L.) Hepper grown in an arsenic-contaminated field. *Journal of General and Applied Microbiology* **54**: 93-99. DOI: https://doi.org/10.2323/jgam.54.93

Meghvansi MK, Prasad K, Harwani D, Mahna SK. 2008. Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. *European Journal of Soil Biology* **44**: 316-323. DOI: https://doi.org/10.1016/j.ejsobi.2008.03.003

Meharg AA, Hartley-Whitaker J. 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytologist* **154**: 29-43. DOI: https://doi.org/10.1046/j.1469-8137.2002.00363.x

Monroy-Ata A, Estevez-Torres J, García-Sánchez R, Ríos-Gómez R. 2007. Plant establishment through the use of micorrhizae and resource island in a disturbed xerophytic scrub. *Botanical Sciences* **80S**: 49-57. DOI: https://doi.org/10.17129/botsci.1756

Montaño NM, Alarcón A, Camargo-Ricalde SL, Hernández-Cuevas LV, Álvarez-Sánchez J, González-Chávez MDCA, Gavito ME, Sánchez-Gallen I, Ramos-Zapata J, Guadarrama P, Maldonado-Mendoza IE, Castillo-Argüero S, García-Sánchez R, Trejo D, Ferrera-Cerrato R. 2012. Research on arbuscular mycorrhizae in Mexico: an historical synthesis and future prospects. *Symbiosis* **57**: 111-126. DOI: https://doi.org/10.1007/s13199-012-0184-0

Montero-García IA. 2002. *Huizachtepetl: Geografía Sagrada de Iztapalapa*. Ciudad de México. ISBN: 968-6789-00-6

Oldroyd GE, Dixon R. 2014. Biotechnological solutions to the nitrogen problem. *Current Opinion in Biotechnology* **26**: 19-24. DOI: https://doi.org/10.1016/j.copbio.2013.08.006

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158-161. DOI: https://doi.org/10.1016/S0007-1536(70)80110-3

Rhoades JD, Mantghi NA, Shause PJ, Alves W. 1989. Estimating soil salinity from saturated soil-paste electrical conductivity. *Soil Science Society of America Journal* **53**: 428-433. DOI: https://doi.org/10.2136/sssaj1989.03615995005300020019x

Rodríguez-Echeverría S, Crisóstomo JA, Freitas H. 2007. Genetic diversity of rhizobia associated with *Acacia longifolia* in two stages of invasion of coastal sand dunes. *Applied and Environmental Microbiology* **73**: 5066-5070. DOI: https://doi.org/10.1128/AEM.00613-07

Romdhane SB, Nasr H, Samba-Mbaye R, Neyra M, Ghorbal MH, De Lajudie P. 2006. Genetic diversity of *Acacia tortilis* ssp. raddiana rhizobia in Tunisia assessed by 16S and 16S-23S rDNA genes analysis. *Journal of Applied Microbiology* **100**: 436-445. DOI: https://doi.org/10.1111/j.1365-2672.2005.02765.x

Sá-Pereira P, Rodrigues M, Castro IVE, Simoes F. 2007. Identification of an arsenic resistance mechanism in rhizobial strains. *World Journal of Microbiology and Biotechnology* **23**: 1351-1356. DOI: https://doi.org/10.1007/s11274-007-9370-2

SEMARNAT [Secretaría del Medio Ambiente y Recursos Naturales]. 2002. Norma Oficial Mexicana NOM-021-RECNAT-2000, Que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis. *Diario Oficial de la Federación*. Segunda sección, 31 de diciembre del 2002.

SEMARNAT. 2007. Norma Oficial Mexicana NOM-147-SEMARNAT/SSA1-2004, Que establece criterios para determinar las concentraciones de remediación de suelos contaminados por arsénico, bario, berilio, cadmio, cromo hexavalente, mercurio, níquel, plata, plomo, selenio, talio y/o vanadio. *Diario Oficial de la Federación*. Segunda sección, 2 de marzo del 2007.

Somasegaran P, Hoben HJ. 1994. *Handbook for rhizobia: methods in legume-Rhizobium technology*. New York: Springer-Verlag New York, Inc. DOI: https://doi.org/10.1007/978-1-4613-8375-8; ISBN: 978-1461383772

Thrall PH, Millsom DA, Jeavons AC, Waayers M, Harvey GR, Bahnall DJ, Brockwell J. 2005. Seed inoculation with effective root-nodule bacteria enhances revegetation success. *Journal of Applied Ecology* **42**: 740-751. DOI: https://doi.org/10.1111/j.1365-2664.2005.01058.x

Toledo I, Lloret L, Martínez-Romero E. 2003. *Sinorhizobium americanus* sp. nov., a new *Sinorhizobium* species nodulating native *Acacia* spp. in Mexico. *Systematic and Applied Microbiology* **26**: 54-64. DOI: https://doi.org/10.1078/072320203322337317

Tovar-Franco J. 2006. Selección en invernadero de inóculos de micorriza arbuscular (MA) para establecimiento de la alfalfa en un andisol de la sabana de Bógota. *Universitas Scientiarum* **11**: 87-103.

