



EFFECT OF MYCORRHIZAL INOCULANTS IN THE DEVELOPMENT OF MEXICAN LANDRACE AVOCADO ROOTSTOCKS

[EFECTO DE INOCULANTES MICORRÍZICOS EN EL DESARROLLO DE PORTAINJERTOS DE AGUACATE CRIOLLO DE LA RAZA MEXICANA]

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SUMMARY

The aim of this work was to assess the effect of two arbuscular mycorrhizal fungi (AMF) inoculants in the development of avocado rootstocks. Seeds of Mexican landrace avocado (*Persea americana* Mill. var. *drymifolia*) were used, with two commercial inoculants: T1 containing *Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, and *Acaulospora scrobiculata*, and T2, containing *G. mosseae* and *G. cubense*. The plants inoculated with AMF showed more rapid growth than the no inoculant control as measured by plant height (50% and 54%), stem diameter (35% and 36%), leaf number (48% and 37%) and length (31% and 40%), and root fresh weight (85% and 59%); however, no significant differences were observed between T1 and T2. The chlorophyll concentration in the leaves from T1 was 16.4% and T2 was 19% higher than the control suggesting a higher photosynthetic capacity in T1 and T2. Finally the shoot/root ratio, as indicator of the potential development of plantations, was 79% and 50% higher in mycorrhizal plants than in the control. In conclusion both T1 and T2 inoculants improved growth rate and vigor of avocado nursery rootstocks producing higher quality plants.

Key words: Arbuscular Mycorrhizal; biofertilizers; *Persea Americana*; rootstocks.

RESUMEN

El objetivo de la investigación fue evaluar el efecto de dos inoculantes de hongos micorrizógenos arbusculares (HMA) en el desarrollo de plantas de aguacate en vivero. Se usaron semillas de aguacate criollo mexicano (*Persea americana* Mill. var. *drymifolia*) y dos inoculantes comerciales: T1 con *Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum* y *Acaulospora scrobiculata*; T2 con *G. mosseae* y *G. cubense*. Las plantas inoculadas con HMA mostraron un mayor desarrollo que las plantas testigo respecto a la altura de planta (50% y 54%), diámetro del tallo (35% y 36%), número (48% y 37%) y longitud de hojas (31% y 40%), así como peso fresco de la raíz (85% y 59%), sin presentar diferencias significativas entre T1 y T2. El contenido de clorofila en las hojas de T1 y T2 fue 16.4% y 19% mayor que en el testigo, respectivamente, sugiriendo una mayor capacidad fotosintética en T1 y T2. Finalmente, el cociente tallo/raíz, como indicador del desarrollo potencial de las plantaciones, fue 79% y 50% mayor en las plantas inoculadas que en el testigo. Se concluye que ambos inoculantes mejoran el desarrollo y vigor de los portainjertos de aguacate en vivero y promueven mayor calidad de las plantas.

Palabras clave: Micorriza arbuscular; biofertilizantes; *Persea americana*; portainjertos.

INTRODUCTION

Avocado production is a major economic activity in Mexico, considered the world's leader in planted surface area, production, consumption and exportation of this fruit crop. More than 74% of the avocado's planted surface area in this country is

located in the State of Michoacan, with an annual production of 1,117,338.5 tons (SIAP, 2012). This agronomic activity encompasses remarkable economic and social impact due to the volume of production and to the large amount of direct and indirect employment it generates (Sánchez, 2007).

The most popular cultivar in Michoacan is “Hass”, grown on the Mexican landrace, *Persea americana* Mill. var. *drymifolia*, as rootstock. Avocado has hairless mangrove-like roots (Salazar-García, 2002) that are highly dependent on mycorrhizal symbiosis (Alarcón *et al.*, 2001). Arbuscular mycorrhizal fungi (AMF) establish symbiotic relationships with plant roots and perform important functions, contributing quite efficiently to the growth and survival of crops due to their capacity to increase water uptake (Augé, 2004; Ruiz-Lozano and Azcón, 2006) and the absorption of sparingly mobile nutrients such as P, Cu and Zn (Krishna *et al.*, 2010; Smith and Smith, 2011). AMF also diminish the incidence of pathogenic diseases in plants (Pozo and Azcón-Aguilar, 2007) and contribute to ameliorate the soil structure (Cuenca *et al.*, 2007).

