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# Identification of arbuscular mycorrhizal fungi in avocado orchards of Uruapan, Michoacán

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## Abstract

The most important agricultural activity in Michoacán, Mexico, is avocado farming, which represents revenues of $30 265 787.40 pesos per year. The crop is extensive and based on conventional agricultural practices that affect the physical, chemical and biological properties of the soil. The objective of this study was to determine the diversity of species of arbuscular mycorrhizal fungi (AMF) in avocado orchards. Soil samples were collected in avocado orchards during the rainy season (August 2016). Soil samples (three subsamples per orchard) were taken from the upper horizon to 30 cm deep. In the laboratory, the spores were isolated from dry soil samples and morphologically identified. 15 morphic species AMF classified into eight genera and four families were recorded and described. 78.5% of the species belong to the families Glomeraceae and Diversisporaceae; the rest to Claroideoglomeraceae and Acaulosporaceae; the species *Sclerocystis sinuosa*, *S. rubiformis*, *Funneliformis geosporum*, *F. mosseae*, *Acaulospora scrobiculata*, *Diversispora spurca* and *Entrophospora infrequens*, previously reported in avocado cultures, were identified, while *Glomus citrícola*, *G. macrocarpum*, *Septoglomus constrictum* and *Claroideoglomus claroideum*, were identified. report for the first time in avocado. The presence of *D. aurantia* and *Tricispora nevadensis* is recorded for the first time in Mexico. Therefore, currently there is a great diversity of AMF in avocado orchards of Uruapan that must be conserved.

**Keywords:** *Persea americana*, monoculture, taxonomy.

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# Introduction

In 2016, the state of Michoacan, Mexico, produced approximately 1 889 353.60 tons of avocado, which represented 80% of the country's total production (SIAP, 2016). Throughout its agri-food chain, this crop generates around 47 thousand direct jobs, 70 thousand seasonal jobs and 187 thousand indirect jobs. The economic and social benefits of this industry contrast with the environmental problems derived from the excessive use of agrochemicals that are used from nursery to the productive stage (De la Tejera-Hernández *et al*., 2013), in addition to the volcanic nature of the soils where this fruit is grown favors the leaching of fertilizers and pollution of groundwater, with the consequent environmental damage and human health (Tapia *et al*., 2012).

There are microorganisms that favor the nutrition and productivity of crops without affecting the ecosystem. Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs of plants that favor root growth, with a consequent increase in the absorption of water and nutrients (Gañán *et al*., 2011). They favor the chlorophyll content and elements in the foliar tissue (Díaz *et al*., 2016). The AMF confer tolerance to salinity (Medina, 2016) and drought by altering the hormonal profiles of the plants.

The diversity of the AMF influences the productivity of the plant communities of natural and agricultural ecosystems (Lovera and Cuenca, 2007). In the production of fruit trees, mineral fertilization can be reduced up to 25% by using beneficial microorganisms (Ramos *et al*., 2013; Simo *et al*., 2015). Apparently, there is no taxonomic specificity in the symbiosis (Lovera and Cuenca, 2007); however, molecular biology studies have shown that some plants are preferentially colonized by certain AMF species (Vandenkoornhuyse *et al*., 2002).

In plants of avocado (*Persea americana* var. *drymifolia*) in nursery, the particular effects of *Acaulospora delicata*, *Acaulospora laevis*, *Glomus badium* and *Rhizophagus fasciculatus* + *Azospirillum* have been demonstrated by doubling the biomass of the root and stem, while *R. irregularis* and *S. pellucida* tripled it (Carreon-Abud *et al*., 2014). Likewise, *D. heterogama*, *C. etunicatum*, *C. claroideum* and *A. scrobiculata* favored the vegetative development of avocado plants (Silveira *et al*., 2003), while *C. claroideum* increased the contents of K, Ca, in the soil. Cu, Zn and Mn and *A. scrobiculata* increased the contents of P, Cu and Zn (Silveira *et al*., 2003).

So the AMF are an important support for agricultural production with a sustainable approach. In avocado orchards it is necessary to know the diversity of this type of microorganisms, knowledge that could contribute to the generation of adequate strategies for their conservation, management and use (Chimal-Sánchez *et al*., 2016).

