



## NATIVE ARBUSCULAR MYCORRHIZAL FUNGI FROM THE RHIZOSPHERE OF *Agave cupreata* AND THEIR EFFECT ON *Agave tequilana* GROWTH

### HONGOS MICORRÍZICOS ARBUSCULARES NATIVOS DE LA RIZÓSFERA DE *Agave cupreata* Y SU EFECTO SOBRE EL CRECIMIENTO DE *Agave tequilana*

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#### SUMMARY

Blue agave (*Agave tequilana* F.A.C. Weber var. Azul) is an important crop for Mexico, from which tequila is made. In 2018 1,138,800 t of agave were produced under a production system where the use of agrochemicals has become widespread; in particular, the excessive use of chemical fertilizers generates problems of environmental impact in the producing areas. An alternative to solve this problem is the use of soil microbial resources to reduce this impact. The use of arbuscular mycorrhizal fungi (AMF) as biofertilizers is a functional alternative in other crops that could be implemented in the production of blue agave. The aim of this research was to evaluate the effect of native mycorrhizal consortia isolated from the rhizosphere of *A. cupreata* from Michoacán on the growth of blue agave. An experiment was conducted under a randomized complete blocks design where eight AMF consortia, a commercial inoculum (*Rhizophagus intraradices*) and a control (without AMF) were evaluated on *A. tequilana* seedlings. The experiment was maintained for 300 d under greenhouse conditions and, at the end, growth and microbiological variables were analyzed; in addition, mycorrhizal dependency and the Dickson index were calculated as parameters of plant quality. Significant differences (Tukey,  $P \leq 0.05$ ) were found in the growth traits of agave plants when inoculated with AMF consortia compared to control plants. The agave plants inoculated with the native consortium Barranca de las Nueces showed greater growth and Dickson index, with a mycorrhizal colonization of 49 % and a mycorrhization dependency of 62 %, as well as the highest spore density (244 spores 100 g<sup>-1</sup> of substrate). Results suggest that AMF could be a biotechnology alternative in the agricultural production process of blue agave.

**Index words:** Tequila agave, mycorrhiza, biofertilizer, maguey, native fungal consortium.

#### RESUMEN

El agave azul (*Agave tequilana* F. A. C. Weber var. Azul) es un cultivo importante para México, a partir del cual se elabora el tequila. En 2018 se produjeron 1,138,800 t de agave bajo un sistema de producción donde se ha generalizado el uso de agroquímicos; en particular, el empleo excesivo de fertilizantes químicos genera problemas de impacto ambiental en las zonas productoras. Una alternativa para resolver este problema es la utilización de recursos microbianos del suelo para disminuir este impacto. El empleo de hongos micorrízicos arbusculares (HMA) como biofertilizantes es una alternativa funcional en otros cultivos que podrían implementarse en la producción del agave azul. El objetivo de este estudio fue evaluar el efecto de consorcios micorrízicos nativos aislados de rizosfera de *A. cupreata* de Michoacán en el crecimiento de agave azul. Se condujo un experimento bajo

un diseño de bloques completos al azar donde se evaluaron ocho consorcios de HMA, un inóculo comercial (*Rhizophagus intraradices*) y un tratamiento control (sin HMA) en plantas de *A. tequilana*. El experimento se mantuvo durante 300 d en condiciones de invernadero y al final se analizaron variables de crecimiento, microbiológicas y se calculó la dependencia micorrízica e índice de Dickson como parámetro de calidad de planta. Se encontraron diferencias significativas (Tukey,  $P \leq 0.05$ ) en las variables de crecimiento de las plantas de agave cuando se inocularon con los consorcios de HMA en comparación con las plantas testigo. Las plantas de agave inoculadas con el consorcio nativo Barranca de las Nueces mostró mayor crecimiento e índice de Dickson, con una colonización micorrízica del 49 % y una dependencia a la micorrización del 62 %, así como la mayor densidad de esporas (244 esporas 100 g<sup>-1</sup> de sustrato). Los resultados sugieren que los HMA podrían ser una alternativa biotecnológica en el proceso de producción agrícola del agave azul.

**Palabras clave:** Agave tequilero, biofertilizante, consorcio nativo de hongos, maguey, micorriza.

#### INTRODUCTION

The genus *Agave* has approximately 200 species, 150 of them are distributed in Mexico (García-Mendoza, 2004). This plant has been used since ancient times as a source of food, fodder, medicine, fiber and as construction material, among other uses. Its main use today is in the production of alcoholic beverages such as pulque, mezcal, bacanora and tequila; the latter of them is produced from the species *Agave tequilana* (Weber) var. Azul (Gentry, 1982). This species is particularly important in Mexico, being the raw material for tequila, which, due to the denomination of origin for tequila, is only produced in Mexico, being an important source of foreign exchange. In 2018, production of tequila in Mexico reached 309.1 million liters, for which 1,138,800 t of *A. tequilana* were required (CRT, 2019).

