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MYCORRHIZAL DEPENDENCY OF AVOCADO AT DIFFERENT LEVELS OF SOIL SOLUTION PHOSPHORUS‡

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ABSTRACT

A greenhouse experiment was carried out to determine the mycorrhizal dependency of avocado (Persea americana) at different levels of soil solution P. The use of arbuscular mycorrhizal fungi (AMF) is a biological alternative to increase plant Pi uptake and growth and reduce the excessive use of P fertilizers. However, in order to incorportae the use of AMF in avocado crops is necessary to know the degree of mycorrhizal dependency (MD) of this plant species and the interaction between the AMF inoculation and the soil P bioavailability. With this purpose avocado seedlings were grown for 150 days in a substrate either inoculated or not with Glomus fasciculatum at three concentrations of soil solution P (0.002, 0.02 and 0.2 mg L^{-1}). The results indicated that avocado cv. Villagorgona was moderately dependent (MD= 25.8%) on the mycorrhizal association. The mycorrhizal inoculation significantly ($P<0.05$) increased shoot dry weight at 0.002 and 0.02 mg L^{-1} by 48 and 35%, respectively, but this decreased at 0.2 mg L^{-1} by 26%. The mycorrhizal colonization decreased when soil solution P increased. These results suggest that avocado seedlings would benefit considerably from AMF inoculation at low concentration of soil available P.

DEPENDENCIA MICORRIZAL DE AGUACATE A DIFERENTES NIVELES DE FOSFORO EN LA SOLUCION DEL SUELO

Palabras claves:

Fosfato
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Persea americana
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RESUMEN

Se realizó un experimento de invernadero para determinar la dependencia micorrizal de plántulas de aguacate (Persea americana) a diferentes niveles de fósforo (P) en la solución del suelo. El uso de hongos micorrizo arbusculares es una alternativa biológica para incrementar la absorción de P y el crecimiento de las plantas y así reducir el uso excesivo de fertilizantes fosfóricos. Sin embargo, para incorporar el uso de estos hongos en cultivos comerciales de aguacate es necesario conocer el grado de dependencia micorrizal (MD) de estas plantas y determinar el efecto de la interacción entre la inoculación micorrizal y la biodisponibilidad de P en el suelo. Con éste propósito plántulas de aguacate cv. Villagorgona crecieron durante 150 días en un sustrato ya sea inoculado o no-inoculado con un inóculo crudo del hongo micorrizal Glomus fasciculatum a tres concentraciones de P en la solución del suelo (0.002, 0.02 y 0.2 mg L^{-1}). Los resultados indicaron que las plántulas fueron moderadamente dependientes (MD= 25.8%) de la asociación micorrizal. La inoculación micorrizal significativamente ($P<0.05$) incrementó la masa seca aérea a 0.002 y 0.02 mg L^{-1} en 48 y 35%, respectivamente; por el contrario, ésta decreció en 26% a la concentración de P disponible de 0.2 mg L^{-1}. La colonización micorrizal decreció cuando el P en la solución del suelo aumentó. Estos resultados sugieren que las plántulas de aguacate se pueden beneficiar considerablemente si se inocula el sustrato de crecimiento con hongos micorrizales cuando la concentración de P biodisponible sean baja (≤0.02 mg L^{-1}).

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INTRODUCTION

The growth and development of avocado (*Persea americana*) seedlings is impaired by the low soil availability of phosphorus (P) in the andean mountains of Colombia (Montoya 2007). The soils of this region are derived from volcanic ashes and characterized by the dominance of allophane in the clay fraction (IGAC 1991). This mineral exhibits a high capacity to sorb P onto its surface (Shoji *et al.* 1993; Osorio 2008), for this reason it is necessary to apply large amounts of P fertilizers, which increases production costs and environmental concerns (Havlin *et al*. 1999).