Traditionally, the taxonomy of AMF has been carried out based on the spore’s morphology (Rivera *et al*., 2016) that considers the size, color, coupling of the supporting hyphae, lipid content and thickness of the cellular wall. The current classification consists of four orders, 11 families, 27 genera and around 261 species (Schüßler and Walker, 2010; Redecker *et al*., 2013).

In Mexico, there are few studies on the identification of AMF genera in the Michoacan avocado producing area, where intensive management agricultural practices may have caused a significant loss of beneficial microorganisms for the plants. The objective of this research was to determine the diversity of AMF species associated with avocado cultivation.

# Materials and methods

## Description of the sampling site

The sampling of rhizospheric soil to obtain AMF spores was made on August 27, 2016, in 10 avocado orchards in the municipality of Uruapan, Michoacan, Mexico, which is located at 19° 22' 41.79" north latitude and 102° 02' 19.11" west longitude, at 1 592 m altitude. The climate is humid temperate with rains in summer; the temperature oscillates between 8 and 37.5 °C and the annual pluvial precipitation of 1 759.3 mm (INAFED, 2016). In this avocado region, the soil type that predominates is Andosol, which covers 10 614.35 ha, but also with a lower proportion are Luvisol, Acrisol and Vertisol types (Gutiérrez-Contreras *et al*., 2010).

## Obtaining and preparing soil samples

From each orchard, three soil samples were collected, from the upper horizon to 30 cm deep, at randomly established points. The samples were homogenized, from this composite mixture, a representative subsample of 2 kg was taken for each orchard, they were labeled according to the sampling order with their geographical location and taken to the laboratory for processing. From each site, a representative sample of 1 kg was taken and the physicochemical properties were determined indicating franc-sandy texture, 50.27 ppm of assimilable N, 28.6 ppm of total phosphorus, 91.4 ppm of potassium, pH of 6.03, electrical conductivity of 1.28 mmhos $cm^{-1}$, organic matter of 11.3% and bulk density of 0.61 g $cm^{-3}$.

The spores were extracted from the soil by means of decantation and wet sieving (Gerdemann and Nicolson, 1963), followed by a gradient centrifugation of 50% sucrose (Daniels and Skipper, 1982). Using a stereoscopic microscope (Nikon Mod. C -PS), the extracted spores were grouped by morphospecies and placed on slides with polyvinyl alcohol lactoglycerol (PVLG), with and without Melzer's reagent (see reaction walls), as assembly medium (Schenck and Pérez, 1990). The morphological characteristics of the AMF spores (size, color, coupling of the supporting hyphae, lipid content, and thickness of the cell wall) were observed in a compound microscope (Nikon H550L) and recorded in photomicrographs.

## Identification of AMF

The taxonomic identification of the AMF was based on the keys of Schenck and Pérez (1990) and the list of species reported worldwide, available in the portals: i) Phylogeny and taxonomy of Glomeromycota ('arbuscular mycorrhizal (AM) and related fungi') (http://www.amf-phylogeny.com); ii) Arbuscular mycorrhizal fungy (Glomeromycota), *Endogone* and *Complexipes* species deposited in the Department of Plant Pathology, University of Agriculture in Szczecin, Poland (Blaszkowski, 2003); and iii) International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM, 2018); iv) The International Bank of the *Glomeromycota* (IBG, 1993); and v) Catalog of Arbuscular Mycorrhizal fungi strains available in the Glomeromycetes *in vitro* Collection (Agriculture and Agri-Food Canada, 2014). The number of spores was expressed as the total number of spores present in 50 g of dry rhizospheric soil, the specimens are deposited in the ENCB herbarium.

The relative abundance was obtained as the number of spores of a species, on the total of the spores isolated, multiplied by 100. The relative richness was determined with the number of species, and for the calculation of the diversity index the proposed method was considered by Shannon and Weaver (1949) obtained with the following formula: $H' = -\sum p_i \ln p_i'$. Where $p_i$ is the number of spores of a species, divided by the total number of spores isolated.

