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Influence of different *Glomus* species on the time-course of physiological plant responses of lettuce to progressive drought stress periods

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Abstract

The effects of different arbuscular-mycorrhizal (AM) fungi of the genus *Glomus* on lettuce (*Lactuca sativa* L. cv Romana) were compared in terms of foliar area and leaf-gas exchange parameters (photosynthetic activity, water use efficiency (WUE), transpiration and stomatal conductance) following progressive drought stress periods. Measurements were made during periods where AM lettuce plants were either well-watered or subjected to successive and progressive drought periods of 1 week duration (−0.06, −0.10 and −0.17 MPa of soil water potential) and rewatered for the last 2 weeks to test their capacity for stress recovery. Plant growth responses to progressive drought were greatly influenced by the *Glomus* species colonizing the roots. Leaf area was reduced in *G. deserticola*-plants by 9% due to drought, while in *G. occultum*-plants the decline was 70%. During the first period of plant growth (6 weeks), before the exposure to drought, the plant physiological parameters were shortly affected among plants colonized by the different endophytes. After the drought exposure, the activity of specific endophytes increased the differences in leaf gas exchange and related parameters among stressed mycorrhizal plants. Mycorrhizal symbiosis by specific *Glomus* spp. exhibited particular abilities (measured as photosynthetic activity, WUE, transpiration and conductance) according to the level of stress applied. Plants colonized by *G. etunicatum*, *G. mosseae* or *G. occultum* had a high sensitivity for decreasing CO_2 assimilation even to light stress (−0.06 MPa) while *G. fasciculatum*-infected plants decreased such parameters only under severe stress (−0.17 MPa). Plants colonized with *G. etunicatum* induced the highest transpiration and conductance values under all conditions and did not reduce water use efficiency until −0.17 MPa (severe stress). *G. deserticola* was the best endophyte in counteracting drought effects on physiological parameters under the drought regimes tested here. Following re-irrigation, *G. deserticola* showed the highest ability to recover CO_2 assimilation, WUE, conductance and transpiration from drought. These results suggest that the ability of different endophytes to protect the host plant against progressive drought stress cannot be ascribed either to a specific physiological mechanism or to the colonizing ability showed by the endophytes. These observations on the particular physiological responses to drought according to endophytes and stress intensity, clarify an important aspect in relation to the biodiversity of *Glomus* spp.

Keywords: Drought stress period; *Glomus* species; Physiological plant response

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1. Introduction

Efficient use of water and enhanced drought resistance are major goals for increasing plant productivity in drought-prone environments. Many factors can be involved in mycorrhizal plant tolerance to water limitation, such as size or architecture of the root system and the efficiency of water and nutrient uptake, among others [11]. However, AM colonization may increase drought resistance of the host plant by mechanisms independent of these. AM fungi show little host specificity but the nutritional and physiological responses as a result of the infection by different AM fungi can vary considerably [5,23,24]. In fact, AM endophytes differ in their tolerance and in their ability to adapt to environmental conditions [27]. Little is still known about the physiological basis for differences in the influence of various endophytes in plant adaptation to drought. Methods are now available for analyzing more precisely the physiological processes likely to be important determinants of symbiotic efficiency [26]. Verification of differences between *Glomus* species in their protective effect against water stress damage suggests the significance of plant physiological studies involved in drought tolerance.

Mycorrhizal fungi are a sink for photosynthetic products, but may also increase CO_2 assimilation [2,19]. Water limitation is one of the most important factors in decreasing photosynthetic rate [6]. A loss of over 10% of carbon from the shoot would be sufficient to cause serious inhibition of plant growth in the absence of a compensating advantage. Photosynthesis can be completely suppressed by severe water stress [12]. AM plants could be stressed more or less severely according to the nature of the stress (intensity and duration) and the mycorrhizal endophyte. Mycorrhizal infection may influence plant resistance to water stress in ways which are apparently unrelated to phosphorus nutrition [24,25]. In fact, the role of AM fungi in drought tolerance is further complicated by the interactions of several mechanisms such as water uptake by the fungal mycelium [15], changes in plant stomatal sensitivity, perhaps by an increase in ABA production, [18] and also in leaf conductance [1].

