

Effects of Arbuscular-Mycorrhizal *Glomus* Species on Drought Tolerance: Physiological and Nutritional Plant Responses

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The tolerance of lettuce plants (*Lactuca sativa* L. cv. Romana) to drought stress differed with the arbuscular-mycorrhizal fungal isolate with which the plants were associated. Seven fungal species belonging to the genus *Glomus* were studied for their ability to enhance the drought tolerance of lettuce plants. These fungi had different traits that affected the drought resistance of host plants. The ranking of arbuscular-mycorrhizal fungal effects on drought tolerance, based on the relative decreases in shoot dry weight, was as follows: *Glomus deserticola* > *Glomus fasciculatum* > *Glomus mosseae* > *Glomus etunicatum* > *Glomus intraradices* > *Glomus caledonium* > *Glomus occultum*. In this comparative study specific mycorrhizal fungi had consistent effects on plant growth, mineral uptake, the CO₂ exchange rate, water use efficiency, transpiration, stomatal conductance, photosynthetic phosphorus use efficiency, and proline accumulation under either well-watered or drought-stressed conditions. The ability of the isolates to maintain plant growth effectively under water stress conditions was related to higher transpiration rates, levels of leaf conductance, and proline, N, and P contents. Differences in proline accumulation in leaves among the fungal symbioses suggested that the fungi were able to induce different degrees of osmotic adjustment. The detrimental effects of drought were not related to decreases in photosynthesis or water use efficiency. Neither of these parameters was related to P nutrition. The differences in P and K acquisition, transpiration, and stomatal conductance were related to the mycorrhizal efficiencies of the different fungi. Our observations revealed the propensities of different *Glomus* species to assert their protective effects during plant water stress. The greater effectiveness of *G. deserticola* in improving water deficit tolerance was associated with the lowest level of growth reduction (9%) under stress conditions. The growth of plants colonized by *G. occultum* was reduced by 70% after a progressive drought stress period. In general, the different protective effects of the mycorrhizal isolates were not associated with colonizing ability. Nevertheless, *G. deserticola* was the most efficient fungus and exhibited the highest levels of mycorrhizal colonization, as well as the greatest stimulation of physiological parameters.

Arbuscular-mycorrhizal (AM) fungi are important in sustainable agriculture because they improve plant water relations and thus increase the drought resistance of host plants (1, 21), they improve disease control (17), and they increase mineral uptake, which reduces the use of fertilizers. Improved plant water status and changes in water relations have been attributed to a wide variety of mechanisms, including some mechanisms not directly related to phosphorus nutrition or water uptake (7, 26). In fact, the inconclusive information that has been obtained suggests that more studies will be required to determine the direct or indirect mechanisms which control plant water relations in AM fungus-plant symbioses. The abilities of specific fungus-plant associations to tolerate drought are of great interest.

Although the relationship between AM fungi and their host plants is usually considered nonspecific, this relationship is tightly regulated at both the structural and physiological levels. The lack of specificity results in considerable variation in symbiotic responses (15). Given the physiological differences within species and even within geographic isolates (6), the biodiversity of AM endophytes is great. Little is known about the physiological specialization and functioning of these soil microorganisms. Knowledge concerning specific relationships between plants and fungi is important for successful utilization of AM fungi under particular conditions. The variability within

endophytes and the different symbiotic strategies that occur in response to drought stress, as well as the compatibility with different environmental conditions, suggest that it may be possible to select effective fungal species.

An efficient organism is an organism whose physiological and biochemical processes are such that it can successfully cope with limiting environmental conditions (26). Generally, the decline in CO₂ assimilation rate associated with a reduction in leaf water status has been attributed primarily to stomatal closure and the resulting increase in leaf epidermal resistance. Transpiration is normally suppressed by water stress concurrently with the suppression of photosynthesis. Thus, particular abilities of AM endophytes to alter physiological plant parameters that enhance adaptation to low soil water content can provide suitable criteria for the selection of inoculants.