## Results and discussion

In all the orchards studied, AMF was found in the rhizosphere of avocado trees. 15 species of AMF were identified, although only one was identified at the genus level. They belong to four families and eight genera of the *Glomerales* and *Diversisporales* orders (Table 1).

**Table 1. AMF species identified in rhizospheric soil of avocado orchards.**

| Order | Family | Genus | Species |
|---|---|---|---|
| Glomerales | Glomeraceae | *Glomus* | *Claroideoglomus etunicatum* Tulasne & Tulasne |
| | | | *Glomus citricola* Tang & Zang |
| | | | *Glomus* sp. |
| | | *Sclerocystis* | *Glomus badium*Gerdemann & Trappe |
| | | | *Sclerocystis sinuosa* Gerdemann & Bakshi |
| | | *Septoglomus* | *Septoglomus constrictum Trappe* |
| | | *Funneliformis* | *Funneliformis geosporum* (Błaszk.) Walker & Schuessler |
| | | | *Funneliformis mosseae* (Nicol. & Gerd.) Gerd. & Trappe |
| | Claroideoglomeraceae | *Claroideoglomus* | *Claroideoglomus claroideum* Schenck & Sm.) Walker & Schuessler |
| | | | *Claroideoglomus clarum* Thaxt (Gerd. & Trappe) Walker & Koske |
| Diversisporales | Acaulosporaceae | *Acaulospora* | *Acaulospora scrobiculata* Trappe |
| | Diversisporaceae | *Diversispora* | *Diversispora aurantia* (Blaszk. V. Blanke, C. Renker & F. Buscot) |
| | | | *Diversispora spurca* (Walker) Walker & Schuessler |
| | | *Entrophospora* | *Entrophospora infrequens* (Hall) R.N. Ames & R.W. Schneid |
| | | | *Tricispora nevadensis* Palenzuela, Barea, Azcon-Aguilar & Fritz |

The largest number of genera corresponded to the family Glomeraceae and the genus *Glomus* was the most abundant with three species, while *Septoglomus* and *Acaulospora*, the latter of the family Acaulosporaceae, were the least common with one species (Figure 1).

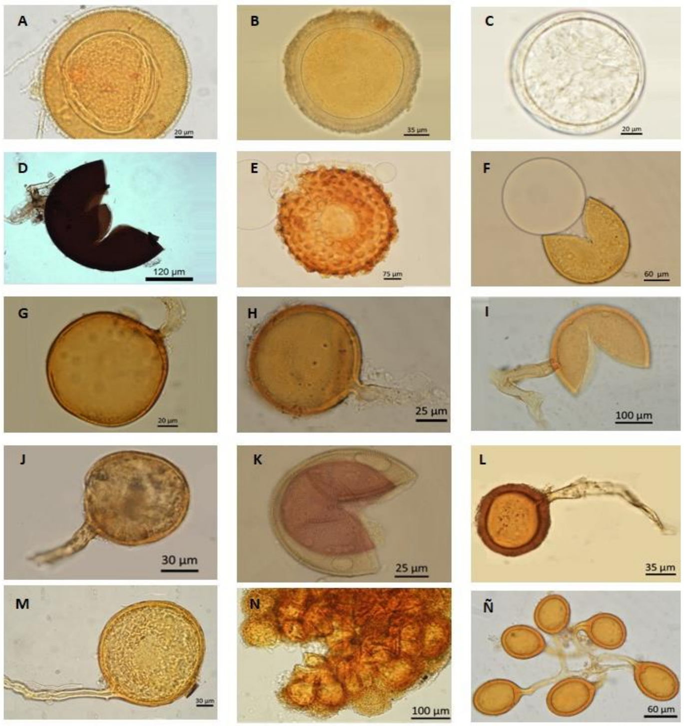

**Figure 1. (A) *Entrophospora infrequens*; (B) *Claroideoglomus clarum*; (C) *Diversispora spurca*; (D) *Septoglomus constrictum*; (E) *Tricispora nevadensis*; (F) *Claroideoglomus claroideum*; (G) *Diversispora aurantia*; (H) *Claroidedglomus etunicatum*; (I) *Glomus* sp.; (J) *Funneliformis mosseae*; (K) *Acaulospora scrobiculata*; (L) *Glomus macrocarpon*; (M) *Funneliformis geosporum*; (N) *Sclerocystis sinuosa*; and (Ñ) *Glomus badium*.**

*Claroideoglomus etunicatum* and *G. macrocarpon* are reported for the first time in the avocado culture were found in the ten sampling sites, so they can be considered as able to survive and form spores under diverse conditions, in addition to compete efficiently and ensure success of colonization, In this regard, Kahiluoto *et al*. (2001); Opik *et al*. (2006) mention that the genus *Glomus* has a wide distribution, so it has been considered as a generalist because its species have a wide range of adaptation in terms of soil type and edaphoclimatic conditions.

