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Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity

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Abstract The purpose of this study was to compare the effect of salinity on the symbiotic efficiencies and mycelial infectivity of two arbuscular mycorrhizal fungi (AMF), one isolated from saline soils (*Glomus* sp.) and the other (*Glomus deserticola*) from nonsaline soils (belonging to the Estación Experimental del Zaidín collection). Lettuce plants inoculated with either of these two fungi or maintained as uninoculated controls were grown in soil with three salt concentrations (0.25, 0.50 or 0.75 g NaCl kg^{-1} dry soil). Both AMF protected host plants against salinity. However, when the results of shoot dry weight and nutrient contents were expressed relative to the total length of mycorrhiza formed, it was found that both AMF differed in their symbiotic efficiencies. These differences were more evident at the two highest salt levels. *Glomus* sp.-colonized plants grew less and accumulated less N and P, whereas they formed a higher amount of mycorrhiza. The mechanism by which *Glomus* sp. protected plants from the detrimental effects of salt was based on the stimulation of root development, while the effects of *G. deserticola* were based on improved plant nutrition. The increase in salinity of soil decreased the hyphal growth and/or viability of *Glomus* sp. to a higher extent than those of *G. deserticola* since the mycelial network generated by *G. deserticola* was more infective than that of *Glomus* sp.

Keywords Arbuscular-mycorrhiza · Infectivity · Fungal isolate · Salt stress · Symbiotic efficiency

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Introduction

Saline soils occupy over 7% of the earth's land surface, and crop production on these areas is relatively low. However, arbuscular mycorrhizal fungi (AMF) in sites affected by salinity may improve early plant tolerance and growth (Jain et al. 1989). Although improved salt tolerance of mycorrhizal plants can be related to enhanced mineral nutrition, particularly that of P (Graham 1986), the salt tolerance showed by AM plants is not only induced by this mechanism. Improvements in physiological processes like photosynthetic activity or water use efficiency have also been evidenced in mycorrhizal plants growing under salt stress conditions (Ruiz-Lozano et al. 1996).

A variety of mechanisms and symbiotic effects on host-plant tolerance to salinity, including improved or suppressed host-plant growth, have been described (Hirrell and Gerdemann 1980; Pond et al. 1984; Poss et al. 1985; Pfeiffer and Bloss 1988; Coperman et al. 1996; Ruiz-Lozano et al. 1996). As mycorrhizal fungi can adapt to edaphic conditions (Brundrett 1991), Coperman et al. (1996) suggested that differences in fungal behaviour and efficiency can be due to the origin of the AMF. They found that fungi from non-saline soil acted as shoot growth promoters, but tended to increase leaf sequestration of Cl^-. Conversely, AMF from saline soil suppressed plant growth but decreased leaf sequestration of Cl^-. They considered that this mechanism could be advantageous for long-term plant survival under salt stress.

Adverse environmental conditions can negatively affect the infectivity and survival of mycorrhizal propagules from one period of root growth to the next (Sylvia and Schenck 1983; Daft et al. 1987; Gazey et al. 1993; Juniper and Abbott 1993). Chambers et al. (1980) and Menge et al. (1987) reported that the addition of various salts to soil negatively influenced mycorrhizal colonization. It has also been demonstrated that salts containing Na and Cl have a negative effect on the in

vitro germination and survival of spores of *Gigaspora margarita* (Hirrell and Gerdemann 1980; Hirrell 1981) or *Glomus mosseae* (Estaun 1989). In addition, McMillen et al. (1998) found that salt inhibited hyphal growth in soil with a subsequent decrease in the spread of mycorrhizal colonization. From a functional point of view, three forms of propagules of AMF could contribute to the colonization of a host plant, namely: (1) AMF spores, (2) mycorrhizal roots or fragments of these, and (3) the mycelia of AMF. The relative importance of spores, mycorrhizal roots and mycelial network as propagule sources strongly depends on the environmental conditions under which they are produced (Jasper 1994). In some cases, it has been demonstrated that the infectivity of the hyphal network can be maintained in the absence of spores (Jasper et al. 1989, 1991). In addition, Requena et al. (1996) reported that mycorrhizal hyphae were the main source of mycorrhizal propagules in a desertified semiarid ecosystem. Thus, loss of viability of these propagules, as occurs under salinity, may be a critical factor in the success and survival of AMF (Dixon et al. 1993).