The positive effect of mycorrhiza on avocado plants has been observed in seedlings and micropropagated plants. Menge *et al.* (1980) reported that *Glomus fasciculatus* increased the growth of ‘Topa Topa’ avocado seedlings, Rivera *et al.* (2011) observed an increase in the development of avocado when seedlings were inoculated with AMF, showing higher results with *G. hoi-like* than with *G. mosseae*, the survival and development of micropropagated avocado plants have shown to be improved when they are inoculated with *Glomus* spp. during the *ex vitro* acclimatization (Azcón-Aguilar *et al.*, 1992; Vidal *et al.*, 1992), additionally mycorrhizal avocado plants are more tolerant to *Phytophthora cinnamomi* damage than the nonmycorrhizal plants (Davis *et al.*, 1978).

Avocado seedlings are grown in local nurseries, on substrates treated with chemical insecticides and fungicides in order to keep them free from plant pathogens, treatments which can negatively affect beneficial AMF. Application of AMF biofertilizers (Gianinazzi and Vosátka, 2004) can reduce the negative effects of pesticide treatments and increase the sustainability of the agricultural production systems. Due to the importance of this association and the benefits that these fungi provide, it is valuable to evaluate how best to use mycorrhizal inoculants at nurseries to prevent and remediate the loss of microorganisms in the substrate, while fostering the rapid growth of seedlings, before they are planted in the field. Herein, we assess the effects of two commercial AMF inoculants on the development of Mexican landrace avocado rootstocks.

MATERIALS AND METHODS

Location

Studies were done in a greenhouse and laboratory facilities of the “Presidente Juárez” Agri-biology

school of the Universidad Michoacana de San Nicolás de Hidalgo, at Uruapan, Michoacan, Mexico.

Biological Material

Since avocado is an allogamous plant with high genetic variability, the seeds to be used were obtained from one single tree. 90 fruits were collected from a Mexican landrace avocado tree (*P. americana* Mill. var. *drymifolia*) located at Tancitaro, municipality in Michoacan. The seeds were separated from the mesocarp of the fruit and 30 seeds with similar size and shape were selected to conduct the experiment.

Two commercial AMF inoculants were used: inoculant-1 is a consortium of five AMF species (*Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum*, and *Acaulospora scrobiculata*) at a density of 20 spores g⁻¹ (Mena *et al.*, 2011) and inoculant-2, which contains 20 to 30 spores g⁻¹ of *G. mosseae* and *G. cubense* species (Rodríguez *et al.*, 2011).

Substrate

A blend of soil from local crop fields, sand and organic matter (pine tree bark) was used at a ratio of 3:1:1 (v:v:v), and was disinfected using wet heat in an autoclave at 121 °C and 15 psi for three cycles of 1 h every 24 h. The blend was air dried for 24 hours and bagged in black polyethylene 3-kg bags.

Treatments

Three conditions were evaluated: T1, avocado seeds coated with inoculant-1; T2, avocado seeds coated with inoculant-2; and T3, avocado seeds not inoculated (control). The experimental unit consisted in two avocado seeds, and five repetitions were considered per treatment, which were randomly distributed. The experiment was conducted during seven months after sprouting.

Inoculation and planting

The selected seeds were dried for 72 hours at room temperature, then the seed coat was removed and seeds were disinfected by submergence in 70% alcohol for 5 min; followed by soaking in 0.8% sodium hypochlorite for 20 min, followed by a profuse rinse with sterile water and left to dry at room temperature. Subsequently disinfected seeds were covered with a fluid paste prepared with 50g of the corresponding, T1 or T2 inoculants and 30 ml of water, as described by Rivera *et al.* (2011), while T3 control seeds were left untreated. Afterwards the seeds were sown one per bag in the sterile substrate

and placed in a greenhouse with partial shade and kept at a mean temperature of 19 °C.

Plant and mycorrhizal response measurements

Plant height, stem diameter, number and length of leaves were measured every month during six months, starting one month after sprouting. A graduated ruler and a Vernier gauge were used to measure plant height from the neck of the first root to the apex; the stem diameter at 5 cm from the soil and the leaf length from the point where the petiole joins the leaf blade.

Chlorophyll concentration was measured six months after inoculation and planting using the third leaf of each plant. Measurements were made with a Minolta Spad 502 meter taken on the same day at 10:00 am-11:00 am. The data were converted into the chlorophyll's concentration rate using the slope and intercept calculated from a standard curve (Figure 2) in which the Spad units of leaves with different green hues were correlated to chlorophyll concentrations measured using an acetone extraction method proposed by Porra *et al.* (1989) and the Arnon's formula (Arnon, 1949).

$$\text{Chlorophyll } (\mu\text{g/g fresh tissue}) = \frac{(\text{CCR}) * (\text{EV})}{\text{Fresh weight (g)}}$$

Where:

CCR= Chlorophyll's concentration rate (μg/ml)

EV= Extract's volume

Root fresh weight (RFW) was recorded as the weight in grams of freshly excised roots.

