ARBUSCULAR MYCORRHIZAL FUNGI IN THE RHIZOSPHERE OF NATIVE PLANTS AND THEIR EFFECT ON POBLANO PEPPER GROWTH

HONGOS MICORRÍZICOS ARBUSCULARES EN LA RIZÓSFERA DE PLANTAS NATIVAS Y SU EFECTO EN EL CRECIMIENTO DE CHILE POBLANO

Juan José Almaraz-Suárez¹, Apolinar González-Mancilla²*, Ronald Ferrera-Cerrato¹, María del Pilar Rodríguez-Guzmán¹, Oswaldo Rey Taboada-Gaytán³, Laura Verónica Hernández-Cuevas⁴, Alejandro Alarcón¹ y Antonio Trinidad-Santos¹†

¹Colegio de Postgraduados (CP), Campus Montecillo, Montecillo, Texcoco, Estado de México, México. ²Universidad Juárez del Estado de Durango, Facultad de Agricultura y Zootecnia, Ejido Venecia, Gómez Palacio, Durango, México. ³CP, Campus Puebla, Santiago Momoxpan, San Pedro Cholula, Puebla, México. ⁴Universidad Autónoma de Tlaxcala, Centro de Investigación en Ciencias Biológicas, Ixtacuixtla, Tlaxcala, México.

*Autor de correspondencia (apolinar.gonzalez@ujed.mx)

SUMMARY

Arbuscular mycorrhizal fungi symbiotically associate with most terrestrial plants, which provide various benefits, including nutrients and water absorption, and higher tolerance to root diseases, promoting greater plant growth. This study aimed to identify and select species of arbuscular mycorrhizal fungi (AMF) native to the rhizosphere of Poblano pepper and to evaluate their effect on its growth. The rhizospheric soil collection was carried out at nine sites where Poblano pepper was being cultivated at the Sierra Nevada region, Puebla, México. An experiment was established under a completely randomized design with 12 treatments and 8 replications. Nine treatments were mycorrhizal consortia from the nine sites (CS1 to CS9) and they were compared with three control treatments, two of these treatments were fertilized according to the recommended dose for Poblano pepper, with phosphorus (120-80-100) (NPK) and without phosphorus (120-00-100) (NK), and an absolute control (no mycorrhiza and no chemical fertilizer) (AC). The species of the AMF consortia were identified and their effect on growth (plant height, leaf area and biomass) and on the phosphorus contents of the plants was evaluated. Three AMF species were found in the nine inoculated treatments: Funneliformis geosporum, Claroideoglomus sp. and C. etunicatum. All the inoculated consortia enhanced growth and were all different from each other (Tukey, P ≤ 0.05), the treatment inoculated with CS2 (F. geosporum + Claroideoglomus sp.) showed greater plant height (20.3 cm), leaf area (100.9 cm²) and higher dry biomass in root (92 mg), leaf (258 mg) and stem (166 mg); treatments with CS3 and CS2 (F. geosporum + Claroideoglomus sp.) induced higher phosphorus content in the plant (1.75 and 1.69 mg). The results indicated that AMF are associated with Poblano pepper plants and can be isolated and inoculated to improve the growth of the Poblano pepper itself.

Index words: Capsicum annuum, Claroideoglomus sp., Funneliformis geosporum, colonization, mycorrhizal, phosphorus, plant height.

RESUMEN

Los hongos micorrízicos arbusculares se asocian simbióticamente con la mayoría de las plantas terrestres, los cuales proporcionan diversos beneficios, que incluyen la absorción de nutrimentos y agua, y mayor tolerancia a enfermedades radicales, promoviendo un mayor crecimiento vegetal. El objetivo de esta investigación fue identificar y seleccionar especies de hongos micorrízicos arbusculares (HMA) nativos de la rizosfera de chile Poblano y evaluar su efecto en el crecimiento del mismo. Se realizó

la colecta de suelo rizosférico en nueve sitios cultivados con chile Poblano en la región Sierra Nevada, Puebla, México. Se estableció un experimento en diseño completamente al azar, con 12 tratamientos y 8 repeticiones. Nueve tratamientos fueron consorcios micorrízicos de los nueve sitios (CS1 a CS9), y se compararon con tres tratamientos testigo, dos fertilizados según la dosis recomendada para chile Poblano, con fósforo (120-80-100) (NPK) y sin fósforo (120-00-100) (NK), y un testigo absoluto (sin micorriza y sin fertilizante químico) (TA). Se identificaron las especies de los consorcios de HMA v se evaluó su efecto en el crecimiento (altura de planta, área foliar v biomasa) y en el contenido de fósforo de las plantas. En los nueve tratamientos inoculados se encontraron tres especies de HMA: Funneliformis geosporum, Claroideoglomus sp. y C. etunicatum. Todos los consorcios inoculados mejoraron el crecimiento y fueron diferentes entre ellos (Tukey, P ≤ 0.05), el tratamiento inoculado con el CS2 (F. geosporum + Claroideoglomus sp.) presentó mayor altura de planta (20.3 cm), área foliar (100.9 cm²) y biomasa seca radical (92 mg), foliar (258 mg) y de tallo (166 mg); los tratamientos con el CS3 y CS2 (F. geosporum + Claroideoglomus sp.) indujeron mayor contenido de fósforo en planta (1.75 y 1.69 mg). Los resultados indicaron que los HMA se asocian con las plantas de chile Poblano y pueden aislarse e inocularse para mejorar el crecimiento del mismo chile Poblano.

Palabras clave: Capsicum annuum, Claroideoglomus sp., Funneliformis geosporum, altura de planta, colonización micorrízica, fósforo.