Salts in the growth medium may induce changes in the length and in other morphological properties of the hyphae, thus affecting their infective capacity (Juniper and Abbott 1993). The progress and range of AM hyphae in soil having a particular characteristic (i.e. salinity level) may be determined by screening a specific length of root-free soil between the AM donor plant and a receiver plant. This method allows one to estimate the rate of mycelial growth throughout the soil and to relate the infective ability of the extraradical fungal phase from a donor plant to the AM development and activity in a receiver plant, as a function of the soil conditions (i.e. increasing salinity level). According to Jakobsen et al. (1992), the spread of AMF within a root system reflects the spread of external hyphae.

The purpose of this study was to determine the effect of a range of salinity on the symbiotic efficiency (in terms of plant growth promotion and nutrient uptake) of two AMF and also on the infective ability of the AM mycelia generated by the fungi. To determine these symbiotic values, two AMF were compared: one isolated from saline soils (*Glomus* sp.) and the other (*Glomus deserticola* Trappe, Bloss and Menge) from a collection; the latter had been previously shown to be an efficient fungal isolate against drought and salt stress (Ruiz-Lozano et al. 1995a, 1995b, 1996).

Materials and methods

Experimental design

Plants were cultivated in containers (Fig. 1) of 21 cm length, 5 cm width and 10 cm depth, which had three adjacent compartments arranged horizontally and separated by a 60-μm nylon mesh. The nylon mesh retains roots but allows AM hyphae to pass (Tobar et al. 1994a, 1994b; Ruiz-Lozano and Azcón 1995) so that hyphae

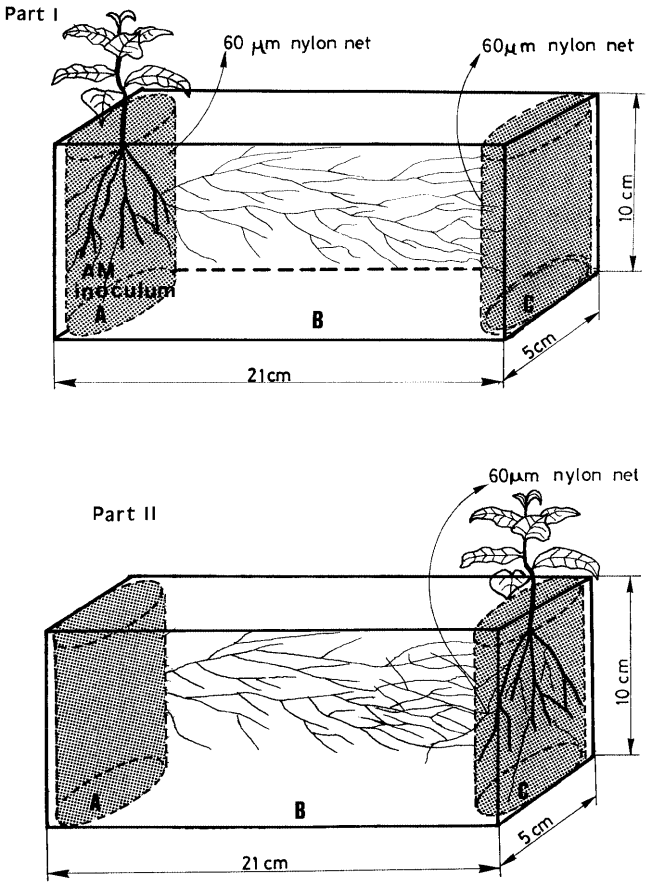

Fig. 1 Schematic representation of containers used to cultivate plants

are able to grow from the root compartment (A) through the hyphal compartment (B) to the root compartment (C). The distance between compartments A and C was 15 cm. The containers were filled with the experimental soil with one of the three salt levels assayed.

