



Survival and growth of

Jacaratia mexicana

seedlings inoculated with arbuscular mycorrhizal fungi in a tropical dry forest

Supervivencia y crecimiento de plántulas de *Jacaratia mexicana* inoculadas con hongos micorrízico arbusculares dentro de un bosque tropical seco

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ABSTRACT

Jacaratia mexicana is not only an endemic and typical tropical dry forest tree of Mexico, it is considered as a direct ancestor of the papaya (*Carica papaya*). Locally it is mainly used in traditional medicine, for human food or for feeding backyard animals (forage plant), but its use value is very restricted or even unknown. Nevertheless, various abiotic and anthropogenic pressures in its Mexican habitat are causing populations of this tree to decline alarmingly. Arbuscular mycorrhizal fungi (AMF) are microorganisms that have an important role for the regeneration of tree species by increasing their ability to absorb water and nutrients. The aim of this study was to determine the effect of AMF on growth and survival of seedlings of *J. mexicana* transplanted within a fragmented area of the remaining dry forest located in the central portion of the state of Veracruz. We measured height, stem diameter, number of leaves, percent seedling survival and mycorrhizal colonization. Results showed increases in all growth-related variables when seedlings were inoculated with AMF. The results suggest that microsymbionts can play a critical role for the regeneration of *J. mexicana* and possibly other species of tree within the dry forest, especially helping this endemic species that is currently surviving in a fragmented environment under unfavorable conditions of low availability of water and nutrients.

KEYWORDS: endangered ecosystem, endemic tree, regeneration of species, tropical dry forest, Veracruz.

RESUMEN

Jacaratia mexicana no es solo un árbol endémico y típico de los bosques tropicales secos de México, sino que se le considera como ancestro evolutivo del papayo (*Carica papaya*). Se le aprecia en la medicina tradicional, para alimentación del ser humano o cría de animales de traspatio (planta forrajera), pero su valor de uso es muy restringido o incluso desconocido. Sin embargo, presiones abióticas y antropogénicas de su hábitat natural están causando una alarmante reducción de las poblaciones naturales de esta Caricaceae. Los hongos micorrízicos arbusculares (HMA) son microorganismos que juegan un papel importante en la regeneración de especies debido al aumento de su capacidad para absorber agua y nutrientes. El objetivo de este estudio fue determinar el efecto de los HMA sobre el crecimiento y supervivencia de plántulas de *J. mexicana* trasplantadas dentro de un área fragmentada del bosque tropical seco ubicado en la parte central del estado de Veracruz. Se determinó en este estudio la altura, diámetro, número de hojas, porcentaje de supervivencia y colonización micorrízica. Los resultados muestran incrementos en todas las variables relacionadas con el crecimiento cuando las plántulas fueron inoculadas con HMA. Los resultados sugieren que estos microsimbiontes pueden desempeñar un papel fundamental para el establecimiento de árboles como *J. mexicana* y posiblemente de otros componentes dentro del bosque tropical seco, los cuales actualmente sobreviven en un entorno fragmentado y bajo condiciones desfavorables de baja disponibilidad de agua y nutrientes.

PALABRAS CLAVE: ecosistema en peligro, árbol endémico, regeneración de especies, bosque tropical seco, Veracruz.

INTRODUCTION

Jacaratia mexicana is an endemic tree of Mexico that is representative of species living in the tropical dry forest (Arias *et al.*, 2012). Its geographical distribution comprises southern and central México (states of Campeche, Chiapas, Colima, Guerrero, Michoacán, Morelos, Oaxaca, Yucatán and Veracruz); in Central America it can be found in Nicaragua and El Salvador (Moreno, 1980; Hernández-Alvarez *et al.*, 2006). In the state of Veracruz the tree can be located in Misantla, Alvarado and near the port of Veracruz (Lascurain *et al.*, 2010). The tree is very popular and is well known and belongs to the Caricaceae family (Ramos-Martínez *et al.*, 2012). It is used locally for food and is also used medicinally, for feeding backyard animals (forage plant), firewood (fuel for cooking), as well as having pharmaceutical and agroindustrial uses (Zulueta, 2003). Nowadays, its silvopastoral potentiality is recognized (Flores and Bautista, 2012). Despite its many and countless utilitarian qualities, populations of *J. mexicana* are at risk because of constant human pressures which unfortunately are accelerating the destruction of vast areas of tropical dry forest (Trejo and Dirzo, 2000) putting at risk the natural populations of this tree (Moreno-Casasola and Paradowska, 2009). Furthermore, the climatic and soil factors prevailing within the tropical dry forest restrict the growth and survival of the tree, mainly because of a decrease in water and nutrient availability, causing its natural regeneration rate to be low. Its loss contributes to the loss of ecosystem services that affect vegetation establishment including the deterioration of ground quality or erosion as well as soil macro components (Meave *et al.*, 2012).

