Proceedings VI World Avocado Congress (Actas VI Congreso Mundial del Aguacate) 2007.
Viña Del Mar, Chile. 12 – 16 Nov. 2007. ISBN No 978-956-17-0413-8.

DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ON AVOCADO ORCHARDS FROM MICHOACAN

A. Bárcenas[1], C. Almaraz[1], L. Reyes[1], L. Varela[2], B. Lara[1], A. Guillén[1], Y. Carreón[3], S. Aguirre[1] and A. Chávez[1].

[1]Facultad de Agrobiología "Presidente Juárez de la UMSNH, Lázaro Cárdenas esq. Berlín s/n, Uruapan, Mich. México. E-mail: abarcenas@prodigy.net.mx; [2]IPN. Hongos y Derivados, SA de CV. E-mail: lvarela@ipn.mx;
[3]Facultad de Biología UMSNH. Edificio B4, Ciudad Universitaria. ycabud@yahoo.com.mx. Teléfono (52+443) 3167412

Avocado trees lack root hairs. It has been demonstrated that arbuscular mycorrhiza forming fungi (AMF) colonize these fruit trees, favour water absorption and nutrient uptake by plants, and also enhance growth and ameliorate plant health. However, the symbiotic associations in the field and the role AMF may play on the production system are not currently well documented; approaches on mycorrhizal symbiosis and its yield effects have been mainly addressed to plant nursery as well as to *in vitro* propagation. This caused the present study, which aims at determining AMF diversity and abundance on avocado orchards soil in Michoacan, Mexico. The experiment was developed in orchards with seven different climates, under two moisture conditions (irrigation and rain fed) and in two times of the year (rainy and low water season). Rhizosphere samples were obtained from soils of 14 orchards; spores from those samples were extracted and mounted on glass slides to identify and quantify them under microscope. 22 morphospecies corresponding to five genera, from three families and two orders, were identified; seven of them had not been previously reported as associated with the avocado culture. In the low water season species, distribution of the three genera was as shown: *Glomus* (39.43%), *Acaulospora* (26.23%) and *Scutellospora* (21%) and while in the rainy season the distribution among these genera differed: *Glomus* (37.56%), *Scutellospora* (29.16%) and *Acaulospora* (23.24%).

Keywords: mycorrhiza, climate, low water and water season, two moisturing conditions.

DIVERSIDAD DE HONGOS MICORRIZÓGENOS ARBUSCULARES EN HUERTOS DE AGUACATE DE MICHOACÁN

A. Bárcenas[1], C. Almaraz[1], L. Reyes[1], L. Varela[2], B. Lara[1], A. Guillén[1], Y. Carreón[3], S. Aguirre[1] y A. Chávez[1].

[1]Facultad de Agrobiología "Presidente Juárez de la UMSNH, Lázaro Cárdenas esq. Berlín s/n, Uruapan, Mich. México. E-mail: abarcenas@prodigy.net.mx; [2]IPN. Hongos y Derivados, SA de CV. E-mail: lvarela@ipn.mx;
[3]Facultad de Biología UMSNH. Edificio B4, Ciudad Universitaria. ycabud@yahoo.com.mx. Teléfono (52+443) 3167412

La raíz del aguacate carece de pelos radicales. Se ha demostrado que los hongos formadores de micorrizas arbusculares (HMA) colonizan las raíces de este frutal, favorecen la absorción de agua y el aprovechamiento de los nutrimentos del suelo por la planta, también propician incrementos de crecimiento y las mantienen saludables. Pero la presencia de la simbiosis en campo y el papel que pueda tener dentro de un sistema de producción no están muy documentados, ya que los trabajos realizados sobre micorrizas en este cultivo en su mayoría son en vivero y propagación *in vitro*. Lo anterior motivó el presente estudio, que tuvo como finalidad determinar la riqueza y abundancia de HMA presentes en huertos de aguacate de la región productora de Michoacán, México, en siete diferentes climas, bajo dos condiciones de humedad (riego y temporal) y en dos épocas (lluvias y estiaje). Para ello se realizaron muestreos de suelo rizosférico de 14 huertos; se extrajeron las esporas del suelo y se montaron en portaobjetos para su conteo e identificación. Se detectaron e identificaron 22 morfoespecies correspondientes a cinco géneros, de tres familias y dos órdenes; siete de ellas no se habían reportado en este cultivo. En la época de estiaje predominaron las especies de los géneros: *Glomus* (39.43%), *Acaulospora* (26.23%) y *Scutellospora* (21%) y en la época de lluvias las de los géneros: *Glomus* (37.56%), *Scutellospora* (29.16%) y *Acaulospora* (23.24%).

Palabras clave: micorrizas, climas, estiaje y lluvias, dos condiciones de humedad.