Part I

The experiment consisted of a completely randomized factorial block with two factors: (1) mycorrhizal inoculation with one of two *Glomus* species plus a nonmycorrhizal treatment, and (2) three salt concentrations (0.25, 0.50 and 0.75 g NaCl kg^{-1} dry soil) added as a water solution. Two seeds of *Lactuca sativa* L. cv. Romana were sown in compartment A and after emergence thinned to 1 seedling pot^{-1}. Four replicates per treatment were made totalling 36 experimental units (1 plant $unit^{-1}$). Plants were grown for 12 weeks.

Part II

The second part of the experiment had the same design as part I, and was designed to evaluate the infectivity of mycelia generated by the donor root grown in compartment A, as affected by the salt level existing in the soil. This part was carried out after completion of part I. Hence the only source of inoculum was the detached mycelia (and eventually spores) developed in hyphal compartment B, which could have also reached compartment C. Two seeds of *L. sativa* cv. Romana were sown in compartment C and after emergence thinned to 1 seedling pot^{-1}. Four replicates for

each treatment gave a total of 36 pots. Plants were grown for 5 weeks.

Soil and biological material

Loamy soil was collected from the grounds of the Estación Experimental del Zaidín (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 for 3 days). The soil had a pH of 8.1; 1.81% organic matter; nutrient concentrations of 2.5 mg N kg^{-1}, 6.2 mg P kg^{-1} ($NaHCO_3$-extractable P, 132.0 mg K kg^{-1}. The soil was composed of 35.8% sand, 43.6% silt and 20.5% clay. The electrical conductivity (EC) was 0.7 dS m^{-1}. Three concentrations (0.25, 0.5 and 0.75 g NaCl kg^{-1} dry soil) of saline solution were applied to the soil/sand mixture by appropriate dilution of 2 M NaCl. These concentrations were selected according to a preliminary test to determine the maximum concentration of salt which does not inhibit lettuce seed germination and the initial growth of seedlings. The EC in the soil after salt application was 1.1, 1.4 and 1.7 dS m^{-1}, respectively. The soil was placed in the containers described in Fig. 1 so that compartments A and C contained 300 g substrate while compartment B contained 600 g.

Mycorrhizal inoculum for each endophyte was bulked in an open pot culture of *Allium cepa* L. and consisted of soil, spores, mycelia and infected root fragments. Two AM species were used as inocula. The first one, belonging to the Estación Experimental del Zaidín collection (Ruiz-Lozano et al. 1995a) was *Glomus deserticola*, isolate IIAG8903, and the second was a *Glomus* species (isolate GPR31, Diaz 1992) obtained from soils with a low level of salinity in southeast Spain (Diaz and Honrubia 1994) and grown in similar soil to the one in which it originates). Five grams of inoculum possessing similar characteristics (an average of 35 spores g^{-1} and 80% of root infected) in the two *Glomus* isolates were added to compartment A at sowing, just below the seeds of *Lactuca sativa* L. cv. Romana. Nonmycorrhizal treatments received the same amount of autoclaved inoculum together with a 2-ml aliquot of a filtrate (<20 μm) of the AM inoculum in order to provide a general microbial population free of AM propagules.

Growth conditions

Plants were grown in a greenhouse with a 16/8 h day/night cycle and 80% relative humidity. Day and night temperatures varied from one day to another, but day temperatures did not exceed 35 °C and night temperatures did not fall below 21 °C. Water was supplied daily to maintain soil at field capacity during the entire period of plant growth.

Plants were fertilized with Hewitt's (1952) nutrient solution (10 ml $week^{-1}$ pot^{-1}) lacking P.

Measurements

At harvest, the root system was separated from the shoot and dry weights were determined after drying in a forced draft oven at 70 °C for 2 days. Concentrations of shoot N (micro-Kjeldahl) and P (Olsen and Dean 1965) were measured and contents calculated according to the shoot dry weight. Shoot N- and P-use efficiency were determined as the ratio of shoot dry weight (milligram) produced per milligram of total shoot N or P content.

