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Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress

Adriana Marulanda, Rosario Azcón and Juan Manuel Ruiz-Lozano*

Departamento de Microbiología del Suelo y Sistemas Simbióticos. Estación Experimental del Zaidín (CSIC), Profesor Albareda n°1, E-18008 Granada, Spain
**Corresponding author, e-mail: juanmanuel.ruiz@eez.csic.es*

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It is currently accepted that, along with nutrients, arbuscular mycorrhizal (AM) fungi also transport water to their host plant. However, the quantity of water supplied and its significance for plant water relations remain controversial. The objective of this work was to evaluate and compare the ability of six AM fungi to alter rates of root water uptake under drought stress conditions. Soil drying rates of uninoculated control plants of comparable size and nutritional status and mycorrhizal plants were recorded daily. *Lactuca sativa* plants colonized by *Glomus coronatum*, *G. intraradices*, *G. claroideum* and *G. mosseae* depleted soil water to a higher extent than comparably sized uninoculated control plants or plants colonized by *G. constrictum* or *G. geosporum*. The differences ranged from 0.6% volumetric soil moisture for *G. mosseae*-colonized plants to 0.95% volumetric soil moisture for *G. intraradices*-colonized plants. These differences in soil moisture were equivalent to 3–4.75 ml plant^{-1} day^{-1}, respectively, and could not be ascribed to differences in plant size, but to the activity of AM fungi. The AM fungi tested in this study differed in their effectiveness to enhance plant water uptake from soil. This ability seems to be related to the amount of external mycelium produced by each AM fungus and to the frequency of root colonization in terms of live and active fungal structures.

Introduction

Drought stress is considered one of the most important abiotic factors limiting plant growth and yield (Kramer and Boyer 1997). Plants can respond to water deficit at morphological, anatomical and cellular levels with modifications that allow the plant to avoid the stress or to increase its tolerance (Bray 1997). Apart from the natural protection systems that plants possess against stress, plants grow in association with a number of soil micro-organisms that can alleviate the stress symptoms. Among those, arbuscular mycorrhizal (AM) fungi are widespread micro-organisms able to establish a symbiotic association with the roots of most terrestrial plants. The fungus gets a protected ecological niche and plant photosynthates whereas plants improve their ability for nutrient uptake and tolerance to biotic and abiotic stresses (Smith and Read 1997).

Several eco-physiological studies investigating the role of AM symbiosis in drought stress protection have demonstrated that the symbiosis often results in altered rates of water movement into, through and out of the host plants, with consequent effects on tissue hydration and plant physiology (Augé 2001). It is becoming accepted that the contribution of AM symbiosis to plant drought tolerance is the result of cumulative physical, nutritional, physiological and cellular effects. The studies carried out so far have suggested several mechanisms by which the AM symbiosis alleviates drought stress in host plants (for reviews see Augé 2001, Ruiz-Lozano 2003). One of these mechanisms is the direct uptake and transfer of water through the fungal hyphae to the host plant. Pioneer studies carried out by Allen (1982) and Hardie (1985) suggested a possible role of AM hyphae in water uptake and transfer to the host plant. AM fungal hyphae, with a diameter of 2–5 μm, can penetrate soil pores inaccessible to root hairs (10–20 μm diameter) and absorb water that is not available to-non-mycorrhizal plants. Allen (1991) estimated that the rate of water

Abbreviations – ALP, alkaline phosphatase; AM, arbuscular mycorrhizal; SDH, succinate dehydrogenase; TB, trypan blue.

transport from extraradical hyphae to the root was 100 nl H_2O h^{-1} per hyphal infection point. This rate was considered enough to alter plant water relations (Allen 1991). Faber et al. (1991) measured rates of water transport in hyphae crossing air gaps between compartments, ranging from 375 to 760 nl H_2O h^{-1} per hyphal section. In contrast, other authors have predicted rates of water uptake by hyphae on the basis of hyphal entry points per unit of root length, hyphal cross-sectional areas and water potential gradients. Their predictions suggested that hyphal water transport rates were negligible (Fitter 1988, George et al. 1992, Koide 1993). As no clear conclusion could be drawn on that topic, new studies were developed. Ruiz-Lozano and Azcón (1995) designed an experiment with lettuce plants grown in containers with a compartment that was only accessible to hyphae. Despite the fact that the two AM species used in the experiment differed in their efficiency for hyphal water uptake and transport, the positive effect of AM fungi was enhanced by water addition to the hyphal compartment, and water uptake by the host plant increased due to the presence of the AM fungi (Ruiz-Lozano and Azcón 1995). Thus, it is currently accepted that mycorrhizal fungi also transport some water to the plant along with the nutrients. However, the quantity of water supplied to the host plant via mycelium is unknown and the possible significance of water transport by AM hyphae for plant water relations remains controversial (Bryla and Duniway 1997a).

The objective of this work was to evaluate and compare the ability of six AM fungi to increase rates of root water uptake under drought stress conditions. Uninoculated control lettuce plants of comparable size and nutritional status to the mycorrhizal plants were established and the bulk soil water content was recorded daily. Soil drying rates in plant/soil systems colonized by each AM fungus were related to several parameters of fungal development in the plant and in the soil.

Materials and methods

Experimental design and statistical analysis

The experiment consisted of a randomized complete block design with an inoculation treatment consisting of: (1) plants inoculated with one of six mycorrhizal fungi; and (2) uninoculated control plants fertilized with two levels of N *and* P. Five replicates of each treatment were performed, totalling 40 pots (one plant per pot).

Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test (Duncan 1955). Percentage values were arcsin-transformed before statistical analysis. Correlations were calculated using SPSS software, version 11.0.1 (LEAD Technologies Inc., Chicago, IL, USA).

Soil and biological materials

Loamy soil was collected from the Zaidin Experimental Station (Granada, Spain), sieved (2 mm), diluted with quartz-sand (< 1 mm) (1 : 1, soil : sand, v/v) and sterilized by steaming (100°C for 1 h per day on three consecutive days). The soil had a pH of 8.1 (water); 1.81% organic matter, and the following nutrient concentrations (mg kg^{-1}): N, 2.5; P, 6.2 ($NaHCO_3$-extractable P); K, 132.0. The soil texture was made up of 35.8% sand, 43.6% silt and 20.5% clay.

Three seeds of *Lactuca sativa* L. cv. Romana were sown in pots containing 500 g of the soil/sand mixture and thinned to one seedling per pot after emergence.

Mycorrhizal inoculum was bulked in an open-pot culture of *Zea mays* L. and consisted of soil, spores, mycelia and infected root fragments. The AM species were isolated from two desert areas in the Alicante and Almeria provinces (Southern Spain). The species were *Glomus coronatum* Giovannetti, isolate EEZ 17, BEG 49; *Glomus intraradices* Schenck and Smith, isolate EEZ 6, BEG 121; *Glomus claroideum* Schenck and Smith, isolate EEZ 23; *Glomus constrictum* Trappe, isolate EEZ 22; *Glomus geosporum* (Nicol. and Gerd.) Walker, isolate EEZ 4 and *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, isolate EEZ 7, BEG 122. Ten grams of each inoculum, with similar colonization potential (an average of 50 propagules per gram according to the most probable number test) were placed below lettuce seeds. This amount of inoculum was selected in preliminary tests as the optimum to produce a good colonization level for the total amount of soil in the pot. Non-mycorrhizal treatments received the same quantity of autoclaved inoculum together with a 2-ml aliquot of a suspension filtrate (< 20μm) of the AM inoculum to restore a general microbial population free of AM propagules.

Growth conditions

Plants were grown in a controlled environmental chamber with 70–80% RH, day/night temperatures of 25/15°C, and a photoperiod of 16 h at a photosynthetic photon flux density (PPFD) of 350 μmol m^{-2} s^{-1} as measured with a light meter (model LI-188B; Licor Inc., Lincoln, NE, USA).

Soil moisture was measured with a ML2x ThetaProbe (AT Delta-T Devices Ltd, Cambridge, UK), which measures volumetric soil moisture content by responding to changes in the apparent dielectric constant of moist soil. The probe generates a 100 MHz sinusoidal signal that is applied to a specially designed internal transmission line that extends into the soil by means of the array of four rods. The changes in the transmission line impedance are dependent almost solely on the soil's apparent dielectric constant. Because the dielectric constant of water (approximately 81) is very much higher than that of soil (typically 3–5) and air (1), the dielectric constant of soil is determined primarily by its water content (Roth et al. 1992, White et al. 1994). Volumetric soil water content is the ratio between the volume of water present and the total volume of the soil sample. It is a dimensionless parameter, expressed either as a percentage (% vol) or as a ratio (m^3 m^{-3}). Water was supplied daily to

maintain constant soil water content close to field capacity (17% volumetric soil moisture) during the first 5 weeks of plant growth. At this time plants were allowed to dry until soil water content reached 80% field capacity (12% volumetric soil moisture) and maintained under such conditions for an additional 12 days. In order to control the level of water stress, the soil water content was measured daily with the ThetaProbe ML2x (at the end of the afternoon) and the amount of water lost was added to each pot in order to maintain soil water content at the desired 12% volumetric soil moisture.

Each week throughout the experiment, uninoculated control plants received 10 ml of Hewitt's nutrient solution (Hewitt 1952), modified to contain either 4 m*M* N + 1 m*M* P (high nitrogen plus phosphorus treatment, HNP) or 2 m*M* N + 0.5 m*M* P (low nitrogen plus phosphorus treatment, LNP). Mycorrhizal plants did not receive nutrient solution. The use of two levels of fertilization for non-mycorrhizal plants was meant to obtain control plants of similar size and nutrient contents to the AM plants tested in this assay.

Parameters measured

Biomass production and nutrient concentrations
Two hours before harvesting (7 week after planting) plants were watered to standardize their water content. The root system was separated from the shoot and its fresh weight recorded. The N (micro-Kjeldahl) and P (Olsen and Dean 1965) concentrations in shoots were measured.

Water consumption
Daily water consumption was measured with the Thetaprobe ML2x by determining the volumetric soil moisture in each pot. To initiate the drought period, plants were allowed to dry until soil water content reached 80% field capacity (12% volumetric soil moisture) and maintained under such conditions for additional 12 days. The reading of the ThetaProbe ML2x before daily re-watering until 12% volumetric soil moisture during these 12 days was recorded to estimate the water consumption per plant and per day. The relative plant water uptake was calculated as the ratio between the decrease of volumetric soil moisture of each treatment and that of the non-mycorrhizal LNP treatment, which was set as 100%.