1. Introduction

In the state of Michoacan, Republic of Mexico, avocado culture has been established along 30 municipalities, the area occupied by this culture on each municipality varies from less than 100 ha, until more than 23,000 ha. 22 of the most important municipalities where avocado is produced cover 94,045.09 ha, with elevations among 1100 and 2900 AMSL, and under 10 different climates; however the greatest cultivated area (74,463.15 ha) is located among 1600 and 2100 AMSL (Lara *et al*., 2005).

Arbuscular mycorrhiza forming fungi (AMF) colonize plant roots forming an extended mycelial net that provides multiple benefits to the host plant: greater water and nutrients uptake (specially P, Cu and Zn); protection and better survival under stress environmental conditions such as salinity, drought, acid soils, presence of toxic elements or root pathogens (Smith and Read, 1997). Most plants naturally present associations with AMF (Bonello, 2001) of no apparent taxonomic specificities. However, molecular techniques recently lend to evidence indicating that plants are mainly conolized by few AMF species based on its differential effects on the plant development (Lovera and Cuenca, 2007).

Avocado trees lack hair roots (Salazar-Garcia, 2002), under such circumstances it is most likely that this tree depends on mycorrhiza to accomplish its nurishment. Ginsburg and Avizohar (1965), observed AMF on avocado roots from Israel and Hass and Menge (1990), characterized AMF in soil samples from orchards where this fruit tree was grown in California, USA and Israel. Presence

and identification of AMF has also been detected in different isolated avocado orchards in Michoacan, Mexico (Bárcenas et al., 2006).

The aim of the present study was to determine AMF diversity on avocado orchards soil from the seven most outstanding climates in which avocado culture is stablished in Michoacan, as well as to ascertain the differences between the former factor and climate, season during the year (rainy and low water season) and moisturing conditions (irrigation and rain fed). The investigation was initiated in February 2006 and culminated in February 2007, its implementation intends to contribute to the development of a sustainable avocado production system, without the excessive application of chemical products, avoiding environmental deterioration and damage to the human health.

2. Matherials y methods

2.1 Site description

The investigation was carried out in 14 orchards established in three municipalities of the avocado production area of Michoacan, with different moisturing conditions (irrigation and temporary), and with the following climates: C1: Aw1(w); C2: (To) C (m)(w), C3: C (m) (w), C4: (A)C(w2)(w), C5: A)C(w1)(w), C6: A(C)(w0)(w) and C7: C (w2) (w) (García, 1973).

2.2 Soil Sampling

Two rhizosphere soil samplings were carried out to a depth of 20 cm, one during the low water season (February-March 2006) and the other in the rainy season (August-September 2006).

2.3 Spore extraction and concentration dominance

Spores of AMF were extracted from the soil by wet sieving and decanting (Gerdemann and Nicolson, 1963), followed by sucrose differential centrifugation. The sifted spores were opted through standard filter paper. The isolated spores were picked up under a dissecting microscope and were mounted both in polyvinyl lactoglycerol (PVLG) and PVLG mixed with Melzer's reagent (1:1 v/v).

A gridded acetate base was glued below the preparations and all the spores were examined using a compound microscope in order to perform the spore counting and taxonomic identification. The former was carried out based on spore shape, size, colour; wall layer thickness, number and ornamentation of cell layers, and hyphal shape and its attachments; the identification manual of Schenk and Perez (1990), each species specific original description, and the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (INVAM, 2006) were used.

2. 4 Richness and abundance

The richness (R) of AMF species was determined considering the number of species; the absolute abundance (Aa) as the total number of spores from all the species in each climate, for each one of the two seasons.

2.5 Statistical Analysis

The data obtained were submitted to analysis of variance; when statistical significance was found, comparison of means was performed through Tukey Test with a significance of 0.05. The values obtained were analyzed using SAS version 9, 2002, FUANL version 2 (Olivares, 1992), and Bio-Dap (Anne Magurran, 1988).

3. Results

3.1 Ocurrence of species and taxonomic diversity of AMF

22 morphospecies, correspongind to five genus, three families and two orders were detected (Table I)

Table I. AMF species identified in 14 avocado orchards of Michoacan, Mexico, established in seven different climates.