Arbuscular mycorrhizal fungi (AMF) are organisms that have a very important role in the functionality and composition of plant communities. AMF already have been used successfully in the regeneration of some tree species of the dry tropics (Zulueta, 2003; Huante *et al.*, 2012). In Mexico, they have been recorded in all ecosystems, from coniferous forests, hills, sand coastlines, to very humid environments like rainforests, as well as present in arid environments such as deserts and tropical dry forests.

Among the main functions of AMF in plants is to increase absorption of water and nutrients, especially those elements limiting plant growth such as phosphorus, sodium, boron, copper, zinc, potassium, iron, magnesium and calcium (Cardoso and Kuyper, 2006; Meding and Zasoski, 2008; Watts-Williams and Cavagnaro, 2014). AMF in plants also have an important role protecting plants against attack of soil pathogens, as well as improving tolerance to drought, heavy metals and also reducing damage caused by salinity (Jung *et al.*, 2012; Porcel *et al.*, 2012; Wilson *et al.*, 2012).

OBJECTIVES

There are few records of the effect of AMF in the regeneration of forest species in the tropical dry forest, thus the objective of the present study was to analyze the effects of mycorrhiza formation in *J. mexicana* seedlings on survival and growth of this specie in its natural habitat.

MATERIALS AND METHODS

Study site

The study was conducted within a fragment of tropical dry forest located in an area called La Bandera (19°27'50" N and 96°33'12" W) in the municipality of Actopan, Veracruz, Mexico, owned by the Facultad de Ciencias Agrícolas de la Universidad Veracruzana, *Campus* Xalapa (FCA-UV). There, a humid tropical climate prevails ($Aw_0[w]$ [i]gw") with an average annual temperature of 24.8 °C and rainfall of around 900 mm a year. The soil type of the study area is a rendzic leptosol, clayey, stony, excessively drained and shallow, containing 8.56% organic matter, low phosphorus (6.78 ppm¹), potassium (0.56 ppm), sodium (1 ppm) and high calcium (60 ppm) and magnesium (5.6 ppm) (Castañeda, 2000).

Mycorrhizal fungi inoculum

The AMF inoculum was provided by the Laboratorio de Organismos Beneficos of the FCA-UV and consisted of

¹ Soil available P in the study area was determined according to Olsen method suggested by SARH (1978).



Acaulospora morrowiae, *A. scrobiculata*, *A. spinosa*, *Claroideoglossum etunicatum*, *Funneliformis mosseae*, *F. geosporus*, *Gigaspora rosea*, *Gi. decipiens*, *Glomus aggregatum*, *G. macrocarpum*, *G. intraradices* and *Scutellospora pellucida*.

Seed collection

Within the study area, fruits were collected of wild specimens of *J. mexicana*. The seeds collected from the fruits were washed with sterile distilled water to remove the sarcotest then allowed to dry for 7 days on sterile paper towels under shade at room temperature. Seeds were disinfected with sodium hypochlorite at 10% for 15 min, rinsed with sterile distilled water and placed in containers of high density polyethylene (35 cm x 48.5 cm x 13.5 cm) with perforations in the base for drainage and aeration. The containers were then maintained in an incubator at low temperature (20 °C) and constant humidity until seedling emergence.

Preparation of the substrate

A substrate containing a mixture of soil, sand and tepezil² 3:1.5:3 (v/v) was autoclaved for 1 h at 120 °C and then aired for 3 consecutive days. We filled black plastic bags of 10 cm x 20 cm and containers of high density polyethylene (35 cm x 48.5 cm x 13.5 cm) with the mixture.

Plant culture and mycorrhizal inoculation

Once the seedlings had their first pair of true leaves and a height of 4 cm, they were planted one per container above 6 g of AMF root inoculum. The bags remained for fifteen days in the nursery of the FCA-UV, and then were subsequently transplanted with a 30 cm distance between seedlings and furrows. The distance is long enough so that the roots of each seedling did not intermingle during the treatment period. In addition, the experiment was performed within the tropical dry forest during the dry season, therefore the seedlings root growth is limited because of water scarcity. Fortnightly, height, stem diameter, leaf

number, and survival percentage were quantified, and at the end of the experiment (75 days after inoculation, DAI) the percentage of mycorrhizal colonization was determined. Fifteen seedlings of *J. mexicana* were used for each treatment and the experiment was repeated twice.