The dominance of *Glomus* in the studied agricultural soils is possibly due to the fact that it has an extraradical mycelium that colonizes, while other genera such as *Gigaspora* frequently develop from spores (Hart and Reader, 2002; Pérez-Luna *et al*., 2012).). The diversity of AMF species found in this study coincides with the values reported by Gonzalez-Cortes *et al*. (2012) in disturbed soils, because originally the vegetation was pine forest, later for corn cultivation and for about 30 years avocado orchards with different agronomic management were established.

*Claroideoglomus etunicatum*, has been reported in soils associated with the cultivation of *Agave cupreata* in mezcal regions of the state of Michoacán, Mexico, the most outstanding characteristics of *C. etunicatum* are its spores, which are born individually in the soil from pale yellow to yellow, globose to subglobose (75-) 95 (-135) µm diameter, occasionally ovoid, 110-160 x 140-180 µm, with an underlying hyphae, the subcellular structure of the spores consists of a spore wall composed of two layers.

The mucilaginous layer 1, rough on the upper surface, hyaline, 0.5-2.5 µm thick, layer 2, smooth on both surfaces, pale yellow to yellow and (4.5-) 5.7 (-6.5) µm thick. However, in the melzer reagent, only layer 1 dyes reddish red to dull red, in contrast *G. macrocarpon* their spores are rarely found individually in the soil, usually in sporocarps containing 2-15 spores distributed at random.

These spores are yellow, globose to subglobose of (110) 115 (-120) micron in diameter, rarely ovoid or pear-shaped, 100-110 x 115-130 microns. Layer one is semi-flexible, hyaline (1.2-) 1.5 (-1.7) µm thick, layer two laminated, smooth, yellow (4.7-) 6.6 (-7.6) mµ thick. Neither of the two layers reacts to the Melzer reagent.

Other AMF species reported for the first time in avocado are: *Claroideoglomus clarum*, *C. claroideum*, *Septoglomus constrictum*; however, these species had already been reported in natural ecosystems and agroecosystems in maize plots, secondary vegetation areas and low deciduous forest (Guadarrama-Chávez *et al*., 2007; Pérez-Luna *et al*., 2012; Chimal-Sánchez *et al*., 2015).

While *Tricispora nevadensis*, *Diversispora aurantia*, and *G. citrus* are reported for the first time for Mexico. The species *D. aurantia* was recognized by the depth of orange-yellow color of its spores, its smooth surface and its relatively thick leathery feel (Walker, 1987), the spores have three layers, of which the third is flexible a semiflexible (Błaszkowski, 2012).

On the other hand, *T. nevadensises* is characterized by its spores of yellow-brown to brown color, 90-115 µm in diameter, with two or three layers, conspicuous, 6-12 µm long, the projections of the spine extending over the outer wall is hyaline to subhyaline, Walls and OWL3 OWL2 layers of reddish-brown stain in Melzer's reagent, while the OTB1 outer layer does not stain or spots slightly pinkish; this species had only been isolated from the rhizosphere of *Plantago nivalis* (Plantaginaceae) and *Alchemilla fontquer* (Rosaceae) in the Sierra Nevada, Andalusia Region, Spain (Palenzuela *et al*., 2010), in an area at 3 000 m altitude, in acid soils (pH from 5.1 to 6.5), high contents of organic matter (7.1 to 22.8%) and high humidity.

These edaphoclimatic conditions coincide with those of the study area, despite being at a lower altitude (1592 m). Figure 1 shows the species *T. nevadensis* that is characterized by forming ornamentation with spiny projections on the outer surface of the spores (Palenzuela *et al*., 2010).