The use of arbuscular mycorrhizal fungi (AMF) can enhance plant P uptake and growth of several plant species (Brundrett *et al*. 1996; Alarcon and Ferrera-Cerrato, 1999) including avocado (Hernandez 2001; Menge *et al.* 1978; Ginsburg and Avizohar-Hersheson, 1965). However, little is known about the mycorrhizal dependency (MD) of local cultivars of avocado commonly grown in Colombia and the interaction of mycorrhizal inoculation and soil P availability in these soils. The use of AMF in avocado crop is very promising because this is an environmental-friendly practice that can reduce P fertilizers and improve plant growth. Our hypothesis is that given the coarse root system of avocado (Avilan *et al*. 1984), this plant species must exhibit certain degree of dependency on the mycorrhizal association and this can be affected by the level of soil P bioavailability. The purpose of this study was to determine the MD of avocado cv. Villagorgona and the interaction between AMF inoculation and soil solution Pi concentration on plant growth.

MATERIALS AND METHODS

The study was conducted in the greenhouse of the Soil Microbiology laboratory (6°15N, 75°35´W, and 1495 m altitude) of the National University of Colombia at Medellin. A sub-superficial (30-50 cm) sample from a Bw horizon of a volcanic-ash soil was collected from the Piedras Blancas Forest Experimental Station. Soil sample was air-dried, sieved through a 4-mm sieve, mixed with quartz (soil:quartz ratio of 7:1), and autoclaved at 120°C and 0.1 MPa for one hour. Based on a chemical soil analysis the following compounds were applied (per kg): 173 mg of ammonium nitrate, 380 mg of potassium nitrate, and 3280 mg of magnesium sulfate. According to the approach of Habte and Manjunath (1991) for MD determination, a soil Pi sorption isotherm (Fox and Kamprath 1970) was conducted to determine the Pi requierement to achieve three levels of soil solution P concentration (0.002, 0.02, and 0.2 mg L^{-1}). As a result of that, calcium phosphate was added at 0.26, 2.52, and 19.41 g kg^{-1}, respectively, and mixed throughout. To balance the level of calcium supply, 13.7 and 12.1 mg of calcium sulphate per kg of substrate were applied at the lowest and medium level of P added, respectively. Plastic bags were filled with 5.6 kg (dry basis) of the amended and sterile mixture of soil and quartz.

The substrate was inoculated (M+) with 75 g of a crude mycorrhizal inoculum, which was mixed with the first 10 cm of depth. The inoculum contained 43 infective propagules of *Glomus fasciculatum* per g, which was determined by the most probable number technique (Porter 1979). The inoculum was multiplied in a mixture of soil: quartz (1:1, m:m) with sorghum as host plant. Uninoculated soil (M-) received 75 g of sterilized substrate and 20 cm^3 of washing of crude inoculum after remotion of mycorrhizal fungi structures by filtration with filter paper Whatman No. 1.

Seedlings of avocado were obtained from seeds and grown in a sterile substrate for 60 days. Then, the seedlings were transplanted into the amended and inoculated substrate. A thin layer of fine quartz was applied on the surface of each pot to prevent contamination by mycorrhizal spores from other treatments. Seedlings were growing under natural light for 150 days after transplanting. The substrate was watered with destilled water to maintain it at 50-60% of the maximum water holding capacity. To prevent nutritional deficiencies in plants, we supplied 50 cm^3 of P-free Hoagland solution to each bag once a week. The bags were moved on a weekly basis to randomize variation of environmental conditions.

A complete randomized experimental design was employed; the treatments were arranged in a factorial combination 3x2: three levels of soil solution P and two levels of mycorrhizal inoculation (M+, M-). Each treatment had five replications.

Plant height was measured at 17, 52, 80, 111, and 150 days after transplanting. At the same dates, foliar P content was monitored using the non-destructive sampling method developed by Aziz and Habte (1987). For this purpose, a leaf-disk (6 mm of diameter) was collected from the youngest mature leaf. The leaf-disk samples were ashed in a muffle furnace at 500°C for three hours, then the ash was dissolved in distilled water. The solution P concentration was measured by the blue-molibdate method (Murphy and Riley, 1962; Habte and Osorio, 2001). At harvesting, the shoot dry weight (SDW) was determined after oven-dry the samples at 70ºC for 72 h. To determine root colonization by the AMF, fine roots were cleaned with water, cleared by immersion in KOH (10 % m/v in water) for 24 h (Phillips and Hayman, 1970), and then bleached by immersion in alkaline H_2O_2 (0.5% NH_4OH