The AM fungi used in this study were seven morphologically distinguishable *Glomus* species. All of these organisms were able to grow in neutral to alkaline mediterranean soils which are prone to water stress. In this study we compared *Glomus* species with each other, with a nonmycorrhizal P-fertilized control, and with an unfertilized control and determined the effects of the fungal isolates on plant growth, mineral uptake, the CO₂ exchange rate, water use efficiency, transpiration, stomatal conductance, photosynthetic P use efficiency, and proline accumulation under well-watered and drought stress conditions.

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TABLE 1. Effects of AM fungi and drought acclimation treatments on lettuce shoot and root dry weights and root/shoot ratios^a

Treatment	Shoot dry wt (g)		Root dry wt (g)		Root/Shoot ratios	
	Well-watered plants	Drought-stressed plants	Well-watered plants	Drought-stressed plants	Well-watered plants	Drought-stressed plants
Control	0.56k	0.15l	0.49f	0.16f	0.87c	1.06a
PO ₄ ³⁻	1.15j	0.26l	0.87e	0.29f	0.75b	1.11a
<i>G. deserticola</i>	6.00a	5.50bc	2.23a	1.66b	0.37efg	0.30fgh
<i>G. etunicatum</i>	5.94ab	4.98d	1.87b	1.44c	0.31fgh	0.29gh
<i>G. intraradices</i>	5.43c	4.52e	1.75b	1.30cd	0.32fgh	0.29gh
<i>G. fasciculatum</i>	5.08d	4.39e	1.68b	1.38d	0.33fgh	0.31fgh
<i>G. mosseae</i>	4.54e	3.87f	1.43cd	1.30cd	0.31fgh	0.34fgh
<i>G. caledonium</i>	3.53g	2.70h	1.46cd	0.97e	0.41e	0.36ef
<i>G. occultum</i>	2.20i	0.67k	1.27d	0.35f	0.57d	0.57d

^a Values are the means of five replications. Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range test.

MATERIALS AND METHODS

Experimental design. The experiment was performed by using nine treatments: seven AM fungus treatments, one P-fertilized non-AM fungus control, and one unfertilized non-AM fungus control. Ten replications were used for each treatment; thus, we used a total of 90 pots. For each treatment, one-half of the plants were maintained at a soil water potential of -0.04 MPa (field capacity) until they were harvested (11 weeks after planting). The other plants were subjected to drought stress conditions as follows: the soil water potential was -0.06 MPa during week 7, -0.10 MPa during week 8, and -0.17 MPa during week 9. After these drought periods, the plants were watered so that the soil water potential was -0.04 MPa for the last 2 weeks to determine their capacity for recovery.

Soil and biological material. The soil which we used was a loam soil that was collected in Granada Province in southern Spain and had a pH of 8.1. This soil contained 6.2 mg of available P (NaHCO₃-extractable P) per g, 1.8 mg of NO₃⁻ N per g, 0.8 mg of NH₄⁺ N per g, 132.0 mg of K per g, 1.81% organic matter, 35.8% sand, 43.6% silt, and 20.5% clay. The soil was sieved (pore size, 2 mm), diluted with quartz sand (particle diameter <1 mm) (1:1, vol/vol), and sterilized by steaming the mixture at 100°C for 1 h on 3 consecutive days. Each pot was filled with 500 g of the sterilized soil-sand mixture. The mycorrhizal inoculum for each endophyte consisted of soil, spores, mycelium, and infected root fragments obtained from an open pot culture of *Allium cepa* L. The AM fungal species used, which were obtained from the Zaidin Experimental Station collection, were *Glomus etunicatum* (Becker et Gerd), *Glomus fasciculatum* (Thax. sensu Gerd.) Gerd. et Trappe, *Glomus mosseae* (Nicol. et Gerd.) Gerd. et Trappe, *Glomus deserticola* (Trappe, Bloss et Menge), *Glomus caledonius* (Nicol. et Gerd.) Trappe et Gerd., *Glomus intraradices* (Schenck et Smith), and *Glomus occultum* (Walker). *Glomus* isolate preparations that had similar characteristics (an average of 30 spores per g and 75% of roots infected) were used as inocula. A 5-mg portion of inoculum was added to each pot at sowing time just below *Lactuca sativa* L. cv. Romana seeds. Controls received sterilized inoculum. Four seeds were sown in each pot, and the seedling were thinned after emergence so that there was one seedling per pot.