Symbiotic development
The roots were carefully washed and then divided into three batches: one was stained by the normal non-vital trypan blue (TB) staining of all fungal tissues (Phillips and Hayman 1970) and the other two were used for histochemical staining [succinate dehydrogenase (SDH) or alkaline phosphatase (ALP) activities] of roots. This method makes it possible to compare directly the total amount of fungal tissue in mycorrhizal root systems (TB staining) and the proportion which is alive (SDH staining) with that associated with an active phosphate metabolism (ALP staining), as proposed by Tisserant et al. (1993).

SDH activity was measured according to the procedure described by Smith and Gianinazzi-Pearson (1990). Briefly, the roots were immersed in a freshly made solution containing 0.2 *M* Tris-HCl pH 7.0, 2.5 *M* sodium-succinate 6-hydrate, 4 mg ml^{-1} nitro blue tetrazolium, 5 m*M* $MgCl_2$. Root fragments were stained overnight at room temperature and then rinsed for 15–20 min in a 3% active chlorine solution of sodium hypochlorite.

ALP was determined according to the procedure described by Tisserant et al. (1993). The roots were immersed in a freshly made solution containing 50 m*M* Tris-citric acid, pH 9.2, 1 mg ml^{-1} alfa-naphthyl acid phosphate (monosodium salt), 0.05% $MgCl_2$ anhydro, 0.05% $MnCl_2$ tetrahydrate and 1 mg ml^{-1} Fast Blue RR salt. Root fragments were stained overnight at room temperature and then rinsed for 15–20 min in a 1% active chlorine solution of sodium hypochlorite.

Mycorrhizal development was evaluated after either non-vital or vital staining procedures by the method of Trouvelot et al. (1986) (for more information visit http://www.dijon.inra.fr/bbceipm/Mychintec/Mycocalc-prg/). The colonization frequency (*F*%) is a ratio between colonized root fragments and total number of root fragments observed. It gives an estimation of the root length colonized by the fungus. The colonization intensity (*M*%) is an estimation of the amount of cortical cells occupied by AM fungal structures. Finally, the arbuscule abundance *A*% gives an estimation of the arbuscule richness in root system. Four replicates per treatment were used.

Production of extraradical mycelium
The extraradical mycelium in the soil was determined as described by Jones and Mollinson 1948) with slight modifications. Briefly, 1 g of dry soil was treated with sodium hexametaphosphate and stained with TB (0.05%) in lactic acid. The sample was heated in a water bath at 90°C for 30 min and then sieved through 50 μm mesh. The remaining mycelium was mixed with bacteriological agar for quantification using a gridline intersection method as described by Newman (1966).

Results

Plant biomass production and nutrient contents

Control treatments fertilized with two different nutrients solutions (HNP or LNP) produced plants of different size (Fig. 1). HNP plants had similar shoot fresh weight as plants inoculated with *G. coronatum, G. intraradices, G. claroideum* and *G. constrictum*. LNP plants showed similar shoot and root fresh weights as plants inoculated with *G. geosporum*. Finally, plants inoculated with *G. mosseae* showed the maximum shoot fresh weight, whereas their root fresh weight was similar to that of HNP plants.

Shoot N concentration (Table 1) was higher in the two fertilized controls than in the six mycorrhizal treatments. The lowest N concentration was found in plants

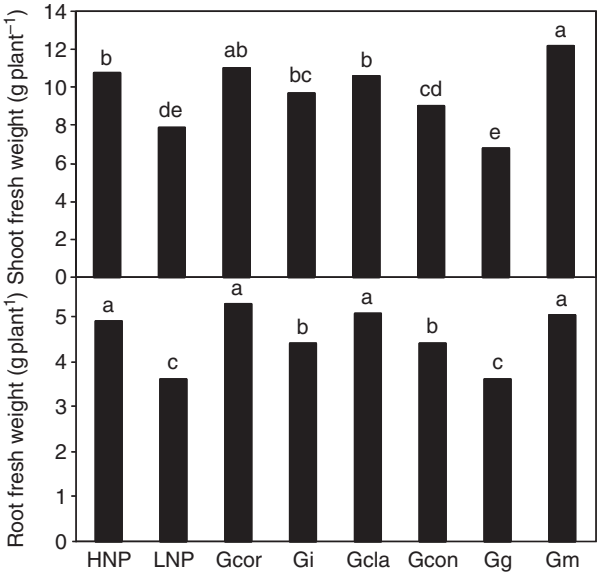

Fig. 1. Shoot and root fresh weight (g plant^{-1}) of uninoculated lettuce plants fertilized with two rates of N *and* P (4 m*M* N + 1 m*M* P, HNP and 2 m*M* N + 0.5 m*M* P, LNP) or mycorrhizal lettuce plants colonized by *G. coronatum* (Gcor), *G. intraradices* (Gi), *G. claroideum* (Gcla), *G. constrictum* (Gcon), *G. geosporum* (Gg) or *G. mosseae* (Gm). Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range test (n = 5).