Foliar dry weight (FDW) and *root dry weight (RDW)* were determined by drying roots and shoots at 60 °C until constant weight was reached (72 hours). These weights were then used to provide the FDW/RDW or Shoot/Root ratio.

Mycorrhizal colonization. A sample of roots was taken from the plants and, each sample was stained as described by Chávez-Bárceñas *et al.* (2013). Stained roots were analyzed under a compounded microscope

at 40X and 100X, quantifying the typical structure of mycorrhizal symbiosis present on the sample roots. The colonization percentage of individual root hair segments was calculated using Phillips and Hayman (1970) formula.

$$\text{Total colonization (\%)} = \frac{\text{N}^\circ \text{ colonized segments}}{\text{N}^\circ \text{ total segments}} * 100$$

Destructive analyses were performed seven months after sowing (six months after sprouting). At that point the roots of the avocado plants were carefully disaggregated from the soil and cut apart from the upper aerial part of the plant to achieve the determinations.

Statistical Analysis

Statistical differences in all the variables determined between the inoculated and not inoculated treatments were assessed by variance analysis and Tukey means comparison test (p<0.05), using the ANOVA module of the statistical package V 2.5 (Olivares, 1994).

RESULTS AND DISCUSSION

Mycorrhizal colonization

Arbuscular mycorrhizal structures were observed in the roots of plants inoculated with T1 and T2, while the roots of plants without AMF did not show any evidence of mycorrhizal colonization (Fig. 1). These results reveal that at least some AMF species from both inoculants are able to develop mycorrhiza with studied avocado roots.

The total colonization percentage in T1 roots was 6% higher than in T2 (Table 1). These results, together with the fact that inoculants in T1 include a wider AMF species diversity and lower spore density than T2, suggest that AMF species within T1 bear a greater capacity to establish symbiosis with avocado roots than AMF species from T2. Nevertheless, more acute and specific experiments should be design to ascertain the colonization capacity of the species within inoculants 1 and 2.

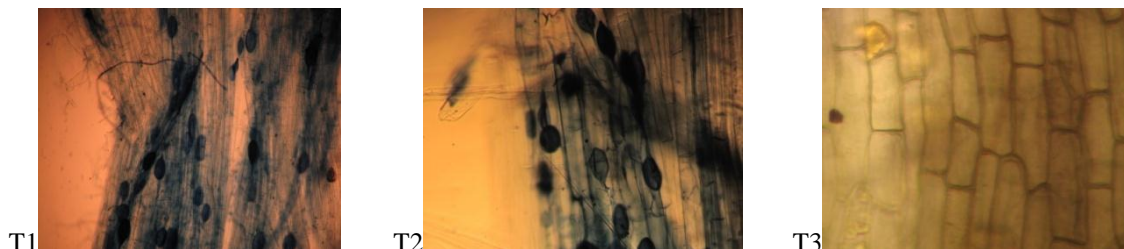


Figure 1. Avocado plant roots colonized with AMF from T1 and T2, and non-colonized roots in T3 control.

Morphological variables

Both AMF treatments developed plants with better morphological traits than the non-inoculated control (Table 1). However, no statistically significant differences were observed between T1 and T2 plants, but T1 and T2 differed significantly of control. Plant height was 50% to 54% higher in AMF treated plants than in the control. Similarly, higher values were registered in AMF inoculated plants as regards to stalk diameter (35% to 36%), leaf number (37% to 48%), leaf length (31% to 40%), and root fresh weight (59% to 85%), when compared to the not inoculated control. These results coincide with previous reports that showed positive effects of different AMF species on the growth and development of avocado plants when compared to a non-mycorrhizal control (Azcón-Aguilar *et al.*, 1992; Reyes *et al.*, 1997; Rivera *et al.*, 2011).