The Shannon-Weaver index obtained was high (3,192), compared with those reported by Perez-Luna *et al*. (2012) in the cultivation of maize under different treatments (1.22 to 1.64) and similarity indexes reported by Gonzalez-Cortes *et al*. (2012) in forest and avocado orchards (0.88) and corn-avocado cultivation (0.65). It can be inferred in this analysis that the dominant populations are in active symbiosis under the evaluated conditions (Table 2) taking into account the above, the dominant genera under edaphic conditions are in their order: *Glomus* and *Claroideoglomus*.

**Table 2. Species and morphospecies of AMF found in the rhizosphere of avocado.**

| Species | No. of spores/50 g of soil | Relative abundance (%) |
|---|---|---|
| *Claroideoglomus etunicatum* | 120 | 4.4 |
| *Glomus citrícola* | 400 | 14.5 |
| *Glomus* sp. | 50 | 1.8 |
| *Sclerocystis rubiformis* | 800 | 29 |
| *Sclerocystis sinuosa* | 90 | 3.3 |
| *Septoglomus constrictum* | 70 | 2.5 |
| *Funneliformis geosporum* | 60 | 2.2 |
| *Funneliformis mosseae* | 90 | 3.3 |
| *Claroideoglomus claroideum* | 350 | 12.7 |
| *Claroideoglomus clarum* | 400 | 14.5 |
| *Acaulospora scrobiculata* | 40 | 1.5 |
| *Diversispora aurantia* | 25 | 0.9 |
| *Diversispora spurca* | 70 | 2.5 |
| *Entrophospora infrequens* | 50 | 1.8 |
| *Entrophospora nevadensis* | 140 | 5.1 |
| Total | 2 755 | 100 |

The taxonomic diversity can be affected by agricultural practices, the application of fungicides, as well as the cultivation of non-mycotrophic species and the establishment of monocultures; these factors cause in a crop the loss or diminution of the AMF diversity due to the selection pressure exerted on them (Varela and Trejo, 2001; Guadarrama-Chávez *et al*., 2007; Oehl *et al*., 2011; Pérez-Luna *et al*., 2012).

In the study area, the antecedent is that, before the avocado cultivation was established as monoculture, the vegetation cover was formed by a mixed pine-oak forest, as well as forests of oyamel, oak and mountain mesophile (Chávez-León *et al*., 2012). Bautista-Cruz *et al*. (2014) point out that the morphospecies of *Glomus*, *Scutellospora*, *Diversispora* and *Gigaspora* were found in mesophilic mountain forests. These forests underwent a change in land use, since in them they established corn crops, which caused an alteration in the AMF communities (González-Cortés *et al*., 2012). In these same soils, avocado cultivation was established.

*Acaulospora scrobiculata* presented a relative abundance of 1.5% (Table 2), which contrasts with that reported by González-Cortés *et al*. (2012), who report high frequency of *A. laevis*, *A. scrobiculata* and *A. spinosa* in sites associated with avocado cultivation. These differences can be attributed to the variation in the characteristics of the soil, which, despite having a similar pH, the content of organic matter was higher in the soils of our study and lower the P-total content. Attributed by the above, these species can be considered dominant.

The environmental conditions and physical-chemical characteristics of the soil can be determinant in the abundance of species, it has been found that in soils with a pH greater than 7 the abundance of species of the genus *Acaulospora* is greater than at lower pH, in which the species *A. scrobiculares* increase their frequency (González-Cortés *et al*., 2012).

## Conclusions

There is a great diversity of AMF in avocado orchards of Uruapan. It is identified 15 morphospecies of AMF in the rhizosphere of avocado trees, the predominant family was *Glomeraceae*, which showed the greatest diversity, followed by *Diversisporaceae*.

The seven new species reported in avocado cultivation are *Claroideoglomus clarum*, *C. claroideum*, *Diversispora aurantia*, *Septoglomus constrictum*, *Entrophospora nevadensis*, *Claroideoglomus etunicatum* and *G. citricola*. The species *Diversispora aurantia* and *Tricispora nevadensisse* register, for the first time, in Mexico.

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