The development of intensive agricultural practices in the production of *A. tequilana* has led to an excessive use of chemical fertilizers, resulting in environmental deterioration and increasing risks to human health, microfauna and wild flora (Gomiero *et al.*, 2011). Using organic resources as microbial biofertilizers could reduce the use of chemical

products and in turn could reduce their environmental impact. A particularly promising alternative is the use of arbuscular mycorrhizal fungi (AMF) as biofertilizer (Harris-Valle *et al.*, 2019). AMF form one of the most important symbiotic associations with plants, the arbuscular mycorrhizae (Schüßler *et al.*, 2007). This association promotes plant growth through different mechanisms of action, such as producing substances that regulate plant growth, improving water absorption, increasing nutrient uptake (especially P, Cu, Zn, N, etc.) (Hodge and Fitter, 2010; Schüßler *et al.*, 2007; Smith and Read, 2008; Smith and Smith, 2012) and increasing tolerance to different abiotic stresses such as drought (Azcón-Aguilar and Barea, 2015) and salinity (Harris-Valle *et al.*, 2012). AMF also help to increase the diversity and population levels of rhizospheric microorganisms by inducing a suppressive effect on soil phytopathogens (De La Noval *et al.*, 2007; Jung *et al.*, 2012; Trinidad-Cruz *et al.*, 2017a) and improving the physical properties of the soil by forming stable aggregates (Rillig and Mummey, 2006).

Other studies have evaluated the effect of AMF on different species of agave plants. Quiñones-Aguilar *et al.* (2016) evaluated the effect of native AMF consortia on the growth and plant quality of *A. inaequidens*; they reported a growth-promoting effect and high quality in agave plants inoculated with native AMF consortia compared to a commercial strain; they also reported an increase larger than 500 % in the total dry weight of plants with mycorrhizal consortia compared to plants without AMF. Trinidad-Cruz *et al.* (2017a) reported a significant increase ( $P \leq 0.05$ ), between 148 % and 239 % in total dry biomass of *A. cupreata* inoculated with native AMF consortia compared to agave plants without inoculum. Pimienta-Barrios *et al.* (2009) evaluated the effect of inoculating *A. tequilana* seedlings with *Glomus fasciculatum* and *G. intraradices* on photosynthesis, foliar development and growth; they reported that inoculation with *G. intraradices* increased photosynthesis and induced an increase in the mesophyll of the plants.

Robles-Martínez *et al.* (2013) evaluated the functional compatibility of different native AMF inocula in *A. angustifolia*; they found that at least one native consortium and one commercial inoculant significantly promoted the accumulation of aerial biomass in the plants, and also increased the number and length of the roots compared to non-inoculated plants. Martínez-López *et al.* (2009) evaluated the dry biomass of *A. americana* inoculated with native strains of *G. intraradices* (*R. intraradices*) from Nuevo León, Mexico, and a commercial INIFAP® strain (different from *G. intraradices*), and found no differences in the biomass of agave plants between the two types of inoculum (commercial or native); thus, the aim of this study

was to assess the effect of different native AMF consortia obtained from the rhizospheric soil of *Agave cupreata* plantations in the state of Michoacán, Mexico on the growth of *A. tequilana* seedlings grown under greenhouse conditions.

## MATERIALS AND METHODS

### Arbuscular mycorrhizal fungi (AMF) and blue agave plants

In order to obtain native AMF consortia, samples of rhizospheric soil were taken from *A. cupreata* plantations at the mezcal denomination of origin region in Michoacán, México. The fungal consortia were obtained from different municipalities and labelled according to the name of the locality in which they were extracted. Consortia were identified as Dominant genera: *Glomus*, *Scutellospora*: El Huizachal (EH); Dominant genera: *Acaulospora*: Las Campesinas (LC), Rancho Carlos Rojas (CR), El Limón (EL), Agua Dulce (AD); Dominant genera: *Acaulospora*, *Glomus*: Paso Ancho (PA), Barranca de las Nueces (BN) and Cerro del Metate (CM). The mycorrhizal consortia were previously propagated in trap pots for eight months using a variety of plants: sorghum (*Sorghum* spp.), alfalfa (*Medicago sativa*), bermuda grass (*Cynodon dactylon*) and agave (*A. cupreata*). The consortia were taxonomically identified and a total of 40 species were found, belonging to 13 genera, nine families and three orders (Trinidad-Cruz *et al.*, 2017b).

The *A. tequilana* seedlings were propagated from bulbils collected from adult plants; the average weight of the seedlings was  $2.8 \pm 0.52$  g at the beginning of the experiment. The bulbils came from blue agave plants from the Arenal region, in the state of Jalisco. The selected plants had produced an inflorescence.