Shoot/root ratio

Shoot/root ratio was higher in T1 (0.74) than in T2 (0.63) and T3 (0.41); the difference between T1 and T3 was significant (Table 1). The shoot/root ratio is an important indicator of “quality” of nursery tree seedlings for achieving high survival and good take-off after transplanting (Larsen *et al.*, 1986; Zandstra and Lyptay, 1999; Dans *et al.*, 1999; Vaario *et al.*, 2009; Negreros-Castillo *et al.*, 2010), denoting equilibrium between the size of the plants aerial parts and their root size, and a balance status of carbon partitioning and allocation (Basil *et al.*, 2009; Landhäusser *et al.*, 2012). The shoot/root ratio is usually higher in more fertile soils and substrates (Lambers *et al.*, 1998; Greenwood *et al.*, 2007). Our results suggest that avocado nursery plants inoculated with AMF are closer to a shoot/root biomass balance, than the non-inoculated plants, this agreed with Navarro *et al.* (2011) who reported a higher balance of root/shoot ratio in mycorrhizal plants of *Arbutus unedo* L. when compared with non-mycorrhizal during the nursery period, Zhang *et al.* (2011) also

showed that AMF affect the interaction root vs. root allocation and thus allometric allocation and biomass-density relationships. Notwithstanding, our results also suggest that these allometric adjustments are also dependent on the AMF species, since both inoculants attained different shoot/root responses compared to the control.

Chlorophyll concentration

Chlorophyll concentrations in leaves of T1 and T2 plants were 16% and 19% higher, respectively, than those of the non-inoculated control (Table 2, Fig. 2). These results are consistent with Azcón-Aguilar *et al.* (1992) and Reyes *et al.* (1997) results, which showed that besides improving the uptake and utilization rates of nutrients in avocado plants, AMF also increased their photosynthetic capacity, promoted higher growth rates and enhanced plant health. This study also agrees with Alarcón and Ferrera-Cerrato (1999), who noted that the symbiotic association of mycorrhizal fungi with plant roots produces several changes and/or modifications at a physiological level; among which an increase in the photosynthetic capacity can be highlighted, due to a greater CO₂ fixation capacity, and the resulting increase in growth and biomass rates.

Moreover, the difference on the chlorophyll concentration between the inoculated treatments T1 and T2 (Table 2) are in agreement with previous studies that have shown a differential response of plants to AMF inoculants within treatments of diverse AMF composition, as well as when compared to non-inoculated, less developed plants (Janousková *et al.*, 2009; Mena *et al.*, 2011; Rivera *et al.*, 2011). Additionally, it is to be noticed that although T1 showed greater mycorrhizal colonization percentage (Table 1), T2 had higher chlorophyll content, suggesting that the mycorrhizal symbiosis with the AMF species in T2 might promote enhancement of the photosynthetic capacity of plants.

Table 1. Mean comparison of the variables measured in avocado nursery plants inoculated or not with AMF.

TREATMENTS	PH (cm)	SD (mm)	NL	LL (cm)	RFW (g)	S/RR	C%
T1	33.6a	8.79a	21.9a	13.3a	31.5a	0.75a	79.5a
T2	34.6a	8.87a	20.3a	14.2a	27.0a	0.63ab	75.0b
T3	22.4b	6.5b	14.8b	10.1b	17.0b	0.42b	00.0c

PH, Plant Height; SD, Stem Diameter; NL, Number of Leaves; LL, Leaf Length; RFW, Root Fresh Weight; S/RR, Shoot/Root ratio; C%, Total Colonization Percentage. The same letters in a column indicate statistically equal values ($p \leq 0.05$)

Table 2. Chlorophyll concentration in avocado nursery plants inoculated or not with AMF.

Treatments	Chlorophyll concentration ($\mu\text{g/g}$ fresh tissue)
T1	1886.9b
T2	1929.1a
T3	1621.0c

The same letters in a column indicate statistically equal values ($p \leq 0.05$)

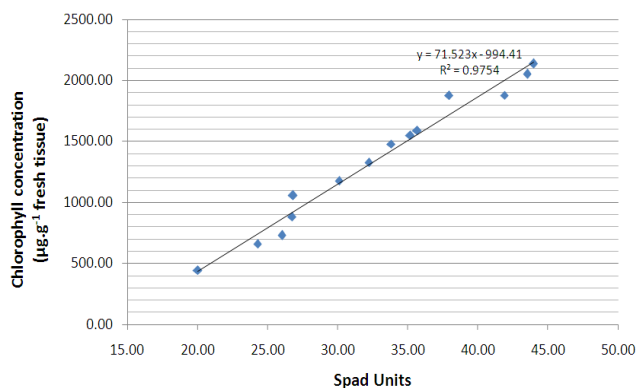


Figure 2. Standard curve of chlorophyll concentration in avocado leaves.