INTRODUCTION

Arbuscular mycorrhizal symbiosis occurs in 70 to 90 % of terrestrial plants in natural and agricultural ecosystems (Smith and Reed, 2008). Pepper roots form these symbiotic associations with arbuscular mycorrhizal fungi (AMF) (Carballar-Hernández et al., 2017; Douds et al., 2012). Poblano pepper (Capsicum annuum L.), whose origin can be traced to the state of Puebla, is widely grown in Mexico, not only for its taste and great culinary tradition in the Mexican cuisine but also for its high nutritional value (Cyphers et al., 2009). In the state of Puebla, this plant is cultivated by small-scale farmers relying on the family workforce (Pérez et al., 2016).

Recibido: 1 de mayo de 2020 **Aceptado:** 21 de junio de 2021

In the state of Puebla, the production of Poblano pepper has decreased in recent years, during the period of 2005 to 2014 the planted area decreased by 4 % and the production volume by 12 % (SIAP, 2021); likewise, Rodriguez et al. (2007) reported a 60 % reduction in the yield of this crop. This results as a consequence of the poor quality of seedlings and the lack of market for the fruit (Garcia et al., 2011), adding the incidence of diseases such as "Damping off" or the pepper wilt disease as it is known in the region, which is originated by phytopathogenic fungi such as Phytophthora capsici, Rhizoctonia solani, Fusarium spp., Pythium spp. and Alternaria spp. (Bautista-Calles et al., 2010); wilt caused by nematodes as Nacobbus aberrans, Meloidogyne incognita, M. arenia, M. javanica and M. hapla (Huerta et al., 2007); viral diseases and physical damages from insects and mites (Crosby, 2008); and water and nutrients availability (Mena-Violante et al., 2006).

These factors have led to the reduction of the planted area, the substitution for other species (maize, beans or fruit crops) and the increased risk of extinction of Poblano pepper local varieties (Pozo, 1983). The use of beneficial microorganisms, as native AMFs of the rhizosphere, may be an alternative to reduce these limiting factors; AMF, through its mycelium network, can form and stabilize soil aggregates (Barbosa *et al.*, 2019), increase exploration volume and optimize the absorption of water and essential nutrients for the plant, mainly nitrogen and phosphorus (Bonfante and Genre, 2010; Smith and Read, 2008).

Several authors have inoculated AMF in *Capsicum* crops, and have found a higher resistance to foliar and root diseases (Felle *et al.*, 2009; Lioussanne *et al.*, 2009), higher content of nutrients in the plants (Boonlue *et al.*, 2012; Carballar-Hernández *et al.*, 2018; Kim *et al.*, 2010) and greater adaptation under conditions of salinity (Cekic *et al.*, 2012) and drought (Mena-Violante *et al.*, 2006), which have resulted in a higher growth and yield of the crop. Therefore, the present study aimed to isolate and identify native AMF from the rhizosphere of Poblano pepper and analyze the response in growth and phosphorus content in Poblano pepper plants, when inoculated with AMF.

MATERIALS AND METHODS

Rhizosphere soil sample collection in Poblano pepper crops

A total of 36 samples were collected from rhizospheric soil from Poblano pepper plants at the fruiting stage. Samples were taken from nine different sites (four samples from each site) located at three municipalities of the state of Puebla, Mexico (San Matias Tlalancaleca, San Lorenzo Chiautzingo and Huejotzingo), where altitude ranges

between 2284 and 2467 meters above sea level (Figure 1). The samples, of approximately 5 kg of rhizospheric soil, were kept in a cooler in order to transport them to the microbiology laboratory. In the sampling sites, pH ranged between 4.9 and 7.6, organic matter content from 0.27 to 1.48% and total nitrogen content from 0.01 to 0.07 %. Phosphorus ranged from 41.1 to 428.8 mg kg⁻¹ and soil texture varied from sandy loam to sandy clay loam to loamy sand. The colonization percentage by AMF in Poblano pepper roots fluctuated between 4.5 and 67.7 %, while the number of spores in 100 g of dry soil ranged between 195 and 896 (Table 1).

Experiment setup

One-hundred g of each sample of the rhizospheric soil collected were used as mycorrhizal inoculant to establish the experiment into a greenhouse. The experiment was set up three days after the collection of the rhizospheric soil; meanwhile, the samples were kept refrigerated. Pots with a capacity of 1 kg were used. A mixture of peat moss, perlite and vermiculite (1:1:1) was used as a substrate, which was properly sterilized in an autoclave at a pressure between 1.26 and 1.40 kg cm⁻². Twelve treatments were evaluated: nine mycorrhizal inocula treatments from different Poblano pepper sites and three control treatments. Two of the control treatments were fertilized according to the recommended dose for Poblano pepper crops in the studied area [one treatment with phosphorus (120-80-100) and another treatment without phosphorus (120-00-100)] and one absolute control (AC) with no fertilizer or inoculum. The AMF inocula treatments were fertilized without phosphorus (120-00-100) and they were identified as mycorrhizal consortia according to the sampled sites (CS1 to CS9) as the taxonomic identification was carried out at the end of the experiment.

The sowing was carried out in a greenhouse, at a temperature of 25-30 °C. Seed from a native variety of Poblano pepper was used, collected by the same producers and selected for more than 20 years. Seeds were disinfected in sodium hypochlorite 1% for 3 min and rinsed three times with sterile distilled water. Seeds were planted directly into the pots, watered with distilled water every third day; to avoid contamination between treatments, a beaker was used; in addition, it was fertilized with the Long Ashton nutrient solution, once a week and in amounts of 11 μg mL⁻¹ of phosphorus, a modified dose for AMF (Hewitt, 1966). For the plants to be colonized by AMF, the experiment lasted 65 days, after this time, plants were harvested to evaluate the effect of the treatments. The experiment was established using a completely randomized design with 12 treatments and 8 replications.