### Experimental conditions

A randomized complete blocks experimental design was used; the treatments consisted of the previously propagated native consortia EH, LC, CR, EL, AD, PA, BN and CM, along with the commercial inoculum MI (Mycorrhiza INIFAP® based on *R. intraradices*) used as positive control and a negative control without AMF. Five replicates were established per treatment. The experimental unit consisted of a pot with a blue agave plant, the roots were directly inoculated with the necessary amount of the corresponding inoculum containing 80 AMF spores. For the control without AMF, 10 g of sterile sand were used as inoculum. Black polyethylene bags (2 kg) were used as pots, each containing 1.5 kg of a sterile mixture of sand and soil (1:1 v/v) sterilized three times for 3 h at 120 °C and 1.03 kg cm<sup>-2</sup> of pressure. The pots were maintained

at field capacity with deionized water. The experiment was established under greenhouse conditions for 300 days after transplantation (dat).

**Evaluation of growth and microbiological variables**

At 90, 180, 270 and 300 dat, the number of leaves and the plant height were recorded. At the end of the experiment, the fresh and dry biomass of the plants were quantified using an analytical balance (Mettler Toledo AT200, Columbus, Ohio, USA). Only half of the total fresh biomass was treated, the rest was used to determine mycorrhizal colonization, head diameter (digital vernier), leaf area (LICOR LI-3100 planimeter, Lincoln, Nebraska, USA), root length (tape measure), and root and foliage volume (water displacement method in a graduated glass test tube). In regard to the microbiological variables, the percentage of root colonization was determined using the root clearing and staining technique (Phillips and Hayman, 1970), and quantified using the method proposed by McGonigle *et al.* (1990). A sample of 100 g of substrate was taken from each pot to determine the number of spores using the wet-sieving and decanting technique (Gerdemann and Nicolson, 1963) in combination with sucrose gradient centrifugation (Walker *et al.*, 1982).

**Data analysis**

The Dickson index (DI), which assesses plant quality based on morphological characteristics, was calculated using the following formula (Dickson *et al.*, 1960):

$$DI = \frac{Total\ DB}{\frac{PH}{HD} + \frac{LDB}{RDB}}$$

Where Total DB is the total plant dry biomass (g), PH is the plant height (cm), HD is the head diameter (mm), LDB is the leaf dry biomass (g) and RDB is the root dry biomass (g).

The relative mycorrhizal dependency index (RMDI), which assesses the increase in dry biomass due to the effect of mycorrhization, was calculated with the following formula:

$$RMDI\ (\%) = \left( \frac{DB^{+AMF} - DB^{-AMF}}{DB^{+AMF}} \right) \times 100$$

Where DB<sup>+AMF</sup> is the dry biomass of mycorrhizal plants (g), DB<sup>-AMF</sup> is the dry biomass of non-mycorrhizal plants (g).

The response variables (fresh and dry biomass of the plants, head diameter, leaf area, root length, root and foliage volume, percentage of root colonization, number of spores, Dickson index and relative mycorrhizal dependency index)

were subjected to analysis of variance; when the variables were significant, a multiple comparison of means test was performed using the Tukey test (P ≤ 0.05). These analyses were performed using the statistical software Statgraphics Centurion XV (StatPoint Inc., 2005).

**RESULTS**

**Blue agave plant growth was stimulated by AMF**

The statistical analysis showed high variability in the different response variables between replicates, which can be attributed to the origin of the bulbils used in the experiment, since not all came from the same mother plant and not all were the same age. There were no significant differences in the number of leaves between the different treatments at the first three samplings; however, from 270 dat there were significant differences between the inocula, and those differences remained until the end of the experiment. The fungal consortium Barranca de las Nueces (BN) induced the largest number of leaves, surpassing by three leaves on average the treatments without mycorrhization, the fungal consortium Las Campesinas (LC) and the commercial inoculum (MI) produced the lowest number of leaves (Figure 1).

Plant height did not show significant differences between treatments during the different samplings (Table 1). In general, the vegetative growth of agave plants inoculated with a native fungal consortium was significantly different (P ≤ 0.05) compared with non-inoculated agave plants (Figure 2), with significant increases (Tukey, P ≤ 0.05; Table 1) in fresh biomass, leaf area, root and foliage volume, number of leaves and head diameter.

Dry biomass, plant height and root length showed no significant differences between inoculated and non-inoculated plants. The results of the positive control (MI) for all evaluated variables were statistically different from those obtained for the treatments with AMF consortia; however, the growth traits of *A. tequilana* plants inoculated with the native fungal consortium Barranca de las Nueces (BN) had the highest values (Table 1).