The percentage of mycorrhizal root colonization was estimated after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v) according to Phillips and Hayman (1970). Quantification was performed using the grid-line intersect method (Giovannetti and Mosse 1980). The total mycorrhizal root was calculated as: % mycorrhizal root length × root dry weight × ratio length/dry weight, and expressed as centimeters of mycorrhiza per plant. Finally, the mycorrhizal effect (expressed as milligrams per unit of mycorrhiza × 10^3) was calculated as milligrams of shoot dry weight or N and P contents relative to the total length of mycorrhiza formed.

Data were subjected to a two-way ANOVA with AM treatments and salinity level as factors. When the main effects were significant ($P<0.05$), differences among means were evaluated for significance by Duncan's multiple range test (Duncan 1955) in an orthogonal design. For the percentage values an arcsin transformation was made before the statistical analysis.

Results

Part I

Mycorrhizal colonization positively affected plant growth at all of the salt levels used (Fig. 2). *Glomus* sp. did not significantly increase shoot biomass production compared to uninoculated controls only under low salinity (1.1 dS m^{-1}). At this salt level, the mycorrhizal effect on growth was 52% in *G. deserticola*-colonized plants, while it was insignificant for plants colonized by *Glomus* sp. At the highest salt level (1.7 dS m^{-1}), the mycorrhizal effect on shoot biomass production was 100% (*G. deserticola*-colonized plants) and 82% (*Glomus* sp.-colonized plants) over controls. In contrast, increasing the salinity from 1.1 dS m^{-1} to 1.7 dS m^{-1} did not affect the growth of control plants.

Both mycorrhizal treatments had increased root development compared to control plants. However, increasing salinity reduced the root weight in control and *G. deserticola*-colonized plants (Fig. 2). Root dry weight decreased by 47% in control plants and by 21% in *G. deserticola*-colonized plants when grown at 1.1 dS m^{-1} relative to plants grown at 1.7 dS m^{-1}. In contrast, roots of *Glomus* sp.-colonized plants increased by 26% and by 57% when grown at 1.1 dS m^{-1} relative to those of plants grown at 1.4 and 1.7 dS m^{-1}, respectively.

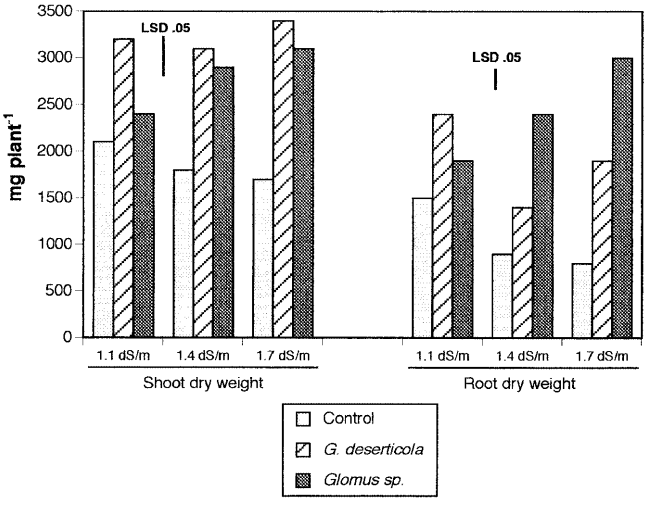

Fig. 2 Shoot and root dry weights (mg $plant^{-1}$) in non-mycorrhizal or mycorrhizal lettuce plants grown at three levels of salinity (1.1, 1.4 or 1.7 dS m^{-1}) in compartment A (part I). *LSD* Least significant difference

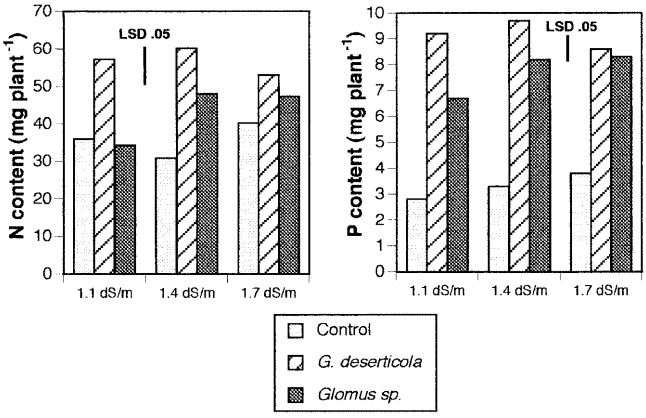

Fig. 3 Shoot N and P contents (mg plant^{-1}) in non-mycorrhizal or mycorrhizal lettuce plants grown at three levels of salinity (1.1, 1.4 or 1.7 dS m^{-1}) in compartment A (part I)