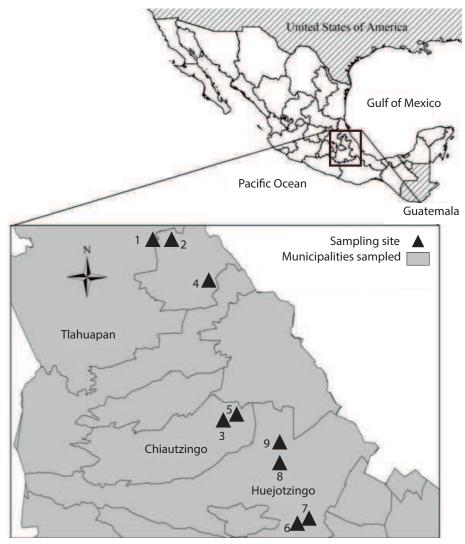


Figure 1. Geographic location of the nine sites where Poblano pepper rhizospheric soil samples were collected at Sierra Nevada, state of Puebla, México.

Table 1. Soil characteristics and mycorrhizal colonization in Poblano pepper, in the nine studied sites of the Sierra Nevada of Puebla, Mexico.

or racina, mexico.										
Sites	Municipality	Altitude (msnm)	рН	OM (%)	TN (%)	P (mg kg ⁻¹)	TC (%)	Spores (100 g s.s.)		
1	SMT	2467	5.5	0.27	0.01	184.7	7.7	195		
2	SMT	2453	5.8	0.54	0.03	224	11.7	477		
3	SMT	2414	6.8	1.48	0.07	314.5	16.1	235		
4	SLC	2425	6.9	0.94	0.05	153.7	23.3	486		
5	SLC	2404	4.9	0.27	0.01	187.1	4.5	654		
6	Н	2313	5.8	0.4	0.02	41.5	43.9	447		
7	Н	2292	6.7	0.94	0.05	428.8	43.0	896		
8	Н	2286	7.3	1.48	0.07	50.1	27.0	355		
9	Н	2284	7.6	0.67	0.03	44.3	67.7	483		

SMT: San Matías Tlalancaleca, SLC: San Lorenzo Chiautzingo, H: Huejotzingo, OM: Organic matter, TN: total nitrogen, P. phosphorus, TC: total colonization.

Table 2. AMF consortia, root colonization (total and vesicles), number of spores in rhizosphere and Poblano pepper growth, 65 days after planting in the greenhouse.

Treatments	AMF consortium	TC	V	S	Н	L	LA
AC	-	0.0 d	0.0 b	0 d	5.2 ef	4.3 e	2.8 d
NK	-	0.0 d	0.0 b	0 d	3.6 f	4.5 e	3.5 d
NPK	-	0.0 d	0.0 b	0 d	7.8d ef	5.8 de	10.9 cd
CS1	Fg + C	10.1 cd	0.0 b	1452 abc	16.5 ab	11.7 abc	49.9 abc
CS2	Fg + C	35.4 a	3.6 a	2002 ab	20.3 a	14.0 ab	100.9 a
CS3	Ce	5.9 d	0.0 b	1835 abc	10.7 cd	8.7 cd	20.6 bcd
CS4	Fg + C	24.1 ab	0.2 b	2590 a	19.8 ab	14.2 ab	92.1 a
CS5	С	4.8 d	0.0 b	639 cd	15.0 bc	10.5 bc	34.0 bcd
CS6	Ce	9.4 cd	0.0 b	1522 abc	9.8 de	6.7 de	9.3 cd
CS7	Fg + C	20.5 bc	1.9 ab	1775 abc	16.1 ab	13.3 ab	56.4 ab
CS8	Fg + C + Ce	14.0 bcd	0.0 b	1024 bcd	18.8 ab	12.8 ab	57.9 ab
CS9	С	19.2 bc	2.5 ab	1058 bcd	17.1 ab	14.3 a	64.4 ab

Means with the same letters in the columns are not statistically different (Tukey, $P \le 0.05$). n = 8. AC: absolute control, NK: fertilized with nitrogen and potassium, NPK: fertilized with nitrogen, phosphorus and potassium, CS1 to CS9: site 1 consortium to site 9 consortium, Fg: Funneliformis geosporum, C: Claroideoglomus sp., Ce: Claroideoglomus etunicatum, TC: total colonization (%), V: vesicles (%), E: spores (100 g s.s.), H: height (cm), L: leaves per plant, LA: leaf area (cm²).

Plant height, biomass and phosphorus content in plant

Plant height was measured with a ruler graduated in cm and the leaf area was determined with a leaf area meter (Model LI-3100, Lincoln, Nebraska, USA). Plants were dissected into stems, leaves and roots; the roots were washed with tap water. To evaluate dry biomass the samples were dried into an oven (FELISA, Model 242-A, Mexico City) at 70 °C for 72 hours; each organ of the plants was weighted separately in an analytical balance (Sartorius, Model Analytic AC 210S, Wood Dale, Illinois, USA).

Phosphorus content in the plant material was determined by phosphovanadomolybdate colorimetry (AOAC, 1980). The material was digested by the wet digestion method (acid digestion). First, 0.25 g of plant tissue were weighted (stem and leaves) and put into flasks with 6 mL of nitric acid solution (HNO₃) and perchloric acid (HClO₄) (ratio 4:2). Flasks were allowed to stand for one day and then dried in an oven at 360 °C for 6 h. After the digestion process (stock solution), the sample was poured into a 25 mL flask and diluted with distilled water. It was filtered with Watman filter paper number 2, then a 10 mL aliquot was poured into a 50 mL flask. From the mixture (2.5 mL of ammonium vanadate, 2.5 mL of ammonium molybdate and 2.5 mL of nitric acid) 7.5 mL were added to the 50 mL flask and diluted with distilled water. It was left to settle for 30 min and aliquots of 150 µL were taken and placed into micro plates to make the reading at 470 nm, on an spectrophotometer (Varian, Model 725-ES(r), Agilent, Santa Clara California, USA).