CONCLUSIONS

AMF from both inoculants used in this essay are able to establish a mycorrhizal symbiosis with Mexican landrace avocado; however inoculant T1 showed higher mycorrhizal colonization rates. Both inoculants promote positive developmental traits on avocado plants, with minor differences between them. Better plant fitness due to higher balance in shoot/root ratio was obtained with inoculant T1, while higher photosynthetic potential due to superior chlorophyll concentration is developed in leaves of plants was observed with inoculant T2. Together our results may have critical implications in the avocado production in Mexico.

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REFERENCES

- Alarcón, A., Almaraz, J.J., Ferrera-Cerrato, R., González-Chávez, M.C.A., Lara H., M.E., Manjarrez M., M.J., Quintero, L.R., Santamaría, R.S. 2001. Manual: Tecnología de hongos micorrízicos en la producción de especies forestales en vivero. Colegio de Postgraduados-PRONARE-CONAFOR. México. pp. 98.
- Alarcón, A., Ferrera-Cerrato, R. 1999. Manejo de la micorriza arbuscular en sistemas de propagación de plantas frutícolas. Revista Terra Latinoamericana. 17:178-191.
- Arnon, D.I. 1949. Enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.
- Augé, R. M. 2004. Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84:373-381.
- Azcón-Aguilar, C., Barceló, A., Vidal, M. T., De la Viña, G. 1992. Further studies on the influence of mycorrhizae on growth and development of micropopagated avocado plants. Agronomie. 12:837-840.
- Basil, G., Mazzarino, M.J., Roselli, L., Letourne, F. 2009. Efecto del compost de biosólidos en la producción de plantines de *Austrocedrus chilensis* (ciprés de la cordillera). Ciencias del suelo. Argentina. 27(1):49-55.
- Cuenca, G., Cáceres, A., Oirdobro, G., Hasmy, Z., Urdaneta, C. 2007. Las micorrizas arbusculares como alternativa para una agricultura sustentable en áreas tropicales. Interciencia. 32:26-29.
- Chávez-Bárceñas, A.T., Lua-Aldama, J., Salmeron-Santiago, I.A., Silva-Adame, M.B., García-Saucedo, P.A., Olalde-Portugal, V. 2013. A modified staining technique for the anatomical observation of mycorrhizal roots of woody trees. African Journal of Microbiology Research. 28:3589-3596.
- Dans del Valle, F., Fernández de Ana M., F.J., Romero G., A. 1999. Manual de silvicultura del pino radiata en Galicia. Escuela Politécnica Superior de Lugo y Asociación Forestal de Galicia. pp. 199.