Udaiyan K, Karthikeyan A, Muthukumar T. 1996. Influence of edaphic and climatic factors on dynamics of root colonization and spore density of vesicular-arbuscular mycorrhizal fungi in *Acacia farnesiana* Willd. and *A. planifrons* W. et. A. *Trees* **11**: 65-71. DOI: https://doi.org/10.1007/PL00009657

Vincent JM. 1970. *A manual for the practical study of the root-nodule bacteria. IBP Handbook No. 15.* Oxford-Edinburgh: Blackwell Scientific Publications. DOI: https://doi.org/10.1002/jobm.19720120524; ISBN 10: 0632064102; ISBN-13: 978-0632064106

Vinuesa P, Silva C, Werner D, Martínez-Romero E. 2005. Population genetics and phylogenetic inference in bacterial molecular systematics: the roles of migration and recombination in *Bradyrhizobium* species cohesion and delineation. *Molecular Phylogenetics and Evolution* **34**: 29-54. DOI: https://doi.org/10.1016/j.ympev.2004.08.020

Xia YS, Chen BD, Christie P, Smith FA, Wang YS, Li XL. 2007. Arsenic uptake by arbuscular mycorrhizal maize (*Zea mays* L.) grown in an arsenic-contaminated soil with added phosphorus. *Journal of Environmental Sciences* **19**: 1245-1251. DOI: https://doi.org/10.1016/S1001-0742(07)60203-4

Xu P, Christie P, Liu Y, Zhang J, Li X. 2008. The arbuscular mycorrhizal fungus *Glomus mosseae* can enhance arsenic tolerance in *Medicago truncatula* by increasing plant phosphorus status and restricting arsenate uptake. *Environmental Pollution* **156**: 215-220. DOI: https://doi.org/10.1016/j.envpol.2008.01.003

Associated editor: Joel Flores

Author contributions: SGA performed the experiments. EAL designed the experiments of AMF and identified them. ETW designed the experiments of rhizobia. FNRO carried out analysis of DNA sequences. JCCD helped with the interpretation of the data and helped to write the first draft of the paper. JACM helped with the interpretation of the data and performed statistical analysis and JJR (ORCID: 0000-0001-6177-0189) analyzed all the experiments and data and wrote the manuscript.

Appendix 1. AMF species found on the soil of Cerro de la Estrella.

Order	Family	Species
Diversisporales	Acaulosporaceae	*Acaulospora laevis*
	Acaulosporaceae	*Acaulospora spinosa*
	Gigasporaceae	*Gigaspora decipiens*
	Gigasporaceae	*Racocetra gregaria*
	Gigasporaceae	*Scutellospora pellucida*
Glomerales	Glomeraceae	*Septoglomus constrictum*
	Glomeraceae	*Funneliformis mosseae*
	Glomeraceae	*Glomus* sp.
	Glomeraceae	*Glomus aurantium*
Paraglomerales	Paraglomeraceae	*Paraglomus occultum*

Appendix 2. Sequence similarities (%) for 16S rRNA, *recA*, *atpD*, and *glnII* genes between the studied strain and the reference strains.

Isolate	Phylogenetic relative	16S rRNA	*recA*	*atpD*	*glnII*
		% Similarity			
ENCBTM1	*E. adhaerens* strain Lc04	99.4	84.6	90.6	–
	S. americanum strain CFNEI 156	99.2	92.7	99.8	–
	S. fredii strain HH103	99.2	90.5	93.1	–
	S. xinjiangense strain LMG 17930	99.2	89.6	93.1	–
ENCBTM31	*S. americanum* strain CFNEI 156	99.9	92.1	100	99.8
	S. fredii strain HH103	99.9	91.2	93.1	93.1
	S. xinjiangense strain LMG 17930	99.9	90.1	93.1	80.0
ENCBTM34	*S. americanum* strain CFNEI 156	99.1	85.2	93.0	93.1
	S. saheli strain LMG 7837	99.6	91.9	91.7	86.5
	S. fredii strain HH103	99.1	90.5	93.1	92.7
	S. xinjiangense strain LMG 17930	99.1	89.5	93.1	78.8
	S. glycinis strain CCBAU 23380	93.0	90.2	92.1	79.8
	E. sojae strain CCBAU 05684	95.4	89.3	91.4	93.2
ENCBTM43	S. americanum strain CFNEI 156	99.9	100	100	97.2
	S. fredii strain HH103	99.9	90.1	93.1	92.7
	S. xinjiangense strain LMG 17930	99.9	91.2	93.1	80.1
ENCBTM45	*S. americanum strain* CFNEI 156	98.9	84.8	92.1	92.0
	S. saheli strain LMG 7837	99.4	90.5	90.3	86.4
	S. fredii strain HH103	98.9	91.2	91.4	93.1
	S. xinjiangense strain LMG 17930	98.9	89.6	91.4	79.8
	S. glycinis strain CCBAU 23380	93.0	87.9	92.4	80.4
	E. sojae strain CCBAU 05684	95.4	86.8	90.8	93.6

Appendix 3. Maximum-likelihood phylogenetic tree based on the 16S rDNA (1411 bp) sequences. The analysis was performed using the nucleotide substitution model GTR +I with −InL = -2594,05137 and *p*-inv = 0.954. Bootstrap values >50% (based on 1000 replications) are shown at each node.