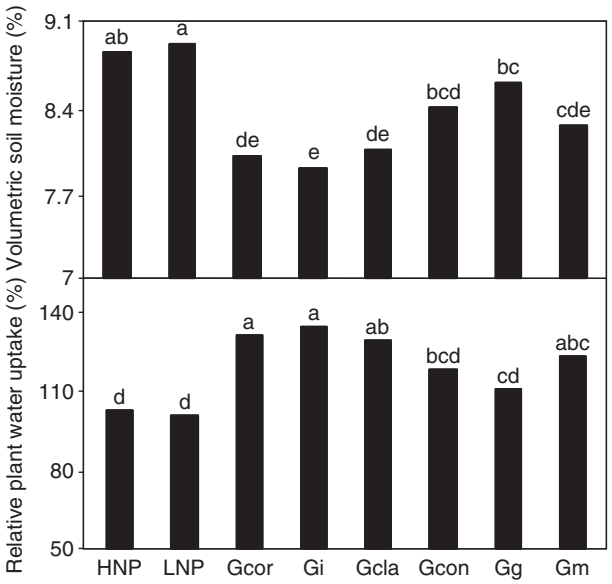

Fig. 2. Volumetric soil moisture (%) and relative plant water uptake in uninoculated lettuce plants fertilized with two rates of N *and* P (4 m*M* N + 1 m*M* P, HNP and 2 m*M* N + 0.5 m*M* P, LNP) or mycorrhizal lettuce plants colonized by *G. coronatum* (Gcor), *G. intraradices* (Gi), *G. claroideum* (Gcla), *G. constrictum* (Gcon), *G. geosporum* (Gg) or *G. mosseae* (Gm). Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range test (n = 60).

colonized by *G. coronatum* and *G. mosseae* (the biggest plants), probably due to a dilution effect. The highest N content was found in HNP plants and the lowest in *G. geosporum*-colonized plants.

The highest P concentration was found in HNP and *G. intraradices*-colonized plants and, the lowest in *G. mosseae*-colonized plants. The P content was similar in HNP plants and most of the mycorrhizal treatments. Only plants colonized by *G. geosporum* had lower P content than LNP plants.

Daily water consumption

Figure 2 shows the volumetric soil moisture recorded before watering. Both control treatments exhibited the highest soil water content (an average of 8.9%), regardless of plant size. Plants colonized by *G. coronatum, G. intraradices or G. claroideum* showed the highest soil water depletion (average of 8% of volumetric soil moisture). *Glomus mosseae*-colonized plants also exhibited a significantly lower soil water content (8.2% of volumetric soil moisture) whereas plants colonized by *G. constrictum* and *G. geosporum* did not show significant differences in soil water depletion when compared with HNP control plants.

The relative plant water uptake was calculated as an index of pot water loss (Fig. 2). Setting the water uptake of the LNP treatment as 100%, there were no significant differences with the HNP, *G. constrictum* or *G. geosporum* treatments. Plants colonized by *G. coronatum, G. intraradices, G. claroideum* and *G. mosseae* significantly increased the daily water loss, ranging from 22% of increase for *G. mosseae*- to 33% of increase for *G. intraradices*-colonized plants.

Table 1. Nitrogen and phosphorus concentration (mg g^{-1}) and content (mg plant^{-1}) in shoots of uninoculated lettuce plants fertilized with two rates of N *and* P (4 m*M* N + 1 m*M* P, HNP and 2 m*M* N + 0.5 m*M* P, LNP) or mycorrhizal lettuce plants colonized by *G. coronatum*, *G. intraradices*, *G. claroideum*, *G. constrictum*, *G. geosporum* or *G. mosseae*. Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range test (n = 5).

Treatment	N (mg g^{-1})	N (mg plant^{-1})	P (mg g^{-1})	P (mg plant^{-1})
HNP	25.0 a	34.2 a	1.8 a	2.3 ab
LNP	24.9 a	25.3 b	1.3 b	1.4 d
G. coronatum	11.3 d	22.0 bc	1.3 b	1.9 bc
G. intraradices	17.7 bc	24.3 b	1.6 a	2.5 a
G. claroideum	15.0 c	25.0 b	1.3 b	2.1 b
G. constrictum	18.1 b	24.1 b	1.3 b	1.7 cd
G. geosporum	20.0 b	19.0 c	1.1 bc	1.0 e
G. mosseae	9.2 d	23.0 b	1.0 c	2.2 ab

Symbiotic development

Fungal colonization was estimated after TB, SDH or ALP staining to measure total (TB), living (SDH) and functional (ALP) fungal development (Smith and Gianinazzi-Pearson 1990, Tisserant et al. 1993). No fungal development was found in any of the uninoculated control treatments. TB staining (Fig. 3) showed that colonization frequency (*F*) and arbuscule richness (*A*) were higher in plants colonized by *G. coronatum, G. intraradices, G. claroideum* and