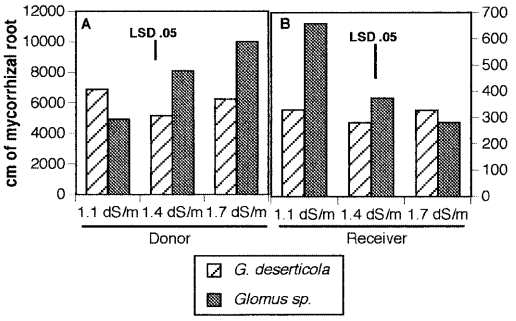

Fig. 4 Total arbuscular mycorrhizal (AM) colonization (cm mycorrhizal root) formed by *Glomus deserticola* and *Glomus* sp. in donor (**A**) or receiver (**B**) plants

Data on N and P contents in shoots (Fig. 3) showed that plants colonized by *Glomus* sp. accumulated these nutrients more efficiently at the highest salt level than under the lowest salt level. In contrast, increasing salinity did not significantly alter N and P accumulation by control and *G. deserticola*-colonized plants. The difference in effectiveness of *G. deserticola* and *Glomus* sp. on N and P uptake and translocation to shoots was evident at the two lowest salt levels (1.1 and 1.4 dS m^{-1}), where *G. deserticola* was the most efficient endophyte (Fig. 3). Increasing salinity had a negative effect on shoot N- and P-use efficiencies in control plants, while these values were unaffected by increasing salinity in mycorrhizal plants (Table 1).

The two AMF actively colonized the root under all salt levels assayed (Table 2). At 1.1 dS m^{-1}, the colonizing ability of the autochthonous fungus (*Glomus* sp.) from saline soil was lower than that of *G. deserticola* when expressed in percentages (Table 2) or total mycorrhizal root formed (Fig. 4A). In contrast, at the highest salt levels, *Glomus* sp. showed a similar percentage of colonization and higher total mycorrhizal root formed (Fig. 4A). This effect is explained by the important stimulation of root development by this AMF.

Figure 5 shows the results of shoot dry weight and nutrient contents (N and P) expressed in relation to the total length of mycorrhiza formed (milligram of shoot dry weight or nutrient contents per unit of mycorrhiza). *G. deserticola* exhibited a higher symbiotic efficiency compared to *Glomus* sp. Both fungi behaved similarly

Table 1 Shoot N- and P-use efficiency in mycorrhizal or non-mycorrhizal lettuce plants grown at three levels of salinity in compartment A (part I) (see Fig. 1 for description). Means followed by the same letter within each calculated efficiency (N or P) are not significantly different ($P<0.05$) as determined by Duncan's multiple-range test ($n=4$)

Treatment	N-use efficiency			P-use efficiency		
	(mg shoot biomass produced mg^{-1} shoot nutrient content)					
	1.1 dS m^{-1}	1.4 dS m^{-1}	1.7 dS m^{-1}	1.1 dS m^{-1}	1.4 dS m^{-1}	1.7 dS m^{-1}
Control	58.0bc	58.2bc	42.2d	750a	545b	447c
Glomus deserticola	55.8c	51.5c	64.3ab	348d	320d	395cd
Glomus sp.	69.9a	60.4abc	65.5ab	358d	354d	374cd

Table 2 Mycorrhizal colonization in mycorrhizal or non-mycorrhizal lettuce plants grown at three levels of salinity in compartment A (part I) or compartment C (part II) (see Fig. 1 for description). Means followed by the same letter for each compartment (part I or part II) are not significantly different ($P<0.05$) as determined by Duncan's multiple-range test ($n=4$)