AMF colonization and taxonomic identification

The AMF colonization percentage in Poblano pepper roots was evaluated with the clearing and trypan blue staining techniques (Phillips and Hayman, 1970) and the colonization percentage was estimated in roots of 1 cm in length expressed in percentage, according to the Biermann and Linderman (1981) method. For spore extraction and quantification, the sieving and decanting technique was used as described by Gerdemann and Nicolson (1963), followed by a sucrose gradient centrifugation (20 and 60 %) (Sieverding, 1983). The number of spores was quantified in 100 g of moist soil and finally expressed in 100 g of dry soil; for this, 10 g of soil were dried in an oven (FELISA, Model 242-A) at 70 °C for 72 hours, the number of spores was multiplied by 100 and divided by the amount of dry soil found in 100 g of moist soil. Spores were separated by their morphological similarities (shape, size, color and presence or absence of supporting hyphae) and placed on glass slides in polyvinyl alcohol-lactic acid-glycerol. Healthy and intact spores were numbered and examined using a Nomarski differential contrast light microscope (Nikon Optiphot II Plus, Tokyo, Japan). For the taxonomic classification of AMF, species were compared and verified with those described by the International Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) (http:// invam.wve.edu/).

Statistical analysis

The data were analyzed using the SAS statistical package for Windows (SAS Institute, 2002). Analysis of variance and

Tukey test ($P \le 0.05$) were applied. The data on percentage of mycorrhizal colonization were subjected to a normality test and transformed to Log (x + 1) (Barbosa *et al.*, 2019).

RESULTS AND DISCUSSION

Identified AMF species

Three AMF species were found in treatments inoculated with consortia from the nine soil samples. Funneliformis geosporum, Claroideoglomus sp. and C. etunicatum. Consortia F. geosporum and Claroideoglomus sp. prevailed on sites 1, 2, 4 and 7; consortia formed by F. geosporum, Claroideoglomus sp. and C. etunicatum was found on site 8; C. etunicatum was found on sites 3 and 6 (Table 2). The predominance of these endomycorrhizal species depends on the presence of Poblano pepper; Carballar-Hernández (2017) also found a predominance of Funneliformis geosporum and Claroideoglomus species in Poblano pepper cultivars. The same authors confirmed that soil pH and phosphorus content are factors that influence the abundance and distribution of mycorrhizae. In this study, mycorrhizal colonization was relatively low in the higher altitude sites that presented acidic pH and lower organic matter content (Table 1). The pH, the texture, the fertility of the soil (mainly phosphorous), the altitude, the geographical location and the intensive management of the soil are factors that affect the abundance and diversity of AMF (Carballar-Hernández et al, 2017; Jansa et al., 2014).

The AMF spores found belong to the order of the glomerals, from the *Glomeraceae* and *Claroideoglomeraceae* families. These families have been found in natural and agricultural ecosystems in Mexico, and coincide with what was observed by Carballar-Hernández *et al.* (2017) in *Capsicum annuum*, Trinidad-Cruz *et al.* (2017) in *Agave cupreata*, Peñuelas-Rubio *et al.* (2021) in *Carica papaya*, and Hernández-Zamudio *et al.* (2017) in *Larrea tridentata*; the abundance of these families is related to their ability to propagate through mycelial fragments, fragments of mycorrhizal roots and the production and germination of spores (Bahadur *et al.*, 2019; Varela-Cervero *et al.*, 2016; Yang *et al.*, 2015).

Claroideoglomus etunicatum is ranked as one of the most common and ubiquitous species worldwide, so it can be considered highly competitive due to its ability to adapt to extremely different conditions from the original sites (Trejo-Aguilar et al., 2013). A study conducted by Carballar-Hernández et al. (2017) in Poblano pepper in the same region of Mexico found a predominance of *F. geosporum*, *C. claroideum* and *C. luteum*; these genera were also identified in the present study, being the dominant species *F. geosporum*, Claroideoglomus sp. and C. etunicatum. In

addition to the soil factors, there are other factors that may influence the predominance of these species; for example, the sampling date and whether if it is the rainy or the dry season (Hernández-Zamudio *et al.*, 2017; Trinidad-Cruz *et al.*, 2017), for this study the sampling was carried out in the month of July, a rainy season.

Total colonization by AMF in root plants found at the end of the experiment fluctuated between 5.9 and 35.4 % in treatments grown under mycorrhizal influence; plants inoculated with the site CS2 were those with the highest root colonization (35.4 %), which represented six times the increase in mycorrhizal colonization than the site CS3 (5.9 %). Mycorrhizal colonization in controls (AC, NK and NPK) was null, since no consortium was applied (Table 2). The percentage of colonization by vesicles was relatively low and excels the mycorrhizal consortium of site 2 with 3.6 %, the lower colonization might be associated to the fact that the plants were evaluated when they were in an active stage of growth; thus, the development and the establishment of mycorrhizal symbiosis was in its early stages. Treatments showed significant statistical differences (Tukey, $P \le 0.05$) in the number of spores, being the consortia of the sites 4 (CS4) and 2 (CS2) the ones with the highest number of spores with 2590 and 2002 spores in 100 g of dry soil, the lowest value was found in site 5 (CS5) with 639 spores in 100 g of dry soil (Table 2); the spores quantified in this study are higher compared to other studies; for example Carballar-Hernández et al. (2018) reported between 10 and 310 spores in Poblano pepper plants 90 days after inoculation, Reyes-Tena et al. (2016) reported between 130 and 406 spores in the same culture but 56 days after transplantation, subsequently Reyes-Tena et al. (2017) reported between 125 and 257 spores in Poblano pepper 86 days after transplantation.