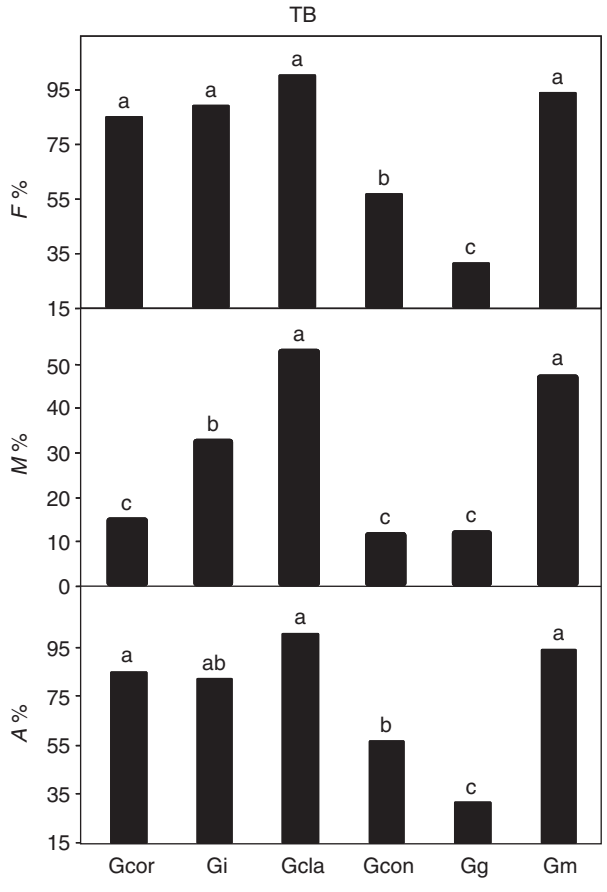

Fig. 3. Colonization frequency (*F*), intensity (*M*) and arbuscule abundance (*A*) after trypan blue (TB) staining of roots from mycorrhizal lettuce plants colonized by *G. coronatum* (Gcor), *G. intraradices* (Gi), *G. claroideum* (Gcla), *G. constrictum* (Gcon), *G. geosporum* (Gg) or *G.mosseae* (Gm). Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range test (n = 4).

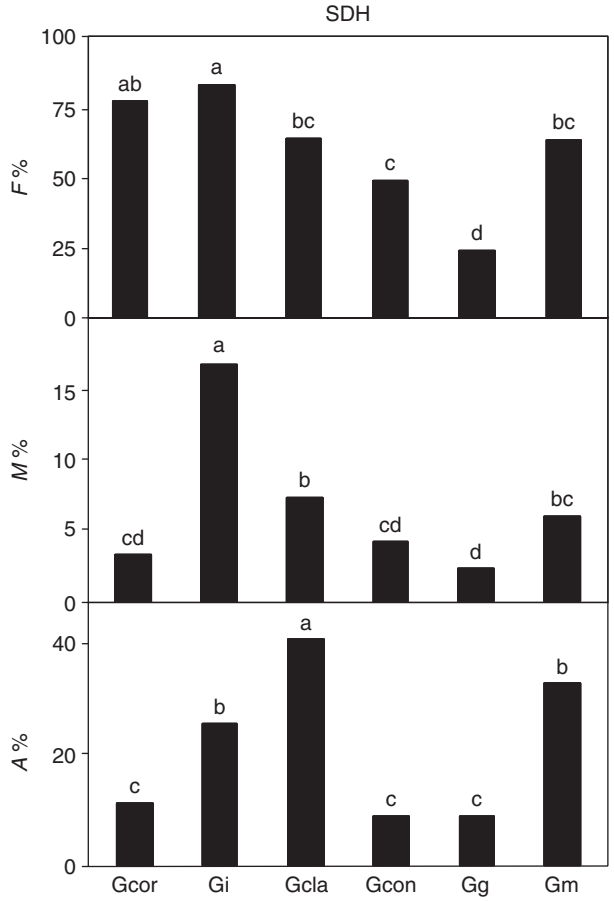

Fig. 4. Colonization frequency (*F*), intensity (*M*) and arbuscule abundance (*A*) after succinate dehydrogenase (SDH) staining of roots from mycorrhizal lettuce plants colonized by *G. coronatum* (Gcor), *G. intraradices* (Gi), *G. claroideum* (Gcla), *G. constrictum* (Gcon), *G. geosporum* (Gg) or *G. mosseae* (Gm). Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range test (n = 4).

G. mosseae than in those colonized by *G. constrictum* and *G. geosporum*. The colonization intensity (*M*) was highest in plants inoculated with *G. claroideum* and *G. mosseae*, followed by those inoculated with *G. intraradices*, whereas plants inoculated with *G. coronatum*, *G. constrictum* and *G. geosporum* showed the lowest *M*-values.

The highest *F*-value after SDH staining (Fig. 4) was observed in plants colonized by *G. coronatum* and *G. intraradices*. Plants colonized by *G. claroideum, G. constrictum* and *G. mosseae* had a medium *F* value, whereas those colonized by *G. geosporum* had the lowest *F*. The value of *M* was highest in *G. intraradices*-colonized plants and there were no significant differences among the other fungal treatments. The *A* value reached the maximum value in *G. claroideum*-colonized plants, followed by those colonized by *G. intraradices* and *G. mosseae*. No significant differences were found among the other three fungal treatments.

The ALP staining (Fig. 5) indicated that *F* was similar in all treatments, except in plants colonized by *G. geosporum,* which exhibited a lower *F* value. No significant differences were found in *M* value among the different fungal treatments, except for a higher value in *G. intraradices*-colonized plants. Finally, *A* was higher in plants colonized by *G. intraradices* and *G. claroideum* than in those colonized by *G. coronatum* and *G. mosseae*. The lowest *A* values were found in plants colonized by *G. constrictum* and *G. geosporum*.

Production of mycelium

Figure 6 shows the amount of external mycelium produced by each AM fungus. *Glomus coronatum* and *G. claroideum* produced the highest amount of mycelium (about 50 cm g^{-1}) followed by *G. intraradices* (34 cm g^{-1}). The lower amount of mycelium was produced by *G. constrictum, G. mosseae* and *G. geosporum*.