Num	Species	Symbol	Num	Species	Symbol
1	*Acaulospora spinosa,* (Walker and Trappe)	*A sp*	12	*Glomus geosporum* (Nicolson and Gerdemann) (Walker)	*Gl g*
2	*Acaulospora scrobiculata* (Trappe)	*A sc*	13	*Glomus constrictum* (Trappe)	*Gl c*
3	*Acaulospora scrobiculata chica*	*A sc ch*	14	*Glomus spinuliferum* (Sieverd and Oehl)	*Gl s*
4	*Acaulospora denticulada* (Sieverding and Toro)	*A d*	15	*Glomus tortuosum* (Schenck and Smith)	*Gl t*
5	*Acaulospora sp*	*A sp*	16	*Glomus sinuosum,* (Gerdemann and Bakshi)	*Gl sin*
6	*Gigaspora aff. gigantea* (Nicolson and Trappe)	*G g*	17	*Glomus sp1*	*Gl sp1*
7	*Scutellospora gregaria* (Schenck and Nicolson) (Walter and Sanders)	*Sc g*	18	*Glomus sp2*	*Gl sp2*
8	*Scutellospora scutata* (Walker and Diederichs)	*Sc sc*	19	*Glomus sp3*	*Gl sp3*
9	*Scutellospora aff. calospora* (Gerdemann and Trappe)	*Sc cal*	20	*Sclerocystis rubiformis,* (Gerdemann and Trappe)	*S r*
10	*Scutellospora verrucosa* (Koske and Walker) (Walter and Sanders)	*Sc v*	21	*Sclerocystis pachycaulis* (Wu and Chen)	*S p*
11	*Scutellospora sp.*	*Sc sp*	22	*Sclerocystis sp*	*S sp*

Species *A sc ch*, *A d*, *Sc cal*, *Gl s*, *Gl sin*, *S p* and *S* sp have not previously been reported as associated to avocado rizhosphere; while species A s*, A sc, A sp*, *G g, Sc g, Sc sc, Sc cal, Sc v, Sc* sp., *Gl g, Gl c, Gl s, Gl t, Gl* sp1*, Gl* sp2*, Gl* sp3

and *S r,* have been already found on seven former studies of avocado orchards in Michoacan, *Gl g* was common among all (Barcenas *et al*., 2006). Hass and Menge (1990), reported the existence of nine species of AMF in California and six in Israel, some of these species were also found in the present study, *A sc* described in California, *Gl g* in Israel and *Gl c* in both countries.

In this study, the species ocurrence was predominantly of genus *Glomus, Acaulospora* and *Scutellospora* (Figure 1), the two first coincide with the few reports on mycorrhiza in avocado on a worldwide basis, and the only two genus recounted by Hass and Menge in California and Israel, where *Glomus* was the genus most frequently found (Hass and Menge, 1990). This two genus have been also reported as the most abundant in all the seven former studies in Michoacan, however, *Scutellospora* and *Gigaspora* are consistent in four and six of these reports, respectively (Bárcenas *et al*., 2006). The presence of the two last genus can be explained as a result of the original microbiota in the soil under study, since orchards in which this research was carried out, as well as most of the avocado orchards located in the state of Michoacan, had been established in territory of a natural forest of pine-evergreen trees and González (2005) has described the presence of *Scutellospora* and *Gigaspora* in addition to *Glomus* y *Acaulospora* as the most abundant genus in these kind of territories.

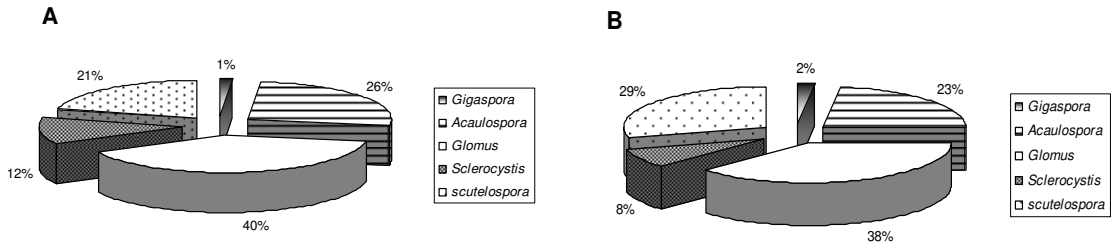


Figure 1. Relative abundance of AMF spores by genus in soil of avocado orchards in Michoacan, Mexico. Rainy season (A) and Low water season (B).

Richness (R) of AMF in the different climates during the low water season, varied between 17 and 22 species and from 20 to 22 species during the rainy season. The climates with highest abundance of spores in the rainy season were: (A)C(w2)(w) warm sub-humid (20%) and C(w2)(w) temperate sub-humid (18%). In the low water season there was greater number of spores in the climate C(w2)(w) temperate sub-humid (21%), followed by spores on climate (A)C(w2)(w) warm sub-humid(19%) (Figure 2).