Regarding the total volume of the plants, an increase was observed when they were treated with any of the AMF inocula, compared to non-inoculated plants, but this increase was only statistically significant in the agave plants inoculated with the fungal consortium Barranca de las Nueces (BN) (Figure 3), which induced by 243 % the volume increase. Even though the statistical analysis showed no significant differences between the fungal consortia CR, EL and AD, they did induce an increase in the total volume of agave plants by more than 100 %, compared

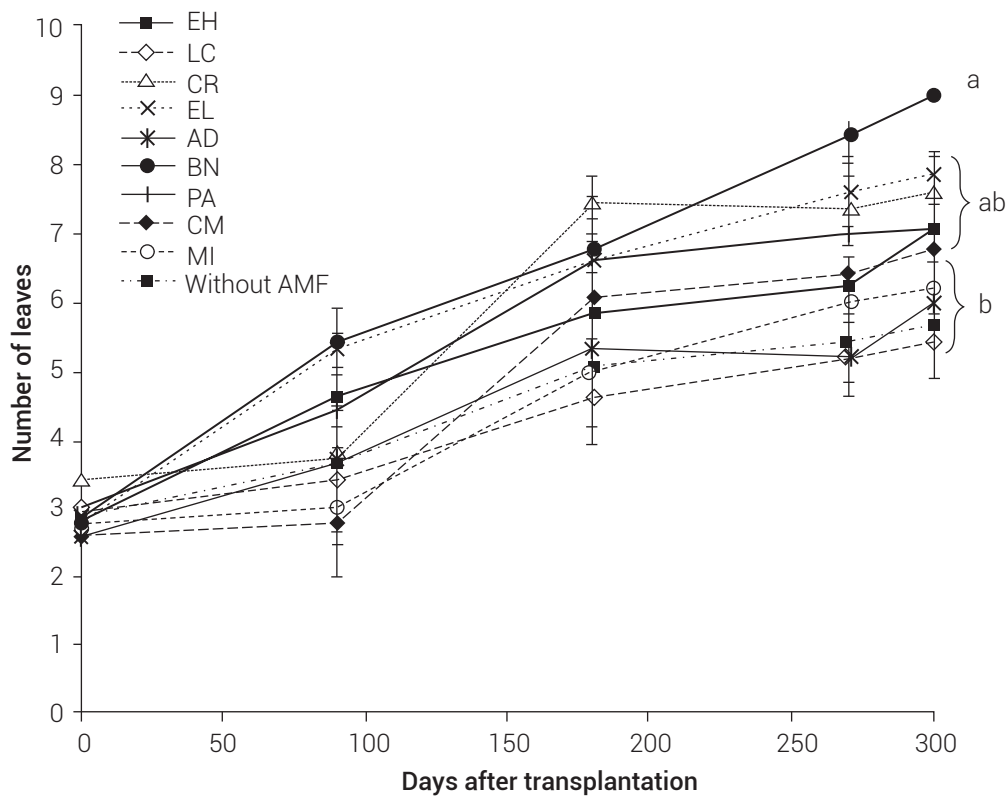
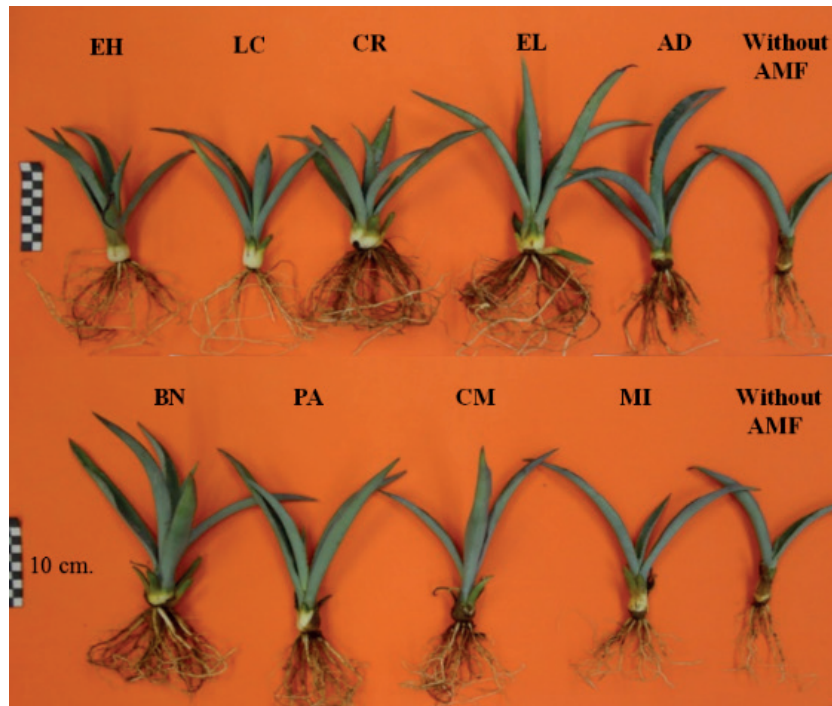


Figure 1. Effect of inoculation with arbuscular mycorrhizal fungi on the number of leaves of *Agave tequilana* plants grown under greenhouse conditions at 300 days after transplantation. Lines with the same letters are not statistically different (Tukey,  $P \leq 0.05$ ). The line on each point indicates the  $\pm$  standard error.