Discussion

After nearly 25 years of research on the water relations of mycorrhizal plants, there is still considerable

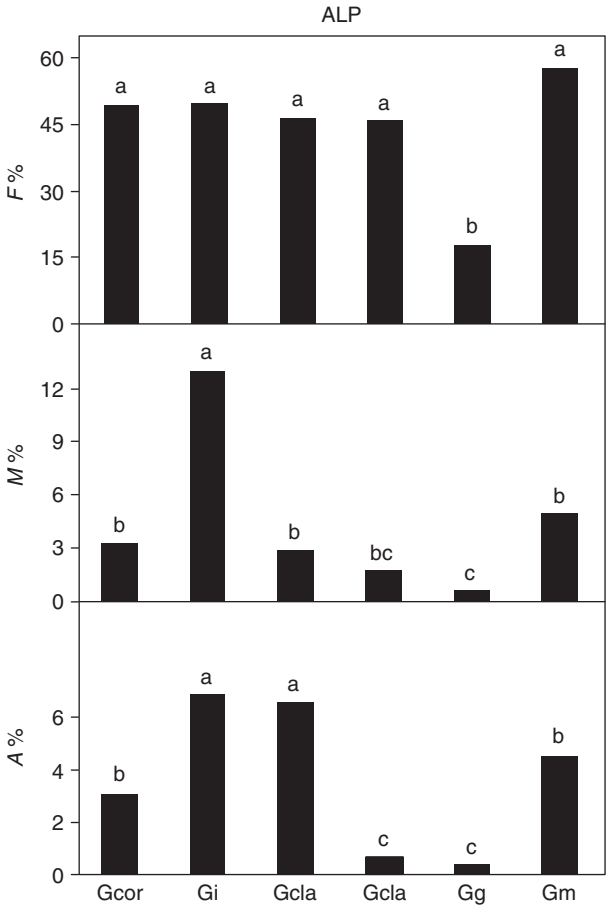

Fig. 5. Colonization frequency (*F*), intensity (*M*) and arbuscule abundance (*A*) after alkaline phosphatase (ALP) staining of roots from mycorrhizal lettuce plants colonized by *G. coronatum* (Gcor), *G. intraradices* (Gi), *G. claroideum* (Gcla), *G. constrictum* (Gcon), *G. geosporum* (Gg) or *G. mosseae* (Gm). Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range test (n = 4).

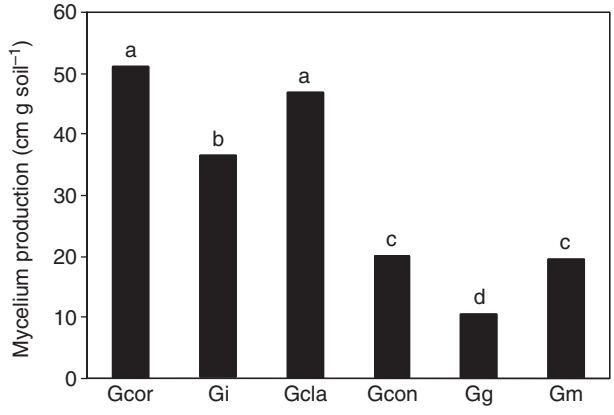

Fig. 6. Extraradical mycelium production (cm g soil^{-1}) by mycorrhizal lettuce plants colonized by *G. coronatum* (Gcor), *G. intraradices* (Gi), *G. claroideum* (Gcla), *G. constrictum* (Gcon), *G. geosporum* (Gg) or *G. mosseae* (Gm). Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range test (n = 4).

disagreement on the significance of the effect of arbuscular mycorrhizas on host water relations and on the mechanisms involved (Bryla and Duniway 1997b). Several studies have suggested that roots colonized by AM fungi can have higher specific water uptake rates (volume of water absorbed per unit root length) than non-colonized roots, independently from direct hyphal water transport (Koide 1993). The usual method to determine the influence of AM fungi on the specific root water uptake of a colonized plant is to measure changes in total weight of a potted plant resulting from transpiration and divide that value by the total root length in the pot (Bryla and Duniway 1997a). In this study we have measured the daily water consumption of lettuce plants infected by six AM fungi or by two uninoculated control treatments, using a soil moisture sensor that responds to changes in the apparent dielectric constant (Roth et al. 1992, White et al. 1994). Read (1992) suggested that hyphal transport might supply adequate water to maintain physiological function when hydraulic conductivity of the soil begins to limit uptake at the root surface. Because of that, plants in our study were subjected to a moderate drought stress so that any increase in water uptake by fungal hyphae would be of greater importance for plant development.

The results showed that plants colonized by four AM fungi depleted soil water to a higher extent than uninoculated control plants (Fig. 2). The differences ranged from 0.6% volumetric soil moisture (22% increase in relative plant water uptake) for *G. mosseae*-colonized plants to 0.95% volumetric soil moisture (33% increase in relative plant water uptake) for *G. intraradices*-colonized plants. The other two AM fungi did not show significant differences when compared with the control plants. According to our calculations, the differences of 0.6–0.95% volumetric soil moisture between AM and control plants would represent 3–4.75 ml plant^{-1} day^{-1}, respectively. Although this amount of water can be seen as small to explain the important differences in water relations between mycorrhizal and non-mycorrhizal plants, we agree with the conclusion of Augé (2001) that mycorrhizal effects on plant water relations are not as dramatic and consistent as those on P acquisition and host growth, but that modest changes, if sustained, can have meaningful effects on plant fitness.