Mycorrhizal colonization

At 75 DAI the extracted roots of five plants from the field were fixed in FAA (formalin: acetic acid: alcohol) and were stained using the method proposed by Phillips and Hayman (1970). Subsequently, the percentage of mycorrhizal colonization was determined following the grid-line intersection method described by Giovannetti and Mosse (1980).

Micrographs from scanning electron microscope

To observe the mycorrhizal colonization of *J. Mexicana*, samples of roots were washed with saline solution and fixed by immersion in 2.5% glutaraldehyde in phosphate buffer at pH 7.4 for 2 h at 4 °C. The preparation methodology of Prasad *et al.* (2013) was used, and the specimens were observed under a scanning electron microscope (Hitachi S-3000N, San Jose, CA, USA).

Statistical analysis

We used the statistical package Statistica v. 10.0 for Windows (StatSoft, 2011). For analysis of data a one-way repeated measures ANOVA result was used for comparison of means of each variable tested since there were only two treatments compared at a time (at a significance level $P < 0.05$).

RESULTS AND DISCUSSION

We observed a positive effect on growth and survival for the plants of *J. mexicana* inoculated with AMF. In terms of height, AMF plants showed an increase of 56.92% compared to plants that were not inoculated; while in stem diameter and number of leaves, AMF plants showed an increase of 123.24% and 102.56%, respectively, compared to the treatment without the microorganisms (Table 1). Of the total seedlings of *J. mexicana* transplanted into

² Tepezil is a sandy-lite inert mineral of fine grain, light weight and low cost.

the tropical dry forest, survival with AMF was 78.58%, and without AMF it was 54.29% at 75 DAI.

The percentage of root colonization was determined to be 53.33%, while in the roots of non-inoculated seedlings; there was no evidence of AMF (Fig. 1). The effect of promoting the growth of AMF to *J. mexicana* is related primarily to an increase in nutrient absorption and water, which are essential for plants that live within the tropical dry forest (Gehring, 2003; Grilli *et al.*, 2013). Zulueta (2003) determined that *J. mexicana* is a species capable of associating with AMF, which may increase its growth under controlled conditions. The association between plants of *J. mexicana* and AMF was instrumental in increasing survival in the field. AMF can alter water movement in host plants and can modify the morphology of root systems (Sharma and Yadav, 2013), allowing plants to tolerate environments where there is limited availability of water and nutrients (Gerhardt, 1998; Pringle *et al.*, 2011).

AMF plays a fundamental role for the regeneration of different tree species that compose dry forests and other habitats (Ceccon and Hernández, 2009; Gómez-Romero

et al., 2013). *J. mexicana* is a species of which the potential for conservation tends to decrease with the relentless destruction of its habitat. The search for alternatives for propagation and improvement of physiological processes aimed at increasing the survival of this species, which naturally has very low rate regeneration, makes the improvement of growth by AMF important in the conservation of this Mexican tree species (Zulueta *et al.*, 2010). *J. mexicana* seedlings that were not inoculated with AMF showed increased mortality and decreased development mainly because of severe water stress that predominated in the study area as is typical of tropical dry forest. Seedlings without AMF have limited ability to absorb water (Tian *et al.*, 2013) and nutrients (primarily phosphorus) which are limiting factors for plant growth (Esmailifar, 2013).

AMF colonization of the roots of seedlings *J. mexicana* was important for their growth and survival. The soil moisture content is a key factor in AMF development and accordingly, their chances for effecting root colonization (Huang *et al.*, 2011). While there are studies that relate the role of mycorrhizal symbiosis and survival strategies deployed by various tree species under conditions of

TABLE 1. Growth variables of *Jacaratia mexicana* with and without arbuscular mycorrhizal fungi (AMF) in the tropical dry forest.