Effect of AMF on Poblano pepper growth

The AMF from different sites improved Poblano pepper growth (plant height, number of leaves, leaf area and biomass); moreover, significant statistical differences between sites were found (Tukey, $P \leq 0.05$). The best results were found on plants treated with the mycorrhizal consortium from sites 2 and 4 (CS2 and CS4, *F. geosporum* and *Claroideoglomus* sp.), which presented, on average, greater plant height (20.3 and 19.8 cm), number of leaves per plant (14 and 14.3) and leaf area (100.9 and 92.1 cm²) (Table 2). Control treatments (AC, NK and NPK) presented the lowest values in plant height (5.2-7.8 cm), number of leaves (4.3-5.8) and leaf area (2.8-10.9 cm²). Plant growth with CS2 represented a 3-6 times more increase in plant height, and 9-34 times more in leaf area, compared to plants with no AMF.

The roots of chili plants form symbiotic associations with arbuscular mycorrhizal fungi (Douds et al., 2012). The inoculation of these microorganisms in C. annuum cultures significantly influences the growth and development of plants under various conditions, mainly under greenhouse conditions (Douds et al., 2012), and the effects are more significant when native AMF are used (Jiménez-Leyva et al., 2017), such as those used in this study. Regarding growth, other studies have reported 26.3 cm in plant height and 139.2 cm² of leaf area in Poblano pepper 90 days after inoculation with various AMF consortia (Acaulospora laevis, A. morrowiae, A. scrobiculata, A. spinosa, Cetraspora pellucida, Claroideoglomus claroideum, C. luteum, Claroideoglomus sp., Diversispora aurantia, Funneliformis geosporus, F. mosseae, Funneliformis sp., Gigaspora candida, Glomus sp., Rhizoglomus fasciculatus y Sclerocystis sinuosa) (Carballar-Hernández et al., 2018). Reyes-Tena et al. (2016) found 18 cm in plant height and 15.7 leaves of Poblano pepper plants after 56 days of inoculation with the AMF consortium formed by Archaeospora schenckii, Acaulospora scrobiculata, Claroideoglomus etunicatum, Diversispora aurantia y Entrophospora infrequens; subsequently Reyes-Tena et al. (2017) when inoculating Acaulospora rehmii, A. excavata, A. mellea, A. scrobiculata, A. spinosa, y Acaulospora sp. in Poblano pepper, found 23.8 cm in height, 33.4 leaves per plant and 78.2 cm² of leaf area.

The dry-weight of leaf, stem and roots of the Poblano pepper plants were significantly affected (Tukey, $P \le 0.05$) by inoculation of different consortia of AMF (Figure 2). All mycorrhizal consortia improved accumulation of dry biomass in the plant; however, CS2 and CS4 presented greater production of root dry biomass (92 and 56 mg), leaf dry biomass (258 and 238 mg) and stem dry biomass (166 and 113 mg). The lowest values were found in the three control treatments and in one treatment inoculated with the mycorrhizal consortium from site CS6. The variables plant height, leaf area and dry biomass presented a highly significant correlation with total root colonization and with the number of spores found in the treatments, which could explain the better growth of inoculated plants with CS2 and CS4. According to Verbruggen et al. (2012), greater mycorrhizal colonization determines better plant growth. In Poblano pepper, Carballar-Hernández et al. (2018) obtained 0.13 and 0.69 g of dry biomass in root and aerial part, 90 days after inoculation with the aforementioned AMF consortia. Likewise, Mena-Violante et al. (2006) found higher production of root dry biomass (4.7 g) and aerial dry biomass (9.8 g) when they inoculated Glomus constrictum, G. geosporum, G. fasciculatum and G. tortuosum; finally Reves-Tena et al. (2017) found 3.02 g of total dry biomass after 86 days of inoculating AMF with the mentioned species in Poblano pepper.

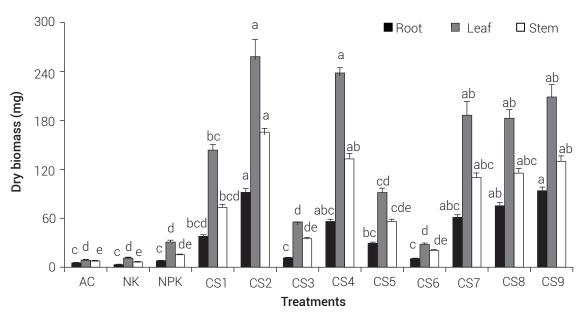


Figure 2. Root, leaf and stem dry weight in Poblano pepper plants, by the inoculation effect of nine AMF consortia (CS), 65 days after planting at the greenhouse. Different letters on the bars indicate significant statistical differences (Tukey, P ≤ 0.05). n = 8, ± EE. TA: absolute control, NK: fertilized with nitrogen and potassium, NPK: fertilized with nitrogen, phosphorus and potassium, CS1 to CS9: site 1 consortium to site 9 consortium.

In this study, plant growth (plant height, number of leaves, foliar area and dry biomass of root, leaf and stems) of Poblano pepper of the treatments inoculated with CS2 and CS4 are possibly related to the efficiency of these to accumulate a larger amount of phosphorus (Figure 3). Several studies have reported that in *Capsicum annuum* AMF allow greater absorption of water and nutrients such as nitrogen, phosphorus, potassium, zinc, calcium and magnesium (Bagyaraj and Sreeramulu, 1982; Carballar Hernández et al., 2018; Kim et al., 2010).