Growth conditions. The plants were grown in a controlled environmental chamber with day and night temperatures of 25 and 15°C, respectively, day and night relative humidities of 70 and 80%, respectively, and a photoperiod of 14 h. The photosynthetic photon flux density was 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Water was supplied daily to maintain the soil moisture level close to field capacity (-0.04 MPa) during the first 6 weeks of plant growth and then again after plants were drought stressed by withholding irrigation (24). The pots were allowed to dry so that the soil water potentials were -0.06 MPa during week 7, -0.10 MPa during week 8, and -0.17 MPa (near the wilting point) during week 9; each drought period lasted 1 week. After the drought periods, the plants were watered so that the soil water potential was -0.04 MPa for the last 2 weeks.

The plants were fertilized with Hewitt's (14) nutrient solution (10 ml per week per pot) lacking P. When P was needed, it was supplied as KH₂PO₄ (7 mg per pot per week) for P-fertilized plants to equalize the sizes and tissue P concentrations in AM fungus-treated and P-fertilized plants.

Measurements. When the plants were harvested (11 weeks after planting), the root system of each plant was separated from the shoot, and dry weights were determined after the preparations were dried for 36 h at 70°C.

The presence of an AM fungus infection was determined visually by clearing washed roots in 10% KOH and staining the preparation with 0.05% (vol/vol) trypan blue in lactophenol as described by Phillips and Hayman (25). Quantities were determined by the line-intercept method of Giovannetti and Mosse (11).

The CO₂ exchange rate, the transpiration rate, stomatal conductance, and water use efficiency were determined at the end of the experiment at a photosynthetic photon flux density of 1,180 $\mu\text{mol m}^{-2} \text{s}^{-1}$; the light was provided by a halogen lamp (18). A model LCA-3 portable, integrated infrared CO₂ analyzer

(Analytical Development Co.) was used for these determinations. Measurements were made 2 h after the light was turned on. Photosynthetic P use efficiency was calculated as the ratio of carbon exchange rate to leaf P concentration. Proline content was determined by colorimetry (4). The concentrations of plant leaf N (micro-Kjeldahl method) and P (22) were also determined. The concentrations of K were determined by flame photometry, and the concentrations of Ca and Mg were determined by atomic absorption spectrometry (16), using a Perkin-Elmer model 5000 spectrophotometer.

Soil water potential was determined with a 15×10^5 -Pa pressure plate apparatus (model 1500 ceramic plate extractor; Soilmoisture Equipment Corp.), and soil water content was determined by weighing samples before and after drying at 110°C for 24 h.

Data were subjected to an analysis of variance. A Two-way analysis of variance with randomized complete blocks was performed by using the following parameters as sources of variation: fungus, water, fungus-water interaction, and error. When the main effect was significant ($P < 0.05$), differences between means were evaluated for significance by using Duncan's multiple-range test (8) in an orthogonal design.

RESULTS

The seven *Glomus* species used in this experiment all increased plant growth under both well-watered and drought stress conditions (Table 1).

The differences in shoot growth stimulation between the least effective fungal isolate (*G. occultum*) and the most effective fungal isolate (*G. deserticola*) ranged from 273% under well-watered conditions to 821% under drought stress conditions. The effects on root growth were similar (Table 1).

Drought decreased shoot biomass by between 9% (in plants colonized by *G. deserticola*) and 70% (in plants inoculated with *G. occultum*). The ranking of *Glomus* species for inducing water stress tolerance, based on the relative decreases in shoot dry weight, was as follows: *G. deserticola* > *G. fasciculatum* > *G. mosseae* > *G. etunicatum* > *G. intraradices* > *G. caledonium* > *G. occultum*. The greater ability of *G. deserticola* to induce tolerance in the host plant resulted in the smallest decrease in growth under stress conditions.