Treatment	DAI*				
	15	30	45	60	75
Foliage height (cm)					
Jm ^α	5.58±0.34a [‡]	6.34±0.27a	6.41±0.82a	6.68±0.74a	7.29±0.95a
Jm+AMF ^β	5.54±0.32a	6.58±0.30a	8.46±0.75b	10.01±0.91b	11.44±0.85b
Diameter (mm)					
Jm	2.21±0.41a	2.81±0.44a	2.89±0.64a	3.56±0.80a	4.08±0.82a
Jm+AMF	2.28±0.42a	2.91±0.38a	3.98±0.34b	4.51±0.91b	5.64±0.62b
Number of leaves					
Jm	2.2±0.59a	2.5±0.71a	3.1±0.74a	3.5±0.71a	3.9±0.84a
Jm+AMF	2.2±0.52a	2.4±0.65a	3.5±0.52a	4.8±0.42b	5.4±0.87b

*DAI days after inoculation with AMF (Arbuscular Mycorrhizal Fungi)

^α *J. mexicana* (Jm) plants not inoculated with AMF

^β *J. mexicana* (Jm) plants inoculated with AMF prior to transplant to the field

[‡] Mean values followed by the same letter in the same column are not significantly different ($P > 0.05$)

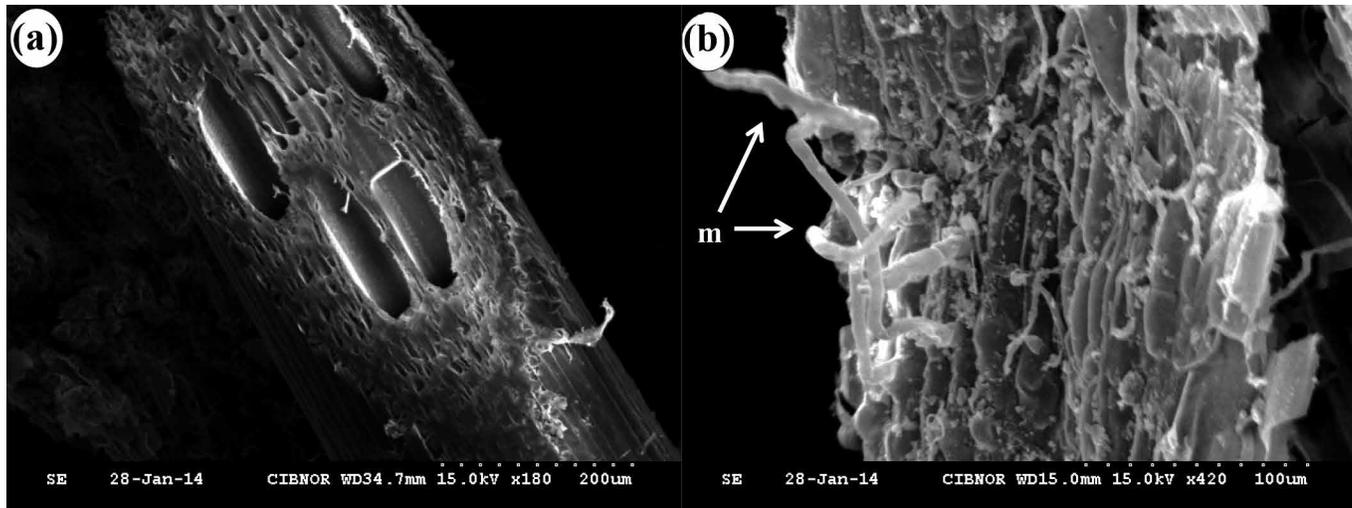


FIGURE 1. Scanning electron microscopy of *Jacaratia mexicana* seedlings roots. (a) Root of *J. mexicana* without arbuscular mycorrhizal fungi (AMF) and, (b) with AMF, m = mycelium.

severe water stress (Sudova, 2009; Grilli *et al.*, 2012), at present there are few studies conducted in tropical dry forest with AMF in Mexico. The seedlings of *J. mexicana* without AMF that were transplanted into field showed no colonization by native AMF as commonly observed in these studies (Allen *et al.*, 2005). The dry season that prevailed throughout the experiment in the tropical dry forest (unusual in this area in the study year) had an influence on the ability to colonize the native AMF to *J. mexicana* plants. Studies have found that the activity, survival and life cycle of the AMF is influenced by soil moisture (Anderson *et al.*, 1984; Camargo-Ricalde and Esperón-Rodríguez, 2005). The lack of water in dry seasons significantly decreases the capacity of the AMF to colonize the roots of host plants (Moreira-Souza *et al.*, 2003; Guadarrama *et al.*, 2014).

CONCLUSIONS

J. mexicana is tree that is considered to be the direct ancestor of the papaya tree. Populations of the tree are presently at risk of disappearing from its native habitat. This ancestral tree has been used historically by local populations for wood and medicinally. We found that seedlings pre-inoculated with AMF had improved survival after

transplant to a dry forest site. In addition, our results show increases in all growth related variables with AMF inoculation. These results suggest that microsymbionts play a critical role in the regeneration of tree species within the dry forest, at least for *J. mexicana*.

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