Richness and abundance of AMF species was greatest in the rainy season as well as in orchards under irrigation, which is consistent with the results reported by Esperón and Camargo (2004) and González (2005) in which the abundance has higher in rainy season compared to low water season (Figure 3)

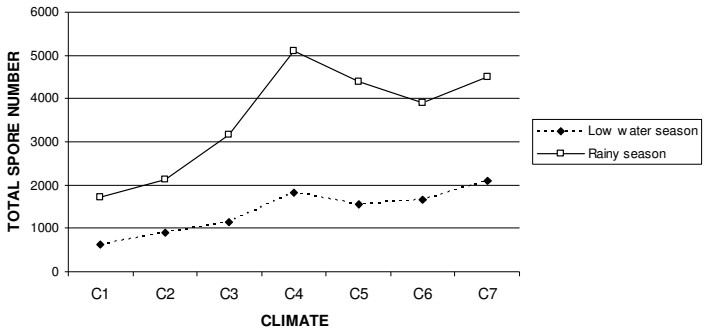
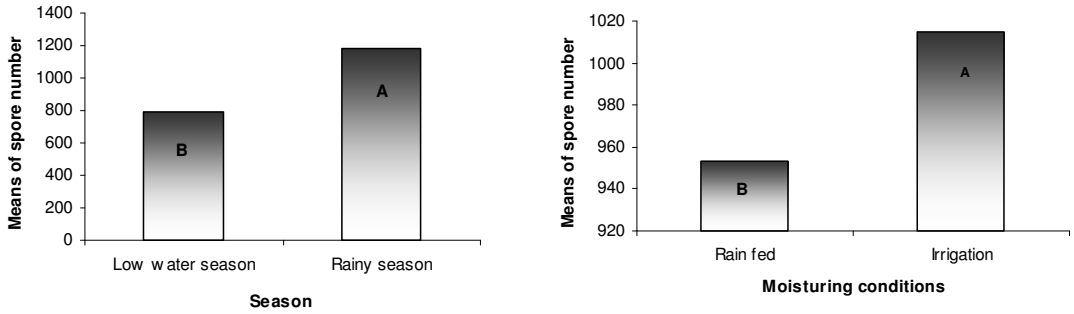


Figure 2. Absolute abundance of AMF spores in soil of avocado orchards with seven climates and during two seasons of the year in Michoacan, Mexico.



Figures 3. Comparison of means of the spore number of 22 AMF species found in avocado orchards in Michoacan, Mexico. Seasons of the year (A) and moisturing conditions (B) ($p < 0.05$).

Acknowledgements

Authors would like to appreciate Dr. Samuel Salazar García for his kind attentions as to have permitted us to work in some of the selected orchards for the project: "Studies on phenology and physiology of avocado cv. Hass in Michoacan", as well as economic support by the Scientific Research Coordination, UMSNH.

References

Bárcenas O., A.E., Varela F.,L., Carreón A., Y., Lara Ch., M.B.N., González C., J.C. and Aguirre P., S. 2006. Estudios sobre hongos micorrizógenos arbusculares en huertos de aguacate *Persea americana* Mill. (RANALES:LAURACEAS). *In*: Memoria del XXIX Congreso Nacional de Control Biológico. SMCB. Manzanillo, Col. pp 1-5.

Gerdeman, J.W. and T.H. Nicolson. 1963. Spores of Mycorhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 46: 235-244.

Ginsburg, O. and Avizohar Z. 1965. Observations on vesicular-arbuscular mycorrhiza associated with avocado roots in Israel. Trans. Brit. Mycol. Soc. 48: pp 101-104.

González C., JC. 2005. Diversidad de hongos micoríizicos arbusculares en un agroecosistema de aguacate (*Persea americana* Mill) comparado con un bosque natural. Masters Thesis. Facultad de Biología. UMSNH. Morelia, Mich. 76 pp.

Hass, H. J. and J.A. Menge. 1990. VA-micorrhizal fungi and soil characteristics in avocado (*Persea americana* Mill) orchard soils. Plant and Soil 127: 207-212.

INVAM. 2006. Internacional Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungy. http://invam.caf.wvu.edu. (2 de enero del 2006)

Lara Ch. B. N., Gutiérrez C. M, Guillen A. H., López M. J. and Vidales F. J. A. 2005. Caracterización agroclimática de la franja aguacatera del Estado de Michoacán, México. Memorias del II Congreso Mexicano y Latinoamericano del Aguacate, Uruapan Mich. México. Pp 2-11

Lovera, M. and Cuenca G. 2007. Diversidad de hongos micorrízico arbusculares (HMA) y potencial micorrízico del suelo de una sabana natural y una sabana perturbada de la Gran Sabana, Venezuela.

Schenk, N.C. and Y. Pérez. 1990. Manual for the Identification of VA Mycorrhizal Fungi. Synergistic Publications. Gainesville, FL, USA.

Smith, S. E. and.J. Read. 1997. Mycorrhizal Symbiosis. Academic Press.