Table 1. Effect of inoculation with native arbuscular mycorrhizal fungi (AMF) on the growth of *Agave tequilana* plants grown under greenhouse conditions at 300 days after transplantation.

Inocula AMF <sup>†</sup>	Response variables										
	HD (mm)	H (cm)	TFB (g)	TDB (g)	LA (cm <sup>2</sup> )	RL (cm)	RV (mL)	FV (mL)	Spores	RMDI (%)	DI
EH	25.1 ab	13.5 a	44.3 ab	6.8 a	95.9 ab	28.0 a	11.4 ab	36.4 b	172 ab	43 ab	2.4
LC	19.2 b	12.9 a	24.9 b	3.6 a	55.8 b	22.3 a	6.4 b	26.0 b	108 ab	<b>-13 b</b>	1.2
CR	23.0 ab	12.8 a	39.9 ab	6.4 a	85.2 ab	<b>31.9 a</b>	13.6 ab	43.2 ab	138 ab	31 ab	2.3
EL	22.5 ab	13.0 a	43.4 ab	7.5 a	95.1 ab	22.9 a	11.4 ab	36.4 b	136 ab	21 ab	2.8
AD	22.5 ab	13.8 a	48.3 ab	6.8 a	99.8 ab	22.2 a	10.4 ab	45.6 ab	200 ab	38 ab	2.4
BN	<b>29.1 a</b>	15.9 a	<b>66.5 a</b>	<b>10.4 a</b>	<b>140.2 a</b>	23.0 a	<b>21.0 a</b>	<b>78.0 a</b>	<b>244 a</b>	<b>62 a</b>	<b>3.6</b>
PA	24.9 ab	<b>16.4 a</b>	44.4 ab	7.1 a	92.1 ab	27.0 a	10.6 ab	31.0 b	239 a	43 ab	2.1
CM	20.8 ab	12.9 a	30.9 ab	4.9 a	68.8 ab	22.7 a	8.0 b	32.6 b	120 ab	11 ab	1.7
MI	21.8 ab	13.3 a	42.8 ab	7.5 a	95.1 ab	27.2 a	10.8 ab	37.4 b	207 ab	39 ab	3.4
WAMF	<b>18.9 b</b>	<b>11.6 a</b>	<b>23.2 b</b>	<b>3.7 a</b>	<b>52.7 b</b>	<b>19.3 a</b>	<b>6.6 b</b>	<b>22.2 b</b>	<b>49 b</b>	---	<b>1.1</b>

HD: head diameter, H: plant height, TFB: total fresh biomass, TDB: total dry biomass, LA: leaf area, RL: root length, RV: root volume, FV: foliage volume, Spores: spores in 100 g of substrate, RMDI: relative mycorrhizal dependency index, DI: dickson index. <sup>†</sup>EH: El Huizachal, LC: Las Campesinas, CR: Rancho Carlos Rojas, EL: El Limón, AD: Agua Dulce, PA: Paso Ancho, BN: Barranca de las Nueces, CM: Cerro del Metate, MI: Mycorrhiza INIFAP<sup>®</sup> based on *R. intraradices*, WAMF: without arbuscular mycorrhizal fungi. Means with the same letters are not statistically different (Tukey,  $P \leq 0.05$ ). The highest and lowest values of each response variable are shown in bold.



**Figure 2. Aspect of *Agave tequilana* plants inoculated with different arbuscular mycorrhizal fungi (AMF) under greenhouse conditions 300 days after transplantation.**

to the non-AMF treatment. The commercial inoculum MI increased by itself the total volume of the plants by 47 % (Figure 3).

**AMF colonization and propagation in agave as a host plant**

Regarding the percentage of mycorrhizal colonization and the production of spores in the growth medium, no significant differences were found between AMF inocula. The colonization percentages ranged between 30 and 49 % (Figure 4), while spore production ranged between 107 and 243 per 100 g of substrate (Table 1).

**Mycorrhizal dependency and blue agave plant quality index**

The mycorrhizal dependency of the blue agave plants showed high variability among the different inocula; there were significant differences only between the fungal consortia BN and LC (Table 1). Agave plants inoculated with the BN consortium showed a 62 % increase in dry biomass compared to agave plants without AMF, whereas the agave plants inoculated with the LC consortium showed a 13 % decrease, accumulating less dry biomass than agave plants without mycorrhization, despite having a mycorrhizal colonization percentage of 35 %.

Regarding the Dickson index, which indicates the quality of a plant as a function of its growth parameters (the higher

the value of the index, the higher the quality of the plant), the different inocula produced plants of different quality (Table 1). Most of the plants inoculated with an AMF had higher quality than the agave plants without mycorrhization (Figure 2), except for the plants inoculated with the fungal consortia CM and LC, which obtained a quality value similar to that of the plants without AMF. Plants inoculated with the BN consortium reached the highest plant quality level, corresponding to the highest values in the different growth variables evaluated.