Phosphorus content in plant

Phosphorus content in plant at the end of the experiment ranged from 0.05 to 1.75 mg per plant, showing significant statistical differences between treatments (Tukey, P ≤ 0.05) (Figure 3). The level of phosphorus in the control treatments AC and NK was 0.05 mg per plant, while it was 0.14 mg per plant in NPK. The content of phosphorus in plants treated with mycorrhizal consortia fluctuated between 0.19 and 1.75 mg per plant, which are higher than the levels found in plants of the control treatments. Plants inoculated with F. geosporum + Claroidoglomus sp. from sites CS2 and CS4 showed a higher content of phosphorus (1.75 and 1.69 mg per plant). In similar studies, with the inoculation of various AMF consortia, Carballar-Hernández et al. (2018) reported phosphorus content in Poblano pepper leaves between 1.4 and 2.8 mg kg⁻¹; Kim et al. (2010) reported 119.5 mg of phosphorus per Poblano pepper plant when these were inoculated with Acaulospora longula, Glomus clarum

and *G. intraradiaces*; Sensoy *et al.* (2007) reported 0.357 and 0.344 % of phosphorus content in Serrano pepper leaves inoculaated with *Gigaspora margarita* and *Glomus intraradices*, although in this study the phosphorus content was reported in mg per plant, the values are similar, since CS2 and CS4 presented phosphorus contents of 0.345 and 0.240 %. AMF are very important for plant nutrition, mainly in terms of phosphorous acquisition (Bonfante and Genre, 2010). In this study, an increase of phosphorus content was observed in plants inoculated with AMF. Bonfante and Genre (2010) suggested that the accumulation of phosphorus and other plant nutrients is due to the network of mycelia that produce AMF, which functions as a transporter and due to an extended exploration of the roots, which influence growth and crop development.

CONCLUSIONS

Arbuscular mycorrhizal fungi (AMF) consortia had a positive effect on the growth of Poblano pepper in the greenhouse compared to the control treatments. The increases were two to four times higher than in the absolute control. Three AMF species were found, *Funneliformis geosporum*, *Claroideoglomus* sp. and *C. etunicatum*, which had a positive effect on plant height, leaf area, dry biomass and a higher concentration of phosphorus in the plant. The best results were found when consortia from sites CS2 and CS4 were inoculated containing the species *F. geosporum* and *Claroideoglomus* sp. These results show the potential of AMF, especially *F. geosporum* and *Claroideoglomus* sp..

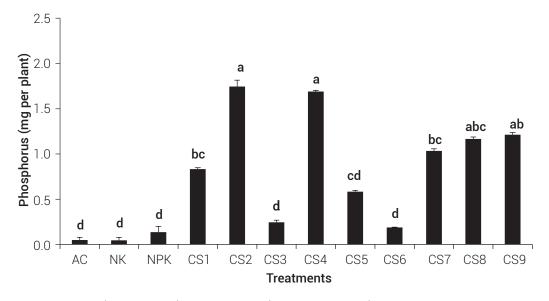


Figure 3. Phosphorus content (mg per plant) in plant tissue (stems and leaves) in Poblano pepper by the inoculation effect of nine AMF consortia (CS), 65 days after planting at the greenhouse. Different letters on the bars indicate significant statistical differences (Tukey, $P \le 0.05$). n = 8, \pm EE. TA: absolute control, NK: fertilized with nitrogen and potassium, NPK: fertilized with nitrogen, phosphorus and potassium, CS1 to CS9: site 1 consortium to site 9 consortium.

when used as inocula in the Poblano pepper crop.

ACKNOWLEDGEMENTS

Thanks to the National Council for Science and Technology (CONACYT-México) for the support given to Apolinar Gonzalez Mancilla, through the scholarship awarded to carry on with doctoral studies. Special thanks to the Postgraduate College, Soil Microbiology Laboratory, where the research was performed. Authors also thank to the Poblano pepper producers for the availability of their plots to carry on the sampling, especially to Mr. Leopoldo Ramírez Morales and to Karen Luévano Gaspar for the translation of this writing.