Two main mechanisms have been put forward in literature to explain how AM fungi might increase root water uptake: (1) AM fungi might indirectly increase water uptake by improving root conductance to water flow (Koide 1993); and (2) extraradical mycorrhizal hyphae might transport water to colonized roots directly (Read 1992, Ruiz-Lozano and Azcón 1995, Bryla and Duniway 1997a). It has been proposed that AM symbiosis may affect root conductance through its effects on plant growth and development (Augé 2001). There are, however, other ways in which colonization of roots by AM fungi might affect root conductance. For example, by modifying the amount and distribution of aquaporins in root membranes. Aquaporins are water channel proteins

Table 2. Statistical correlation among volumetric soil moisture in mycorrhizal treatments and extraradical mycelium production and fungal development within roots (TB, SDH and ALP stainings). *$P < 0.05$; **$P < 0.01$.

		TB staining			SDH staining			ALP staining		
	Mycelium	F(%)	M (%)	A (%)	F (%)	M (%)	A (%)	F (%)	M (%)	A (%)
Pearson correlation coefficient	−0.87*	−0.84*	−0.50	−0.54	−0.97**	−0.68	−0.53	−0.71	−0.70	−0.85*
Significance level	0.02	0.03	0.31	0.27	0.00	0.13	0.28	0.11	0.13	0.03

that when opened facilitate the passive movement of water molecules down a water potential gradient (Tyerman et al. 2002). Studies on aquaporins have suggested that they are important for the bulk flow of water in whole plant (Johansson et al. 2000). It has been reported that AM symbiosis increases the expression of some aquaporin-encoding genes (Roussel et al. 1997, Krajinski et al. 2000). Hence, it has been recently proposed to investigate the contribution of aquaporins to the enhanced plant tolerance to osmotic stresses (Ruiz-Lozano 2003).

In relation to hyphal water transport, Bryla and Duniway (1997a) previously investigated the role of AM fungi in water uptake from well watered to severely droughted conditions. They concluded that mycorrhizal colonization did not affect the rates at which roots extracted water from soil. In contrast, Bethlenfalvay et al. (1987) found that mycorrhizal soybean plants depleted soil water to a greater extent than non-mycorrhizal soybean. Faber et al. (1991) showed that when AM plants were grown in containers constructed with a barrier that excluded roots but not mycorrhizal hyphae from a portion of the soil volume, the soil containing only hyphae had a significant reduction in soil water content that could not be attributed solely to the evaporation. Furthermore, if the hyphae crossing the root barrier were severed, plant water uptake declined by 35% in comparison with intact mycorrhizal plants. Ruiz-Lozano and Azcón (1995) also showed that water uptake by host plants increased due to colonization by AM fungi.

In some of these studies, however, mycorrhizal plants were more intensely rooted and might have simply explored a greater soil volume than non-mycorrhizal plants. Alternatively, mycorrhizal plants were bigger than non-mycorrhizal ones. When large plants are constrained to the same restricted soil volume as small plants in potted experiments, the higher transpiration rates of the larger plants will result in a more intense soil drying (regardless of mycorrhizal symbiosis). However, in the present study, the two fertilized control treatments (HNP and LNP) matched the plant size (shoot and root) of the mycorrhizal treatments (Fig. 1), and still four of the mycorrhizal treatments depleted more soil water content than comparable non-mycorrhizal plants (Fig. 2). Moreover, the best treatment in terms of biomass production was inoculation with *G. mosseae*, and this treatment did not show the maximum soil water consumption. In addition, uninoculated control plants had similar or better nutritional status (N and P) than mycorrhizal plants (Table 1). The differences found between mycorrhizal and non-mycorrhizal plants in soil water depletion cannot be ascribed therefore to differences in plant size or in nutritional status. They should be attributed to the activity of the AM fungal mycelium.

Individual hyphae probably transport limited amounts of water. However, there is a prolific number of hyphae extending from mycorrhizal roots into the surrounding soil. For example, Miller et al. (1995) measured external hyphal lengths as high as $111\,m\,cm^{-3}$ of soil in the tallgrass prairie community and $81\,m\,cm^{-3}$ of soil in a cool-season pasture community. In our study, the most effective AM fungi for soil water depletion produced also higher amounts of mycelium than the other fungi that were less effective in water depletion (Fig. 6). There was a significant correlation between volumetric soil moisture and the amount of mycelium produced by each AM treatment (Table 2). It is known that mycelia of AM fungi increase soil aggregation (Miller and Jastrow 1990, Jastrow and Miller 1991) and that hyphal aggregation binds soil to roots preventing air gaps and preserving hydraulic continuity as soil dries (Davies et al. 1992, 1993). The staining of roots with vital and non-vital techniques indicated that higher colonization frequency (F), intensity (M) and arbuscule abundance (A) was reached by the four AM fungi (*G. coronatum, G. intraradices, G. claroideum* and *G. mosseae*) that depleted more soil water (Figs 3, 4 and 5). In contrast, the AM fungus less effective for water uptake (*G. geosporum*) also showed the lowest values of colonization (F, M and A). There was a significant correlation also (Table 2) between volumetric soil moisture and colonization frequency (F) after TB and SDH stainings, as well as with the A value after ALP staining (active fungal arbuscules).

In conclusion, this study shows that AM fungi increase the rate of plant water uptake from soil. The different fungal species differed in their effectiveness to enhance plant water uptake from soil, and it seems that this ability is related to the amount of external mycelium produced by each AM fungus and to the frequency of root colonization in terms of alive and active fungal structures.