and 0.5% H_2O_2 v/v) for 30 minutes (Brundrett *et al*. 1996). Root samples were stained with triplan blue (0.025 %) (Kormanik *et al*. 1980) and to quantify the presence of AMF structures we used the method of grid line intersection (Giovannetti and Mosse 1980). The MD was evaluated as proposed by Plenchette *et al*. (1983) as the difference between shoot dry mass of inoculated and uninoculated plants, expressed as a percentage of shoot dry mass of inoculated plants. The mean value of MD obtained at the soil solution P of 0.02 mg L^{-1} was compared with the categories proposed by Habte and Manjunath (1991). The data were statistically analyzed with SAS (SAS Institute, Inc.) employing analysis of variance and LSD test (*P*-value ≤ 0.05).

RESULTS

Only those plants grown in the inoculated substrate exhibited mycorrizal colonization. This ranged between 57 and 77% and was not affected by the level of soil solution P. At low concentration of soil solution P there was abundance of mycorrhizal vesicles and arbuscules, while at the highest levels of P there a predominance of intra and extraradical hyphae. The plant height of avocado seedlings was unaffected by the treatments at the three first dates of observation (17, 52, and 80 days) (Figure 1). However, at the last two dates of observation, the plant height was significantly increased by the mycorrizal inoculation only at the soil solution P of 0.002 mg L^{-1} by 8%. Conversely, at 0.2 mg L^{-1} the mycorrhizal inoculation significantly reduced the plant height by 15%.

The inoculation with *G. fasciculatum* did not affect the leaf-disk P concentration at any level of soil solution P and sampling date (data not shown). The leaf-disk P concentration ranged from 0.15 to 0.4%.

The SDW of avocado seedlings was significantly (*P*≤0.05) affected by the interaction between soil solution P and AMF inoculation (Figure 2). The inoculation with *G. fasciculatum* significantly increased the SDW of avocado seedlings grown at the soil solution levels of 0.002 and 0.02 mg L^{-1} by 48 and 35%, respectively. By contrast, at 0.2 mg L^{-1} the mycorrizal inoculation significantly reduced the SDW by 26%.

The MD of avocado cv. Villagorgona decreased as the soil solution P increased (Figure 3). At the lowest level of soil solution P the value of MD was 32.2%, while at 0.02 it was 25.8%. According to the classification of MD proposed by Habte y Manjunath (1991) this cultivar is moderately dependent on the mycorrizal association. At the highest level of soil solution P (0.2 mg L^{-1}) the MD had a negative value (-35.7%).

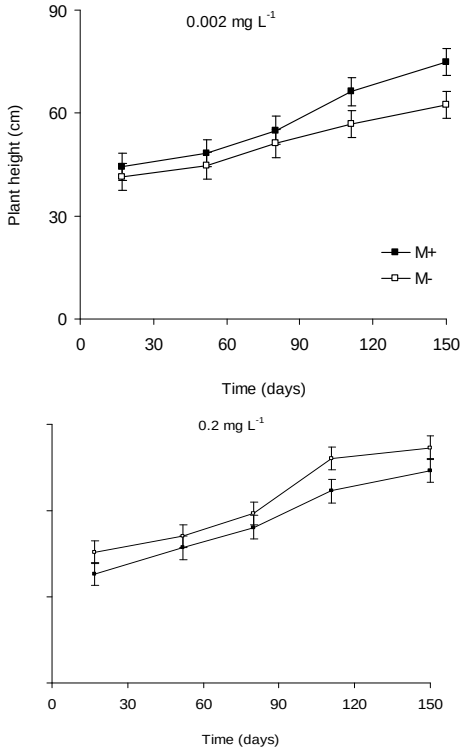

Figure 1. Effect of mycorrizal inoculation with *G. fasciculatum* and three levels of soil solution P on plant height of avocado cv. Villagorgona at different dates after transplanting (M- uninoculated, M+: inoculated).