Mycorrhizal alteration of physiological processes, such as the capacity of a particular endophyte to increase photosynthesis, transpiration, stomatal conductance and WUE in the course of plant and mycorrhizal development are important determinants of symbiotic efficiency under stress conditions [3].

Ruiz-Lozano et al. [23] reported the particular effects of seven fungal isolates on growth, mineral uptake, CO_2-exchange parameters and proline accumulation after subjecting well-watered plants to drought stress conditions. Since altered plant water relations by AM colonization have important consequences for drought tolerance and acclimatisation, how plants maintain their water status and the leaf gas exchange along successive and progressive drought periods it is of interest. In the present study we compared the effect of these AM fungi on the time-course of physiological plant responses to drought as water stress built up. The main objective of this work was to elucidate the evolution of the physiological mechanisms involved in the protection of AM plants from light, moderate or severe water stress. Additionally our purpose was to determine the rate at which mycorrhizal plants recovered from the water stress.

2. Materials and methods

2.1. Experimental design

Lettuce (*Lactuca sativa* L. cv Romana) plants were colonized by one of seven isolates of AM fungi. One control treatment without mycorrhiza was also used. These treatments were replicated 10 times for a total of 80 pots arranged in a randomized block design (one plant per pot). During the first 6 weeks plants were irrigated by weight daily to field capacity (−0.04 MPa). When the plants were 6 weeks old, one half was maintained at field capacity and the other half was subjected to drought stress by exposing it to successive and progressive drought stress periods (−0.06, −0.10 and −0.17 MPa of soil water potential). After these drought periods, plants were rewatered to −0.04 MPa for the last 2 weeks to determine their capacity to recover photosynthetic parameters.

2.2. Soil and biological materials

The loamy soil collected from Granada province (southern Spain) had a pH of 8.1, contained 6.2 $mg \cdot kg^{-1}$ of available P ($NaHCO_3$-extractable), total N of 1.8 mg NO_3^- kg^{-1} and 0.8 mg NH_4^+ kg^{-1}, 132.0 $mg \cdot kg^{-1}$ of K, 1.81% of organic matter, 35.8% sand, 43.6% silt and 20.5% clay. The soil was sieved (2 mm), diluted with quartz-sand (<1mm) (1:1, soil/sand, v/v) and sterilized by steaming (100°C for 1 h on 3 consecutive days). Pots were filled with 500 g of the sterilized soil/sand mixture. All the AM species belonged to the collection of the Estación Experimental del Zaidín (Ruiz-Lozano et al., 1995), *Glomus etunicatum* (Becker and Gerd), *G. intraradices* (Schenck and Smith) and *G. occultum* (Walker) were from the Instituto Venezolano de Investigaciones Científicas (IVIC); *G. fasciculatum* (Thax. sensu Gerd.) Gerd. and Trappe came from Dijon (INRA); *G. deserticola* (Trappe, Bloss and Menge) was sent from the Instituto de Investigaciones Agrobiologicas de Galicia (CSIC) and *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe and *G. caledonium* (Nicol. and Gerd.) Trappe and Gerd. from Rothamsted Experimental Station. All mycorrhizal inocula consisted of soil, spores, mycelium and infected root fragments from an open pot culture of *Allium cepa L.* Five grams of inoculum possessing similar characteristics (an average of 30 spores g^{-1} and 75% of root infected) in the seven *Glomus* isolates were added to each pot at sowing time just below the seeds of *Lactuca sativa* L. cv. Romana. Control treatment received the same amount of autoclaved inoculum together with a 2-ml aliquot of a filtrate (<20 μm) of the AM fungal inoculum to provide the same microbial population. Four seeds were sown, and thinned after emergence to one seedling per pot.

2.3. Growth conditions

The plants were grown in a controlled environmental chamber with a day/night temperature cycle of 25/15°C, 70/80% relative humidity (RH) and a photoperiod of 14 h. Photosynthetic photon flux density was 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During the first 6 weeks of plant growth, water was supplied daily to maintain soil moisture close to field capacity (−0.04 MPa) and then again after plants were drought-stressed by withholding irrigation [22]. Plants were allowed to dry until soil water potentials reached three different levels consecutively: −0.06, −0.10 and −0.17 MPa (close to wilting point), with each drought period lasting 1 week. After drought periods, plants were watered to −0.04 MPa for the last 2 weeks to allow them to recover from drought.