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References

Allen MF (1982) Influence of vesicular-arbuscular mycorrhizae on water movement through *Bouteloua gracilis* Lag ex Steud. New Phytol 91: 191–196

Allen MF (1991) The Ecology of Mycorrhizae. Cambridge University Press, Cambridge, UK

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3–42
Bethlenfalvay GJ, Brown MS, Mihara KL, Stafford AE (1987) The *Glycine-Glomus-Bradyrhizobium* symbiosis V. Effects of mycorrhizal on nodule activity and transpiration in soybean under drought stress. Plant Physiol 85: 115–119
Bray EA (1997) Plant responses to water deficit. Trends Plant Sci 2: 48–54
Bryla DR, Duniway JM (1997a) Water uptake by safflower and wheat roots infected with arbuscular mycorrhizal fungi. New Phytol 136: 591–601
Bryla DR, Duniway JM (1997b) Growth, phosphorus uptake, and water relations of safflower and wheat infected with an arbuscular mycorrhizal fungus. New Phytol 136: 581–590
Davies FT, Potter JR, Linderman PG (1992) Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independent of plant size and nutrient content. J Plant Physiol 139: 289–294
Davies FT, Potter JR, Linderman PG (1993) Drought resistance of mycorrhizal pepper plants independent of leaf P concentration-response in gas exchange and water relations. Physiol Plant 87: 45–53
Duncan DB (1955) Multiple range and multiple F-tests. Biometrics 11: 1–42
Faber BA, Zasoski RJ, Munns DN (1991) A method for measuring hyphal nutrient and water uptake in mycorrhizal plants. Can J Bot 69: 87–94
Fitter AH (1988) Water relations of red clover *Trifolium pratense* L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. J Exp Bot 39: 595–603
George E, Häuser KU, Vetterlein D, Gorgus E, Marschner H (1992) Water and nutrient translocation by hyphae of *Glomus mosseae*. Can J Bot 70: 2130–2137
Hardie K (1985) The effect of removal of extraradical hyphae on water uptake by vesicular-arbuscular mycorrhizal plants. New Phytol 101: 677–684
Hewitt EJ (1952) Sand and Water Culture Methods used in the Study of Plant Nutrition. Technical Communication 22. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, UK
Jastrow JD, Miller RM (1991) Methods for assessing the effects of biota on soil structure. Agric Ecos Environ 34: 279–303
Johansson I, Karlsson M, Johanson U, Larsson C, Kjelbom P (2000) The role of aquaporins in cellular and whole plant water balance. Biochem Biophys Acta 1465: 324–342
Jones PCT, Mollinson JE (1948) A technique for the quantitative estimation of soil micro-organisms. J Gen Microbiol 2: 54–69
Koide R (1993) Physiology of the mycorrhizal plant. Adv Plant Pathol 9: 33–54
Krajinski F, Biela A, Schubert D, Gianinazzi-Pearson V, Kaldenhoff R, Franken P (2000) Arbuscular mycorrhiza development regulates the mRNA abundance of *Mtaqp1* encoding a mercury-insensitive aquaporin of *Medicago truncatula*. Planta 211: 85–90
Kramer PJ, Boyer JS (1997) Water Relations of Plants and Soils. Academic Press, San Diego, CA, USA
Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungi interactions with soil aggregation. Soil Biol Biochem 22: 579–584
Miller RM, Reinhardt DR, Jastrow JD (1995) External hyphal production of vesicular-arbuscular mycorrhiza propagules during topsoil storage. J Appl Ecol 22: 259–266
Newman EI (1966) A method of estimating the total length of root in a sample. J App Ecol 3: 139–145
Olsen SR, Dean LA (1965) Phosphorus. In: Black CA, Evans DD, White JL, Ensminger LE, Clark FE, Dinauer RC (eds) Methods of Soil Chemical Analysis, Part. 2. American Society of Agronomy, Madison, WI, pp. 1035–1049
Phillips JM, Hayman DS (1970) Improved procedure of clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55: 159–161
Read DJ (1992) The mycorrhizal mycelium. In: Allen MF (ed) Mycorrhizal Functioning. An Integrative Plant-Fungal Process. Chapman & Hall, New York, pp. 102–133
Roth CH, Malicki MA, Plagge R (1992) Empirical evaluation of the relationship between soil dielectric constant and volumetric water content as the basis for calibrating soil moisture measurements. J Soil Sci 43: 1–13
Roussel H, Bruns S, Gianinazzi-Pearson V, Hahlbrock K, Franken P (1997) Induction of a membrane intrinsic protein-encoding mRNA in arbuscular mycorrhiza and elicitor-stimulated cell suspension cultures of parsley. Plant Sci 126: 203–210
Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza, in press.
Ruiz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95: 472–478
Smith SE, Gianinazzi-Pearson V (1990) Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L. Effects of photon irradiance and phosphate nutrition. Aust J Plant Physiol 17: 177–188
Smith SE, Read DJ (1997) Mycorrhizal Symbiosis. Academic Press, San Diego, CA, USA
Tisserant B, Gianinazzi-Pearson V, Gianinazzi S, Gollotte A (1993) In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. Mycol Res 97: 245–250
Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and Genetical Aspects of Mycorrhizae. INRA Press, Paris, France, pp. 217–221
Tyerman SD, Niemietz CM, Bramley H (2002) Plant aquaporins: multifunctional water and solute channels with expanding roles. Plant Cell Environ 25: 173–194
White I, Knight JH, Zegelin SJ, Topp GC (1994) Comments to 'Considerations on the use of time-domain reflectometry (TDR) for measuring soil water content' by WR Whalley. J Soil Sci 45: 503–508

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