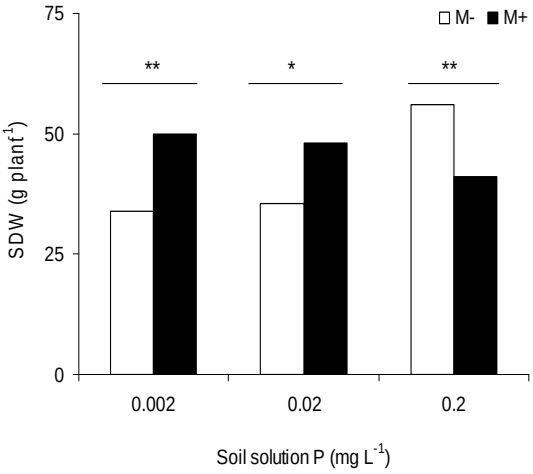

Figure 2. Shoot dry weigh (SDW) of avocado seedlings (cv. Villagorgona) as a function of the interaction between the micorrizal inoculation with *Glomus fasciculatum* and the soil solution P. M-: uninoculated; M+: inoculated. The asterisks indicate significant difference between M+ and M- at the respectivel level of soil solution P (**P≤0.01; * P≤0.05) (LSD $_{0.05}$ = 9.6).

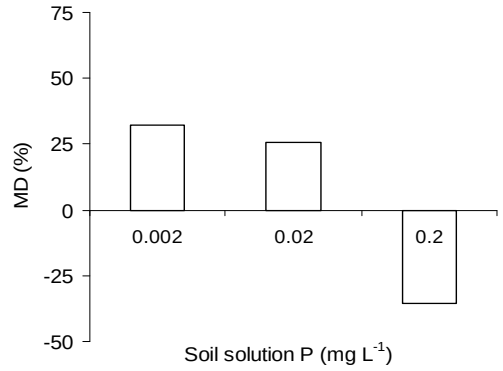

Figure 3. Mycorrizal dependency (MD) of avocado cv. Villagorgona as affected by the soil solution P concentration.

DISCUSION

The results clearly showed that avocado seedlings exhibit MD and this is significantly affected by the soil solution P concentration. As the soil available P increased the dependency of avocado on the mycorrhizal association decreased. In fact, at the highest levels of soil solution P (0.2 mg L^{-1}) the effect of the mycorrhizal inoculation was depressive on avocado seedlings growth. It has been amply reported that the soil solution P is key factor that controls the benefits of the mycorrhizal inoculation. The response to this inoculation is higher at low concentrations of P. This contrasts a little with previous findings in other plant species that showed a better response to mycorrhizal inoculation at 0.02 than at 0.002 mg L^{-1} (Gonzalez and Osorio, 2008; Diez *et al*., 2006).

It is possible that at the lowest soil P level, the P requirement of avocado seedling was partially satisfied by the P reserve of the large seed in addition to the P supply via the mycorrhizal hypha. However, at higher levels of soil available P, the excess of this nutrient might interfere with the assimilation of other nutrients (e.g., Cu, Zn).

The response to the mycorrhizal association is controlled by the exchange of carbonaceous compounds by P between plant and fungus (Fitter, 1991). The plant gives up to 20 to 30% of the C fixed in the photosynthesis to the fungus (Marschner 1997), it is used to satisfy its C requirement. As the fungal mycelium grews it can capture the scarce available P, which is then translocated into the root. If this nutrient exchange is balanced the mycorrhizal relationship is favorable for the host plant. However, at high concentrations of soil available P the mycorrhiza-free roots can uptake enough P from the soil solution. Likely, under this condition the plant does not require the fungus in its roots, but since it is present its nutritional needs constitutes a C-drainage for the host plant without an apparent benefit (Harrier, 2001; Harrison, 1997).

We consider that it is necessary to screen for better combinations between avocado and other AMF species to improve plant growth (Menge *et al*., 1980; Silva and Siqueira, 1991; Smith and Read, 1997). Similarly, the interest of obtaining avocado seedlings by *in-vitro* procedures indicates the need of a new study of the MD of these plants (Hooker *et al*., 1994; Vidal *et al.*, 1992). Under such conditions it is logical to expect a higher dependency on the mycorrhizal association given the absence of the P reserves commonly found in the seeds.

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