The proline content of leaves of inoculated lettuce plants appeared to be a good parameter to measure water stress. Drought increased the concentration of this osmotic regulator (Table 2), especially in plants colonized by *G. deserticola*.

Plant nutrient uptake was strongly influenced by the mycosymbiont involved in the association (Table 3). The period of drought acclimation did not significantly affect the nutrient contents of plants colonized by the most efficient endophytes (*G. deserticola* and *G. etunicatum*); however, nutrient acquisition by plants associated with *G. occultum* was limited. While the P and K contents were kept at constant levels by the most efficient fungus, they were reduced by the less active fungi under adverse conditions. The differences in P contents between AM fungus treatments ranged from 224 to 369% de-

TABLE 2. Effects of AM fungi and drought treatments on root colonization and leaf proline contents of lettuce plants^a

Treatment	% Infected		Proline content (nmol/g [fresh wt])	
	Well-watered plants	Drought-stressed plants	Well-watered plants	Drought-stressed plants
Control	0	0	12.3g	16.2g
PO ₄ ³⁻	0	0	14.8g	16.4g
<i>G. deserticola</i>	92.3a	94.1a	79.3bcd	119.6a
<i>G. etunicatum</i>	59.4d	65.5cd	62.8cde	93.4ab
<i>G. intraradices</i>	86.5ab	86.5ab	57.8def	40.8ef
<i>G. fasciculatum</i>	68.5cd	63.2cd	50.0def	94.7ab
<i>G. mosseae</i>	69.7c	79.4b	38.3ef	79.4bcd
<i>G. caledonium</i>	32.6f	27.2f	30.5f	42.6ef
<i>G. occultum</i>	42.0e	32.0f	50.8def	87.9bc

^a See Table 1, footnote a.

pending on soil water availability. *G. intraradices* significantly increased the Ca and Mg contents of plants under any water regime.

The greatest CO₂ exchange rates and water use efficiency values were observed in plants colonized by *G. deserticola*. The effects of the other isolates were highly variable (Table 4); the other isolates did not have consistent effects on drought tolerance in relation to these two parameters. In the case of transpiration and stomatal conductance, the values varied, as did mycorrhizal efficiency.

In response to fungal endophytes we observed increases of 409% (CO₂ exchange rate), 422% (water use efficiency), 248% (transpiration), and 247% (stomatal conductance) when we compared the values obtained with the most and least efficient *Glomus* species. Inoculation with *G. deserticola* maximized the values for these parameters under stress conditions as well as nonstress conditions. Photosynthetic P use efficiency was altered by mycorrhizal fungi (*G. caledonium* and *G. occultum* under well-watered conditions) and under stress conditions (*G. deserticola*, *G. intraradices*, and *G. occultum*) (Table 4).

DISCUSSION

In this study we determined how diverse AM fungal endophytes ameliorate drought stress in colonized plants. Some fungal isolates were more effective than others at increasing host plant drought tolerance. The AM fungi which we used were *Glomus* species that are predominate organisms in neutral to alkaline mediterranean soils of agricultural interest (13). In these arid to semiarid soils, soil moisture affects the movement of nutrients in the soil. Extraradical AM fungal mycelia extend the root surface area and enhance the acquisition of nutrients and water by the roots (5). Effects of AM fungi on plant water status have been associated with improved host nutrition, particularly P nutrition (11, 12). However, it has also been reported that the effect of AM fungi on drought resistance may be independent of P uptake (3, 5, 27).

AM fungal efficiency can be measured in terms of host plant growth under different environmental conditions. The two most efficient endophytes used in this study were *G. deserticola* and *G. etunicatum* when the organisms were not under stress conditions. As Table 1 shows, drought stress reduced the growth of plants colonized by *G. deserticola* by 9% and the growth of plants colonized by *G. etunicatum* by 17%. Nutrient contents did not significantly change in plants colonized by *G. deserticola* and *G. etunicatum*. Nevertheless, CO₂ exchange rates and water use efficiency values were significantly lower in plants colonized by *G. etunicatum* than in plants colonized by *G. deserticola*. The different effects of these fungi on alleviating stress appeared to be based on physiological processes rather than nutrient uptake by the host.