**DISCUSSION**

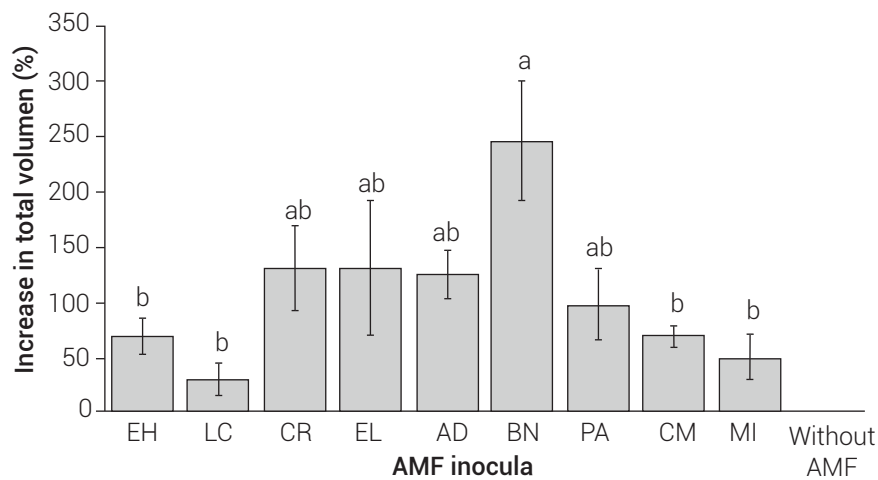
It has been demonstrated in many plant species [papaya (*Carica papaya*), maize, pepper (*Capsicum annuum*), etc.] that AMF promote vegetative growth through various mechanisms, perhaps the most important is the improvement of plant nutrition (Quiñones-Aguilar et al., 2014) by increasing the acquisition of nutrients through the activity of the hyphae of mycorrhizal fungi (Smith and Read, 2008). In the present study all the AMF consortia evaluated promoted vegetative growth of *A. tequilana*, and in some cases, the increase was statistically significant compared to non-mycorrhized agave plants. Similar results have been reported for *A. tequilana* (Ruiz et al., 2011), *A. inaequidens* (Quiñones-Aguilar et al., 2016), *A. cupreata* (Trinidad-Cruz et al., 2017a) and other agave species such as *A. deserti* (Cui and Nobel, 1992), *A. angustifolia* (Robles-Martínez et al., 2013) and other species with CAM metabolism such

as *Ferocactus acanthodes*, *Opuntia ficus-indica* (Cui and Nobel, 1992) and *O. albicarpa* (Estrada-Luna and Davies, 2008), among others. This increase in growth could be attributed in part to an increase in the photosynthetic rate of agave plants or to an improvement in the acquisition of water and nutrients due to the activity of mycorrhizae, which act as an extension of the roots (Fernández-Lizarazo and Moreno-Fonseca, 2016; Pimienta-Barrios *et al.*, 2009; Smith and Read, 2008).

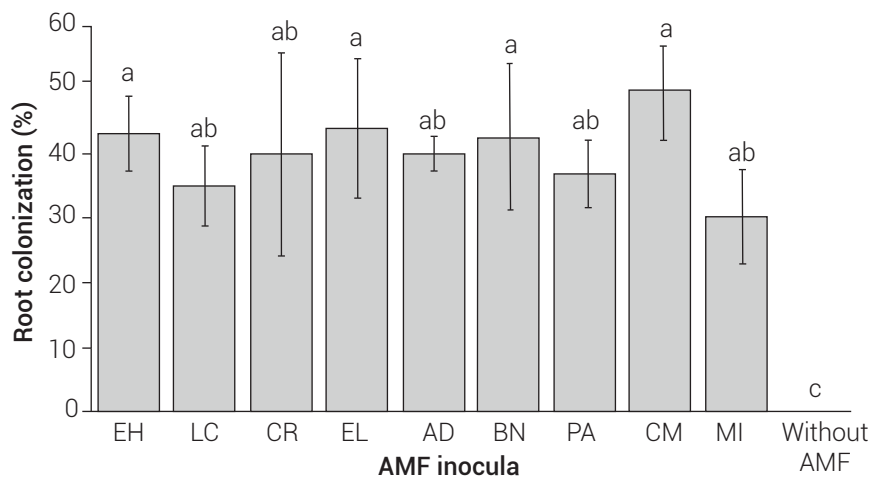
Cui and Nobel (1992) studied the nutritional status, water acquisition capacity and gas exchange capacity of *A. deserti* plants inoculated with AMF and their results showed an increase in the phosphorus content in roots and leaves, higher hydraulic conductivity in roots and

better CO<sub>2</sub> absorption, which was reflected in the larger size of mycorrhized plants compared to non-mycorrhized. The fact that at least one native fungal consortium induced a higher increase in some of the evaluated growth traits compared to the commercial inoculum (MI) could be related to the diversity of AMF species present in the native consortia, as indicated by Trinidad-Cruz *et al.* (2017b).