Treatment	Mycorrhizal colonization (% root length)					
	Part I			Part II		
	1.1 dS m^{-1}	1.4 dS m^{-1}	1.7 dS m^{-1}	1.1 dS m^{-1}	1.4 dS m^{-1}	1.7 dS m^{-1}
Control	0e	0e	0e	0d	0d	0d
G. deserticola	61c	78a	70b	75ab	64c	69bc
Glomus sp.	55d	72b	71b	80a	65c	73ab

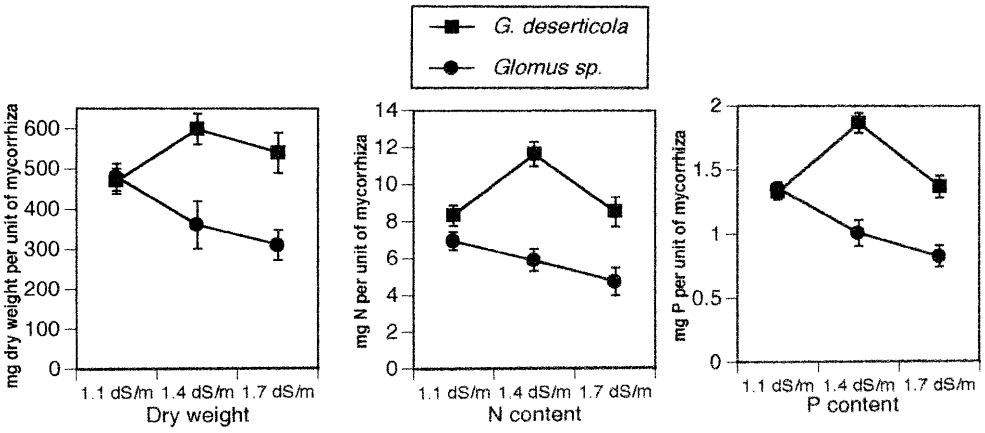

Fig. 5 Mycorrhizal effect on shoot dry weight and N and P contents (mg unit mycorrhiza$^{-1} \times 10^3$) in lettuce plants inoculated with *G. deserticola* or *Glomus* sp. and grown at three levels of salinity (1.1, 1.4 or 1.7 dS m^{-1}) in compartment A (part I)

at 1.1 dS m^{-1}, but differences became evident when plants were cultivated at 1.4 and 1.7 dS m^{-1}.

Part II

In this part we evaluated the effect of salinity on external growth and/or viability of mycelia generated by each fungus during the first plant growth period. To do so, we determined the percentage (Table 2) and total length of mycorrhizal root (Fig. 4B) formed on a receiver host plant by the extraradical mycelia detached from a donor plant, as an estimate of the proportion of active extraradical mycelium. Results in Fig. 4B demonstrated that the colonizing ability of the external mycelium from each fungus was differently affected by increasing salinity. A similar length of mycorrhiza formed in donor roots colonized by *G. deserticola* at each salt level also resulted in equal lengths of mycorrhiza formed at each salt level in receiver plants. In contrast, an increasing length of mycorrhiza formed in donor roots colonized by *Glomus* sp. at increasing salinity resulted in decreased total mycorrhiza formation in receiver plants.