Net photosynthesis declines as leaf water status decreases if water stress is sufficiently severe. In this study, plants were affected by stress during a short period of time (3 weeks), and CO₂ assimilation was not sensitive to reductions in leaf potential until a CO₂ exchange rate threshold value was reached.

Increased CO₂ assimilation has been considered a plant strategy for drought stress tolerance (10). Similarly, changes in stomatal conductance (2) and transpiration have been reported to be mechanisms by which AM fungus-colonized plants increase drought resistance. No close relationship between CO₂ exchange rate in AM fungus-colonized plants and

TABLE 3. Effects of AM fungi and drought acclimation treatments on N, P, K, Ca, and Mg contents of lettuce plants^a

Plants	Treatment	N content (mg/plant)	P content (mg/plant)	K content (mg/plant)	Ca content (mg/plant)	Mg content (mg/plant)
Well watered	Control	18.1ij	0.83fg	57.0f	15.3h	4.9ef
	PO ₄ ³⁻	21.0i	1.12f	63.4ef	15.7h	6.2e
	<i>G. deserticola</i>	37.7ef	3.61a	97.6a	29.1cde	8.4d
	<i>G. etunicatum</i>	40.6bcdef	3.55ab	90.6abc	31.1cd	10.1cd
	<i>G. intraradices</i>	33.8g	2.90cd	98.8a	52.2a	16.9a
	<i>G. fasciculatum</i>	43.6b	2.88cd	99.8a	34.3bc	8.8d
	<i>G. mosseae</i>	42.7bc	2.56d	83.1cd	23.9efg	10.4cd
	<i>G. caledonium</i>	41.5bcde	1.93e	89.2abc	31.2cd	10.0cd
	<i>G. occultum</i>	37.1fg	1.61e	70.1e	19.1gh	6.1e
	Drought stressed	Control	15.3j	0.64g	51.0f	13.9h
PO ₄ ³⁻		20.3i	0.87fg	60.1ef	14.1h	4.9ef
<i>G. deserticola</i>		39.5cdef	3.51ab	91.0abc	28.8cde	9.1d
<i>G. etunicatum</i>		42.6bcd	3.18bc	84.2bcd	28.5cdef	8.8d
<i>G. intraradices</i>		38.1def	2.40d	88.9abc	39.0b	12.4bc
<i>G. fasciculatum</i>		46.8a	2.76cd	92.9ab	38.5bc	12.7b
<i>G. mosseae</i>		41.0bcde	2.00e	70.1e	20.6fg	8.3d
<i>G. caledonium</i>		41.7bcd	1.60e	75.0de	25.5def	8.1de
<i>G. occultum</i>		26.3h	0.95f	38.1g	13.3h	3.9f

^a See Table 1, footnote a.

TABLE 4. Effects of AM fungi and drought acclimation treatments on CO₂ exchange rate, water use efficiency, transpiration, stomatal conductance, and photosynthetic P use efficiency in lettuce plants^a