Similar results were reported by Quiñones-Aguilar *et al.* (2016) with *A. inaequidens* and by Trinidad-Cruz *et al.* (2017a) with *A. cupreata* inoculated with native consortia. In this regard, Gavito and Varela (1995) and Bashan *et al.* (2000) reported that inocula consisting of more than one AMF species provide higher benefits to their hosts. It is also known that native mycorrhizal consortia tend



**Figure 3.** Increase in the total volume of *Agave tequilana* plants inoculated with different arbuscular mycorrhizal fungi (AMF), compared to plants without AMF 300 days after inoculation. Bars with the same letters are not statistically different (Tukey,  $P \leq 0.05$ ); the line at the upper part of the bar indicates the  $\pm$  standard error.



**Figure 4.** Root colonization in *Agave tequilana* plants inoculated with different arbuscular mycorrhizal fungi (AMF) 300 days after inoculation. Bars with the same letters are not statistically different (Tukey,  $P \leq 0.05$ ); the line at the upper part of the bar indicates the  $\pm$  standard error.

to be more effective than consortia constituted by exotic species or by a single species (Gavito and Varela, 1995; Requena *et al.*, 2001); the reason for this is that fungi adapt to certain specific environmental conditions and introducing them into different environments can cause some maladjustments (Hodge, 2000).

Robles-Martínez *et al.* (2013) evaluated the effect of different native AMF consortia on *A. angustifolia* and found that at least one native consortium promoted the increase of aerial biomass compared to non-inoculated plants. Quiñones-Aguilar *et al.* (2016) and Trinidad-Cruz *et al.* (2017a) studied native AMF consortia isolated from the rhizosphere of *A. cupreata* inoculated into *A. inaequidens* and *A. cupreata*. Mycorrhized agave plants with native consortia showed higher growth, plant quality and resistance to *Fusarium oxysporum* while plants of *A. angustifolia* inoculated with AMF showed an increase in the number and length of roots; however, Martínez-López *et al.* (2009) found no differences in the increase of biomass induced by the inoculation of *R. intraradices* strains of different origin (commercial and native) into *A. americana* plants.

The discrepancy between the results of this study and those reported by Martínez-López *et al.* (2009) may be explained by the fact that in the present study, the native inocula used were consortia, rather than single-species inocula, and the interaction between one or more species present in the consortia could yield better results, compared to commercial strains (Robles-Martínez *et al.*, 2013). Even though there is no plant-fungus specificity, the effect of each fungus on its host can vary; it can promote growth, increase nutrient and water uptake capacity, or induce resistance to biotic or abiotic stresses, among other benefits (Smith and Gianinazzi-Pearson, 1988); thus, fungi have differential effectiveness (Hodge, 2000; Quiñones-Aguilar *et al.*, 2016; Robles-Martínez *et al.*, 2013).

Another possible explanation of the increased growth of agave plants inoculated with native consortia is that, due to the way in which the evaluated consortia were propagated (trap pots), other beneficial microorganisms, such as plant growth-promoting rhizobacteria, likely multiplied and contributed to promote the growth of the agave plants. De la Torre-Ruiz *et al.* (2016), studying *A. americana* plants inoculated with *Rhizobium daejeonense*, *Acinetobacter calcoaceticus* and *Pseudomonas mosselii*, observed a significant effect ( $P \leq 0.05$ ) on growth and sugar content. Ruiz *et al.* (2011), working with *A. tequilana* seedlings inoculated with *G. intraradices* and *Gluconacetobacter diazotrophicus*, reported a higher dry weight compared to treatments where more than one bacterial strain were inoculated.

In the present study, there might have been positive effect caused by the interaction between AMF and rhizobacteria in the treatments with native inocula, but this effect would have been unlikely to occur in plants without AMF because the growth medium was previously sterilized, nor could it have occurred in the treatment with the monospecific inoculum (MI).

Regarding the microbiological variables, the results obtained here showed a mycorrhizal colonization percentage of 49 %. In another study on *A. inaequidens* inoculated with native AMF, Quiñones-Aguilar *et al.* (2016) reported mycorrhizal colonization percentages ranging from 20 to 45 %. Pimienta-Barrios *et al.* (2009) reported colonization percentages of more than 70 % in young *A. tequilana* plants inoculated with AMF (*G. fasciculatum* and *G. intraradices*); Cui and Nobel (1992) found mycorrhizal colonization percentages of up to 64 % in *A. desertii* plants inoculated with a native AMF consortium (*G. epigaeum*, *G. fasciculatum* and three other species of *Glomus* spp.). In contrast, Robles-Martínez *et al.* (2013) found colonization percentages of 35 % in *A. angustifolia* plants inoculated with a native AMF consortium native to the Tlacolula district in the state of Oaxaca.