BIBLIOGRAPHY

- AOAC, Association of Official Analytical Chemists (1980) Official Methods of Analysis of the Association Chemists. 13th edition. Association of Official Analytical Chemists. Madison, Wisconsin, USA.1018 p.
- Bagyaraj D. J. and K. R. Sreeramulu (1982) Preinoculation with VA mycorrhiza improves growth and yield of chilli transplanted in the field and saves phosphatic fertilizer. *Plant and Soil* 69:375-381, https://doi.org/10.1007/BF02372458
- Bahadur A., Z. Jin, S. Jiang, Y. Chai, Q. Zhang, J. Pan, ... and H. Feng (2019)
 Arbuscular mycorrhizal spores distribution across different
 ecosystems of the Qinghai Tibetan Plateau. *Pakistan Journal*of Botany 51:1481-1492, https://doi.org/10.30848/PJB20194(10)
- Barbosa M. V., D. F. Pedroso, N. Curi and M. A. C. Carneiro (2019) Do different arbuscular mycorrhizal fungi affect the formation and stability of soil aggregates? *Ciência e Agrotecnologia* 43:e003519, https://doi.org/10.1590/1413-7054201943003519
- Bautista-Calles J., R. García-Espinosa, R. Montes-Belmont, E. Zavaleta-Mejía, J. Pérez-Moreno, R. Ferrera-Cerrato, ... y M. Huerta-Lara (2010) Disminución de la marchitez del chile por introducción de antagonistas en cultivos de rotación. *Interciencia* 35:673-679.
- Biermann B. and R. G. Linderman (1981) Quantifying vesiculararbuscular mycorrhizae: a proposed method towards standardization. New Phytologist 87:63-67, https://doi. org/10.1111/j.1469-8137.1981.tb01690.x
- Bonfante P. and A. Genre (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nature Communications* 1:48, https://doi.org/10.1038/ncomms1046
- Boonlue S., W. Surapat, C. Pukahuta, P. Suwanarit, A. Suwanarit and T. Morinaga (2012) Diversity and efficiency of arbuscular mycorrhizal fungi in soils from organic chili (*Capsicum frutescens*) farms. *Mycoscience* 53:10-16, https://doi.org/10.1007/s10267-011-0131-6
- Carballar-Hernández S., L. V. Hernández-Cuevas, N. M. Montaño, J. Larsen, R. Ferrera-Cerrato, O. R. Taboada-Gaytán, ... and A. Alarcón (2017) Native communities of arbuscular mycorrhizal fungi associated with Capsicum annuum L. respond to soil properties and agronomic management under field conditions. Agriculture, Ecosystems and Environment 245:43-51, https://doi.org/10.1016/j.agee.2017.05.004
- Carballar-Hernández S., L. V. Hernández-Cuevas, N. M. Montaño, R. Ferrera-Cerrato and A. Alarcón (2018) Species composition of native arbuscular mycorrhizal fungal consortia influences growth and nutrition of poblano pepper plants (*Capsicum annuum* L.). Applied Soil Ecology 130:50-58, https://doi.org/10.1016/j.apsoil.2018.05.022
- Cekic F. O., S. Unyayar and I. Ortas (2012) Effects of arbuscular mycorrhizal inoculation on biochemical parameters in *Capsicum annuum* grown under long term salt stress. *Turkish Journal Botany* 36:63-72, https://doi.org/10.3906/bot-1008-32

- Crosby K. M. (2008) Pepper. In: Vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae. Handbook of Plant Breeding 2. J. Prohens-Tomás and F. Nuez (eds.). Springer. New York, USA. pp:221-248, https://doi.org/10.1007/978-0-387-74110-9_6
- Cyphers A., P. Escalante G., J. García-Bárcena, B. García M., L. López L., E. Matos M., ... and M. T. Uriarte C. (2009) Los chiles de México. Catalogo visual. *Arqueología Mexicana* 32:39-74.
- Douds Jr. D. D., J. Lee, L. Rogers, M. E. Lohman, N. Pinzon and S. Ganser (2012) Utilization of inoculum of AM fungi produced on-farm for the production of Capsicum annuum: a summary of seven years of field. Biological Agriculture & Horticulture 28:129-145, https://doi.org/10.1080/01448765.2012.693362
- Felle H. H., F. Waller, A. Molitor and K. H. Kogel (2009) The mycorrhiza fungus *Piriformospora indica* induces fast rootsurface pH signaling and primes systemic alkalinization of the leaf apoplast upon powdery mildew infection. *Molecular Plant-Microbe Interactions* 22:1179-1185, https://doi.org/10.1094/MPMI-22-9-1179
- García M. C., O. R. Taboada G., H. López S., P. A. López, G. Mora A. y B. Tlapal B. (2011) Calidad de plántulas de chile 'Poblano' en la Sierra Nevada de Puebla, México. *Revista Fitotecnia Mexicana* 34:115-121, https://doi.org/10.35196/rfm.2011.2.115
- Gerdemann J. W. and T. H. Nicolson (1963) Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society 46:235-244, https://doi.org/10.1016/S0007-1536(63)80079-0
- Hernández-Zamudio G., J. Sáenz-Mata, A. Moreno-Reséndez, G. Castañeda-Gaytán, A. Ogaz, S. Carballar-Hernández y L. Hernández-Cuevas (2017) Dinámica de la diversidad temporal de los hongos micorrícicos arbusculares de *Larrea tridentata* (Sesse & Mocino ex DC) Coville en un ecosistema semiárido. *Revista Argentina de Microbiología* 50:301-310, https://doi.org/10.1016/j.ram.2017.07.007
- Hewitt E. J. (1966) The composition of the nutrient solution. In: Sand and Water Culture Methods Used in the Study of Plant Nutrition. E. J. Hewitt (ed.). Commonwealth Bureaux of Horticulture and Plantation Crops. East Malling, Maidstone, England. pp:187-246
- Huerta P. A., S. Fernández R. y I. Ocampo F. (2007) Manual de Chile Poblano, Importancia Económica y Sociocultural. Colegio de Postgraduados Campus Puebla, Fundación Produce Puebla, A. C. Puebla, México. 80 p.
- Jansa J., A. Erb, O. Hans-Rudolf, P. Šmilauer and S. Egli (2014) Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. Molecular Ecology 23:2118-2135, https://doi.org/10.1111/mec.12706
- Jiménez-Leyva J. A., A. Gutiérrez, J. A. Orozco, G. Vargas, M. Esqueda, A. Gardea, ... and E Muñoz (2017) Phenological and ecophysiological responses of *Capsicum annuum* var. *glabriusculum* to native arbuscular mycorrhizal fungi and phosphorus availability. *Environmental and Experimental Botany* 138:193-202, https://doi.org/10.1016/j.envexpbot.2017.03.008
- Kim K., W. Yim, P. Trivedi, M. Madhaiyan, H. P. D. Boruah, M. R. Islam, ... and T. Sa (2010) Synergistic effects of inoculating arbuscular mycorrhizal fungi and Methylobacterium oryzae strains on growth and nutrient uptake of red pepper (Capsicum annuum L.). Plant and Soil 327:429-440, https://doi.org/10.1007/s11104-009-0072-4
- Lioussanne L., M. Jolicoeur and M. St-Arnaud (2009) Role of the modification in root exudation induced by arbuscular mycorrhizal colonization on the intraradical growth of *Phytophthora nicotianae* in tomato. *Mycorrhiza* 19:443-448, https://doi.org/10.1007/s00572-009-0257-y
- Mena-Violante H., O. Ocampo-Jimenez, L. Dendooven, G. Martínez-Soto, J. González-Castañeda, F. T. Davies and V. Olalde-Portugal (2006) Arbuscular mycorrhizal fungi enhance fruit growth and quality of chile ancho (*Capsicum annuum* L. cv San Luis) plants exposed to drought. *Mycorrhiza* 16:261-267, https://doi.org/10.1007/s00572-006-0043-z
- Peñuelas-Rubio O., D. Trejo-Aguilar, R. G. Cervantes-Gámez y I. E. Maldonado-Mendoza (2021) Análisis microbiómico de hongos micorrízicos arbusculares asociados a papaya, inoculada con consorcios