Discussion

Mycorrhizal symbiosis is a key component in helping plants cope with adverse environmental conditions. Previous observations have demonstrated the beneficial effect of mycorrhiza on the growth of salt grass (Allen and Cunningham 1983), onion (Hirrell 1981) or lettuce (Ruiz-Lozano et al 1996) under salt stress conditions. In this study, both AMF assayed efficiently protected the host plant against the detrimental effects of salt. Mycorrhizal plants were not negatively affected by increasing salinity in growth or in nutrient acquisition. However, when the mycorrhizal responses are expressed per unit of mycorrhiza formed, both AMF differed in their symbiotic efficiencies. These differences are more evident at the two highest salt levels. In general, *Glomus* sp.-colonized plants grew less and accumulated slightly less N and P, whereas they formed a higher amount of mycorrhiza. Regarding the different salt levels assayed, except for 1.1 dS m^{-1}, the total mycorrhizal root length was highest in *Glomus* sp.-colonized donor plants, but mycorrhizal responses increased in *G. deserticola*-colonized plants. This superior ability of *G. deserticola* for improving plant growth and mineral nutrition could be due to a higher rate of spread of extraradical mycelium than that of *Glomus* sp. Specific and individual behaviour of AMF have been described in terms of induced host-plant tolerance to extreme soil temperatures, pH, moisture and salinity (Menge 1983). In fact, the presence of each AMF in the roots could alter the osmotic balance of leaves, and each fungus could therefore influence the composition of carbohydrates (Augé et al. 1987) and proline (Ruiz-Lozano et al. 1995a) in the host plant in a particular way. The differing behaviour of AMF was also evidenced by Ruiz-Lozano et al. (1996) in lettuce plants subjected to salt stress and inoculated with three different AMF. In that study, the effect of *G. mosseae* and *G. fasciculatum* on salt tolerance seemed to be based on increased gas exchange (increased photosynthetic rate, transpiration, stomatal conductance and water use efficiency) rather than on nutrient uptake (N or P). *G. deserticola*, in contrast, seemed to protect host plants against salt stress by increasing P uptake, in addition to the above-mentioned physiological processes. In the present study, *Glomus* sp. (from saline soils) protected the host plant against salinity by stimulating root growth, while in the case of *G. deserticola,* the increase in N and P accumulation is the basis for plant protection against salinity, in agreement with previous results (Ruiz-Lozano et al. 1996).

The differing behaviour of both AMF species found in this study also suggests that the beneficial effect of AMF on host plants under saline conditions is not necessarily related to the level of salinity existing in the habitat in which the fungal isolates originate. This idea is in agreement with results by Poss et al. (1985) recorded for tomato plants inoculated with isolates of *G.*

mosseae and *G. fasciculatum* isolated from saline soils. They found that colonized plants grew less under both low and high salt concentrations in the medium. Coperman et al. (1996) also found salt stress alleviation in plants inoculated with AMF from nonsaline soil, but growth suppression in plants inoculated with AMF from saline soil.

In this study we measured the effect of salinity on the infectivity of the external mycelium as the length of mycorrhizal root formed in a receiver plant in relation to the total mycorrhiza existing in the donor plant. This type of estimate is more realistic than the quantification of total hyphae because most of the methods used make no distinction between active and inactive mycelium. In fact, AM colonization in the receiver host plant serves as a measure of the potential of each AM fungi to perform under saline conditions. Results showed that soil salinity levels negatively affected the growth of hyphae and/or viability of *Glomus* sp. to a higher extent than those of *G. deserticola*.

Our results are an indication of the varying potential of AMF to traverse and grow through zones of root-free soil under saline conditions. The distance that each AMF can cross into the experimental soil (15 cm) depends to some extent on the capacity of the hyphae to advance from donor to receiver roots under increasing salt levels in the medium. It is likely to depend on specific fungal compatibilities as well as on environmental conditions. This idea also agrees with results by McMillen et al. (1998) on germinating spores in saline soil. The colonizing ability of the extraradical mycelium formed by *G. deserticola* was more efficient than that formed by *Glomus* sp. since a larger proportion of mycorrhiza formed in receiver plants from donor roots colonized by *G. deserticola*. The characteristics and limitations of AMF must be considered in order to determine the effectiveness of a particular fungal species in the colonization of a new habitat where it has to cope with particular stress conditions. The results obtained contribute to the understanding and determination of the differences between fungal isolates of AMF according to changes in environmental conditions. A fungus like the present isolate of *G. deserticola* seems to be an excellent candidate for inoculation purposes in saline soils. Attempts to predict the behaviour of a fungus under field conditions require an understanding of its interactions and compatibility with the environmental conditions.

In conclusion, the results from this study suggest that the isolate IIAG8903 of *G. deserticola* is a more efficient AMF under saline conditions than the autochthonous fungus from a saline soil. In spite of the fact that plant growth and nutrient uptake improvement in response to both fungi was similar, *Glomus* sp. needed to form a higher amount of mycorrhiza than *G. deserticola* to achieve a similar symbiotic effect on the host plant. In addition, the mycelial network generated by *G. deserticola* was more infective than that of the other *Glomus* sp.

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