The observed differences in mycorrhizal colonization may be explained by the conditions and duration of the experiments, the AMF species used, the origin of the inocula, and the affinity of the host for the AMF species. Daniels Hetrick and Bloom (1986) evaluated the influence of different plant hosts as marigold (*Calendula officinalis*), red clover (*Trifolium pratense*), sorghum, tomato (*Solanum lycopersicum*) and asparagus (*Asparagus officinalis*) on the production of AMF spores (*G. fasciculatum*, *G. macrocarpum* and *G. mosseae*) and on mycorrhizal colonization; these authors mentioned that there may be different affinity levels between hosts and AMF, even at the lowest amount of inoculated spores. Likewise, Ochoa-Meza *et al.* (2009) reported colonization percentages of up to 24.9 % in *A. angustifolia* plants grown under field conditions and 32 AMF spore morphotypes associated with the rhizosphere of these plants, corresponding to the following genera: *Glomus* (17), *Acaulospora* (10), *Archeospora* (2), *Diversispora* (1) and *Pacispora* (2).

The inocula used in the present study also contained a great diversity of AMF species (10 on average) associated with the rhizosphere (Trinidad-Cruz *et al.*, 2017a), which might indicate that the roots of agave plants are likely colonized by several species at the same time. The results of this study showed a spore density of up to 243 spores in 100 g of substrate, a relatively lower value than those reported by Ochoa-Meza *et al.* (2009), who found between 400 and 700 spores per 100 g of *A. angustifolia* rhizosphere

soil in the mountainous area of Sonora, Mexico.

The relatively low density of spores found in this study could be due to the conditions of the growth medium, the environment, the age of the plants and the AMF species used. It has also been shown that sporulation is associated with the propagation substrate or growth medium (Douds and Schenck, 1990; Ferrera *et al.*, 1993). Camacho-Camacho *et al.* (2013) evaluated the efficiency of different substrates for obtaining AMF spores from native fungal consortia under greenhouse conditions and reported that the production of spores varied according to the substrate mixture used. A substrate composed of a mixture of sand, peat moss and compost, with sorghum as trap plant, yielded the largest number of spores (866 in 100 g of substrate), while the lowest number was obtained with sand as the substrate (98 spores in 100 g of substrate). Based on these results, the authors indicated that organic matter probably stimulates the production of AMF spores. Since the growth medium used in this study was poor in both organic matter (0.98 %) and nutrients (5, 15 and 35 ppm N, P and K respectively), this could explain the low levels of spore production.

The relative mycorrhizal dependency index showed values from 11 to 62 %; this variation can be attributed to the diversity of AMF species in each consortium and their affinity for the corresponding plant species. Some plant species are heavily reliant on mycorrhizals. Quiñones-Aguilar *et al.* (2014) report a RMDI of up to 99 % for papaya plants inoculated with *Glomus* sp. In agave plants, a wide range of mycorrhizal colonization values have been reported, depending on the growing conditions, the AMF species used and the variety of each agave plant, among other factors; however, if the colonization values found in this study are compared to those reported by Pimienta-Barrios *et al.* (2009) for the same species (less than 60 %) and by Quiñones-Aguilar *et al.* (2016) for *A. inaequidens* (more than 79 %), the mycorrhizal dependency observed in the present study could be classified from medium to low.

In this study agave plants inoculated with the LC consortium showed a negative RMDI, indicating that agave plants inoculated with this particular consortium yielded less dry biomass than non-inoculated agave plants. Smith and Smith (2012) and Johnson *et al.* (1997) pointed out that in some cases mycorrhizal can act as a parasite, taking carbon compounds from plants while providing them with few benefits, reflecting that their growth is equal to or less than that of non-mycorrhized plants. This could have occurred in this study at agave plants inoculated with the LC consortium.

## CONCLUSIONS

Inoculation with native AMF consortia obtained from the rhizosphere of *A. cupreata* promoted the growth of *A. tequilana* plants compared to non-inoculated plants. The native consortium BN (*Ambispora appendicula* (Spain, Sieverding & Schenck) C. Walker, *Acaulospora denticulata* Sieverding & S. Toro, *Acaulospora scrobiculata* Trappe, *Acaulospora spinosa* C. Walker & Trappe, *Acaulospora* sp. 4, *Claroideoglossum etunicatum* (W. N. Becker & Gerd.) C. Walker & A. Schüßler, *Diversispora aurantia* (Błaszk., Blanke, Renker & Buscot) C. Walker & A. Schüßler, *Glomus deserticola* Trappe, Bloss & J. A. Menge, *Glomus* sp. 1, *Rhizophagus diaphanum* (C. Cano & Y. Dalpé) C. Walker & A. Schüßler) was associated with the highest vegetative growth and the highest quality of plants. Native LC consortium could be acting as a parasite, since agave plants inoculated with this consortium did not show an increase in growth. The use of mycorrhizal consortia on *A. tequilana* plants in a nursery could represent an alternative to the use of chemical fertilizers for the production of the raw material for tequila.

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