- Davis, R.M., Menge, J.A., Zentmyer, G.A. 1978. Influence of vesicular-arbuscular mycorrhizae on *Phytophthora* root rot of three crops plants. *Phytopathology* 68:1614-1617.
- Gianinazzi, S., Vosátka, M. 2004. Inoculum of arbuscular mycorrhiza fungi for production systems: science meets business. *Canadian Journal of Botany* 82:1264-1271.
- Greenwood, D.J., Mckee, J.M.T., Fuller, D.P., Burns, I.G., Mulholland, B.J. 2007. A novel method of supplying permits predictable shoot growth and root : shoot ratios of pre-transplant bedding plants. *Annals of Botany* 99:171-182.
- Janousková, M., Seddas, P., Mrnka, L., van Tuinen, D., Dvoracková, A., Tollot M, Gianinazzi-Pearson, V., Vosátka, M., Gollotte, A. 2009. Development and activity of *Glomus intraradices* as affected by co-existence with *Glomus claroideum* in one root system. *Mycorrhiza* 19:393-402.
- Krishna, H., Das, G., Attri, B. L., Grover, M., Ahmed, N. 2010. Suppression of *Botryosphaeria* canker of apple by arbuscular mycorrhiza fungi. *Crop Protection* 29:1049-1054.
- Lambers, H., Chapin III, F. S., Pons, T. L. 1998. Plant Physiological Ecology. *Journal of Agronomy and Crop Science*. 184:143-144.
- Landhäusser, S.M., Pinno, B.D., Lieffers, V.J., Chow, P.S. 2012. Partitioning of carbon allocation to reserves or growth determines future performance of aspen seedlings. *Forest Ecology and Management* 275:43-51.
- Larsen, H.S., South, D.B., Boyer, J. M. 1986. Root growth potential, seedling morphology and bud dormancy correlate with survival of loblolly pine seedlings planted in December in Alabama. *Tree Physiology* 1:253-263.
- Mena, E.A., Fernández S.K., Jerez M.E., Olalde P.V., Serrato R. 2011. Influencia de la inoculación con *Glomus hoi-like* y un conglomerado de especies de HMA en el crecimiento de plantas de sorgo sometidas o no a estrés hídrico. *Cultivos Tropicales* 32:16-27.
- Menge, J.A., LaRue, J., Labanauskas, C.K., Johnson, E.L.V. 1980. The effect of two mycorrhizal fungi upon growth and nutrition of avocado seedlings grown with six fertilizer treatments. *Journal of the American Society for Horticultural Science* 105:400-404.
- Navarro, G.A., Bañón, A.S.P., Morte, A., Sánchez-Blanco, M.J. 2011. Effects of nursery preconditioning through mycorrhizal inoculation and drought in *Arbutus unedo* L. plants. *Mycorrhiza* 21:53-64.
- Negreros-Castillo, P., Apodaca-Martínez, M., Mize, C. W. 2010. Efecto de sustrato y densidad en la calidad de plántulas de cedro, caoba y roble. *Madera Bosques*. 16:7-18.
- Olivares S., E. 1994. Paquete de diseños experimentales FAUANL. Versión 2.5. Facultad de Agronomía. Universidad Autónoma de Nuevo León. Marín, Nuevo León. México.
- Olivares S E (1994) Paquete de Diseños Experimentales FAUANL. Versión 2.5. Facultad de Agronomía de la UANL. Marín, N.L., México.
- Porra, J. R., Thompson, W. A., Kriedemann, P. E. 1989. Determination of accurate extinction coefficients and simultaneous equations assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophylls standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta*. 975:384-394.
- Pozo, M., Azcón-Aguilar, C. 2007. Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* 10:393-398.
- Reyes A., J. C., Alarcón, A., Ferrera C., R. 1997. Aspectos relacionados sobre el uso de la endomicorriza arbuscular en aguacate (*Persea americana* Mill). In Memoria 1997. Fundación Salvador Sánchez Colín. CICTAMEX S.C. Coatepec Harinas, México. pp. 83-94.
- Rivera E., R., Martín C., J. V., Calderón P., A., Torrez H., A. 2011. Utilización de cepas eficientes de hongos micorrízicos

- arbusculares en el desarrollo de portainjertos de aguacate en un sustrato suelo-cachaza. Cultivos Tropicales. 32:172-183.
- Rodríguez, Y., Dalpé, Y., Séguin, S., Fernández, K., Fernández, F., Rivera, R. A. 2011. *Glomus cubense* sp. nov., an arbuscular mycorrhizal fungus from Cuba. Mycotaxon. 118:337-347.
- Ruiz-Lozano, J.M., Azcón R. 2006. Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiologia Plantarum 95:472-478.
- Salazar-García. S. 2002. Nutrición del Aguacate, Principios y Aplicaciones. INIFAP INPOFOS. México. pp. 165.
- Sánchez R., G. 2007. El Cluster del aguacate de Michoacán, desarrollo regional para el liderazgo global. Fundación produce Michoacán. pp. 241.
- SIAP. 2012. Servicio de Información Agroalimentaria y Pesquera. Producción anual, cierre de la producción agrícola por cultivo. Accessed 30 July 2013 In: http://www.siap.gob.mx/index.php?option=com_wrapper&view=wrapper&Itemid=350.
- Smith, S.E, Smith, F.A. 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Review of Plant Biology 62:227-250.
- Vaario, L.-M., Tervonen, A., Haukioja, M., Pennanen, T., Timonen, S. 2009. The effect of nursery substrate and fertilization on the growth and ectomycorrhizal status of containerized and outplanted seedlings of *Picea abies*. Canadian Journal of Forest Research 39:64-75
- Vidal, M.T., Azcón-Aguilar C., Barea J.M. 1992. Mycorrhizal inoculation enhances growth and development of micropropagated plants of avocado. HortScience 27:785-787.
- Zandstra, J.W., Liptay, A. 1999. Nutritional effects on transplant root and shoot growth – a review. Acta Horticulturae 504:23-31.
- Zhang, Q., Zhang, L., Weiner, J., Tang, J., Chen, X. 2011. Arbuscular mycorrhizal fungi alter plant allometry and biomass-density relationships. Annals of Botany 107:407-413.

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