- de dos ecosistemas naturales. Scientia Fungorum 51:e1292, https://doi.org/10.33885/sf.2021.51.1292
- Pérez C. L. J., M. A. Tornero C., J. S. Escobedo G. y E. Sandoval C. (2016) El chile poblano criollo en la cultura alimentaria del Alto Atoyac. Estudios Sociales 27:47-66.
- Phillips J. M. and D. S. Hayman (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, https://doi.org/10.1016/S0007-1536(70)80110-3
- Pozo C. O. (1983) Logros y aportaciones de la investigación agrícola en el cultivo del chile. Publicación Especial. Instituto Nacional de Investigaciones Agrícolas. México, D. F. 20 p.
- Reyes-Tena A., E. E. Quiñones-Aguilar, G. Rincón-Enríquez y L. López-Pérez (2016) Micorrización en *Capsicum annuum* L. para promoción de crecimiento y bioprotección contra *Phytophthora capsici* L. *Revista Mexicana de Ciencias Agrícolas* 7:857-870, https://doi.org/10.29312/remexca.v7i4.260
- Reyes-Tena A., G. Rincón-Enríquez, L. López-Pérez and E. E. Quiñones-Aguilar (2017) Effect of mycorrhizae and actinomycetes on growth and bioprotection of *Capsicum annuum* L. against *Phytophthora capsici*. *Pakistan Journal of Agricultural Sciences* 54:513-522, https://doi.org/10.21162/PAKJAS/17.4245
- Rodríguez J., B. V. Peña O., A. Gil M., B. Martínez C., F. Manzo and L. Salazar L. (2007) Rescate *in situ* del chile poblano en Puebla, México. *Revista Fitotecnia Mexicana* 30:25-32.
- SAS Institute (2002) The SAS System for Windows version 9.0. SAS Institute Inc., Cary, North Carolina, USA.
- Sensoy S., S. Demir, O. Turkmen, C. Erdinc and O. B. Savur (2007) Responses of some different pepper (Capsicum annuum L.) genotypes to inoculation with two different arbuscular mycorrhizal fungi. Scientia Horticulturae 113:92-95, https://doi.org/10.1016/j. scienta.2007.01.023
- SIAP, Servicio de Información Agroalimentaria y Pesquera (2021) Anuario

- estadístico de la producción agrícola. Servicio de Información Agroalimentaria y Pesquera. Secretaría de Agricultura y Desarrollo Rural. Ciudad de México. https://nube.siap.gob.mx/ cierreagricola/ (Noviembre 2021).
- Sieverding E. (1983) Manual de Métodos para la Investigación de la Micorriza Vesículo-Arbuscular en el Laboratorio. Centro Internacional de Agricultura Tropical. Cali, Colombia. 121 p.
- Smith S. E. and D. J. Read (2008) Mycorrhizal Symbiosis. 3rd edition. Academic Press. New York, USA. 800 p.
- Trejo-Aguilar D., L. Lara-Capistrán, I. E. Maldonado-Mendoza, R. Zulueta-Rodríguez, W. Sangabriel-Conde, M. E. Mancera-López, ... and I. Barois (2013) Loss of arbuscular mycorrhizal fungal diversity in trap cultures during long-term subculturing. *IMA Fungus* 4:161-167, https://doi.org/10.5598/imafungus.2013.04.02.01
- Trinidad-Cruz J. R., E. E. Quiñones-Aguilar, L. V. Hernández-Cuevas, L. López-Pérez y G. Rincón-Enríquez (2017) Hongos micorrícicos arbusculares asociados a la rizosfera de *Agave cupreata* en regiones mezcaleras del estado de Michoacán, México. *Scientia Fungorum* 45:13-25, https://doi.org/10.33885/sf.2017.0.1164
- Varela-Cervero S., A. López-García, J. M. Barea and C. Azcón-Aguilar (2016)
 Differences in the composition of arbuscular mycorrhizal fungal
 communities promoted by different propagule forms from a
 Mediterranean shrubland. *Mycorrhiza* 26:489-496, https://doi.
 org/10.1007/s00572-016-0687-2
- org/10.1007/s00572-016-0687-2

 Verbruggen E., M. G. A. Van Der Heijden, J. T. Weedon, G. A. Kowalchuk and W. F. M. Röling (2012) Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils.

 Molecular Ecology 21:2341-2353, https://doi.org/10.1111/j.1365-294X.2012.05534.x
- Yang Y., Y. Song, H. V. Scheller, A. Ghosh, Y. Ban, H. Chen and M. Tang (2015) Community structure of arbuscular mycorrhizal fungi associated with *Robinia pseudoacacia* in uncontaminated and heavy metal contaminated soils. *Soil Biology and Biochemistry* 86:146-158, https://doi.org/10.1016/j.soilbio.2015.03.018