Plants	Treatment	CO ₂ exchange rate (nmol m ⁻² s ⁻¹)	Water use efficiency (mmol of CO ₂ /mol of H ₂ O, 10 ³)	Transpiration (μmol m ⁻² s ⁻¹)	Stomatal conductance (mmol m ⁻² s ⁻¹)	Photosynthetic P use efficiency (nmol m ⁻² s ⁻¹ mg ⁻¹)
Well watered	Control	34.9f	47.4fg	11.4g	1.7ij	42.0c
	PO ₄ ³⁻	45.3f	43.1fg	13.5g	1.7ij	40.4c
	<i>G. deserticola</i>	150.6b	248.6ab	54.3a	5.2a	41.6c
	<i>G. etunicatum</i>	94.4cde	147.9de	51.7a	4.9ab	26.5de
	<i>G. intraradices</i>	97.9cde	172.7cde	43.8b	4.5cd	18.8e
	<i>G. fasciculatum</i>	97.9cde	116.6ef	43.2b	4.1de	27.8de
	<i>G. mosseae</i>	125.2bc	245.0ab	32.8d	3.1g	48.9bc
	<i>G. caledonium</i>	36.4f	58.9f	29.1de	2.7gh	19.1e
	<i>G. occultum</i>	65.0def	100.3ef	21.9f	2.1i	66.4ab
Drought stressed	Control	30.8f	43.0fg	9.2g	1.3jk	48.1bc
	PO ₄ ³⁻	40.1f	37.9g	9.9g	1.5j	44.7bc
	<i>G. deserticola</i>	198.8a	287.5a	54.1a	5.0ab	56.5b
	<i>G. etunicatum</i>	91.9cde	127.9de	50.5a	4.7bc	28.9cd
	<i>G. intraradices</i>	123.3bc	189.8bcd	40.8bc	3.8ef	51.6bc
	<i>G. fasciculatum</i>	104.2cd	160.3cde	43.5b	4.0ef	37.1cd
	<i>G. mosseae</i>	89.4cde	129.8def	39.0c	3.6f	44.6bc
	<i>G. caledonium</i>	65.3def	144.3de	27.8e	2.5h	63.0ab
	<i>G. occultum</i>	70.8def	108.4ef	11.5g	1.1k	74.1a

^a See Table 1, footnote a.

resistance to drought was found in this study. The protection of mycorrhizal plants against water stress was related to the effects that the endophytes had on increasing leaf conductance and transpiration as well as P and K uptake. Potassium plays a key role in plant water stress and has been found to be the cationic solute which is responsible for stomatal movement in response to changes in bulk leaf water status. Accordingly, the response of *Glomus*-infected plants to stress and K content are closely related.

The adjustment of leaf osmotic potentials requires intracellular osmotic balance (19, 20). Proline is accumulated in the leaves and enhances osmotic adjustment. In our study the proline content was greater in drought-stressed plants than in well-watered plants. As relievers of stress, *G. deserticola* and *G. etunicatum* increased host proline contents by only 50% as a consequence of drought, while *G. occultum*, *G. fasciculatum*, and *G. mosseae* enhanced proline production by 73, 89, and 107%, respectively, to achieve the same effect. A lower proline content is an indication of better tolerance to drought.

Host response differs not only with fungal species, but also with geographic isolates of the same species (6). The response range may be due to changing efficiencies of mineral uptake and also to physiological interactions between the symbionts, since fungal efficiency in a physiological comparison depends on the amount of nutrient transferred per unit of carbohydrate utilized by the AM fungus-plant symbiosis. Mycorrhizal fungi require carbon from the host and increase below-ground respiration (23), which represents a carbohydrate cost for the plant, since the C compounds supplied to the fungus are not available for plant biomass production. Levels of colonization and strategies of infection may also affect the physiological equilibrium in the interaction. The levels of CO₂ exchange in colonized plants ranged from 36.4 nmol m⁻² s⁻¹ for *G. caledonium* to 150.6 nmol m⁻² s⁻¹ for *G. deserticola*. The levels of mycorrhizal colonization differed among the endophytes and were highest in plants colonized by *G. deserticola*.

The high level of infectivity of *G. deserticola* despite the low soil water content suggests that this species was the best adapted and/or most aggressive colonizer under drought con-

ditions. *G. deserticola* was also the most effective fungus for increasing drought tolerance of the host plant both in terms of maintaining growth under stress conditions and in permitting more efficient use of water.

Amelioration of drought stress by different AM fungal species can be ascribed to specific physiological (CO₂ fixation, transpiration, water use efficiency) and nutritional (P and K) mechanisms according to the fungus involved in the symbiotic association. Our results support the hypothesis that there are differences in the symbiotic physiology of different host-endophyte associations.

Selection of AM fungi for introduction into dry environments to address specific problem situations is a promising but usually neglected strategy. Suitably adapted AM fungal isolates are potentially important for maintaining and restoring the plant-soil equilibrium in sustainable agriculture situations.

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