Plants were fertilized with P-free Hewitt's [16] nutrient solution ($10 ml \cdot week^{-1} \cdot pot^{-1}$).

2.4. Measurements

Except for control plants, the first determinations of CO_2 exchange rate, transpiration rate, stomatal conductance and WUE (ratio of net CO_2 assimilation per unit water transpired) were done when plants were 6 weeks old and subsequently at weeks 7, 8, 9 and 11 at 1180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (photosynthetic photon flux density) using a halogen lamp [7]. A portable, integrated infrared CO_2 analyzer (Analytical Development Company, model LCA-3) was used for these determinations. Measurements were made 2 h after the light was turned on.

Total leaf area and root length were determined by harvesting the plants after a growth of 11 weeks. Roots were cleared and stained according to Phillips and Hayman [21]. Colonized root length was determined by the gridline-intersect method [13].

Soil water potential was determined by pressure plate apparatus (Soilmoisture Equipment Corp., 15 Bar Ceramic Plate Extractor, Cat. No 1500) and soil water content measured by weighing before and after drying at 110°C for 24 h [3].

Data were subjected to analysis of variance. When the main effect was significant ($P < 0.05$) differences between means were evaluated for significance by using a LSD technique with the Duncan's multiple range test [9]. For the percentage values an Arc Sen transformation was made before the statistical analysis.

3. Results and discussion

Plant growth responses to progressive drought periods were influenced by the AM endophyte colonizing the roots. The largest leaf area was reach-

Table 1
Leaf area (cm^2), root length (cm), mycorrhizal root length (cm) and percentage of infected roots of lettuce plants unstressed and subjected to successive progressive drought periods

Treatment	Leaf area			Total root length			Mycorrhizal root length			% Root mycorrhizal		
	Well watered	Drought vs. well-watered	Drought stressed	Well watered	Drought vs. well-watered	Drought stressed	Well watered	Drought vs. well-watered	Drought stressed	Well watered	Drought vs. well-watered	Drought stressed
G. deserticola	875.9^{a}	91%	797.1^{bc}	9262^{a}	82%	7609^{c}	8549^{a}	84%	7160^{b}	92^{a}	102%	94^{a}
G. etunicatum	793.7^{b}	84%	665.1^{d}	8438^{b}	82%	6958^{de}	5012^{cd}	91%	4557^{d}	59^{d}	112%	66^{cd}
G. intraradices	747.2^{c}	83%	621.6^{e}	7864^{c}	84%	6586^{def}	6802^{b}	86%	5875^{c}	87^{ab}	100%	87^{ab}
G. fasciculatum	634.0^{e}	86%	547.7^{f}	7894^{c}	85%	6734^{def}	4807^{d}	89%	4259^{d}	69^{cd}	91%	63^{cd}
G. mosseae	645.7^{e}	85%	550.1^{f}	6961^{de}	92%	6388^{ef}	4852^{d}	105%	5072^{cd}	70^{c}	113%	79^{b}
G. caledonium	461.1^{g}	78%	361.5^{h}	7006^{d}	88%	6189^{f}	2284^{ef}	74%	1683^{f}	33^{f}	82%	27^{f}
G. occultum	451.1^{g}	30%	135.3^{i}	6958^{de}	32%	2234^{g}	2943^{e}	24%	713^{g}	42^{e}	76%	32^{f}
Control	230.1^{j}	22%	50.6^{k}	4731^{h}	33%	1577^{i}	0	—	0	0	—	0

Within each pair of columns, means followed by the same letters are not significantly different ($P < 0.05$) using Duncan's multiple range test.

Table 2
Evolution in photosynthetic activity (nmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) during 5 weeks under well-watered conditions (−0.04 MPa) or under progressive water stress periods (−0.06, −0.10 and −0.17 MPa) and after rewatering in plants colonized by seven *Glomus* species or control

Treatment	Week 6	Week 7			Week 8			Week 9			Week 11		
	−0.04 MPa	−0.04 MPa	−0.06 vs. −0.04	−0.06 MPa	−0.04 MPa	−0.10 vs. −0.04	−0.10 MPa	−0.04 MPa	−0.17 vs. −0.04	−0.17 MPa	−0.04 MPa	Rewatered vs. −0.04	Rewatered −0.04 MPa
G. deserticola	17.4^{a}	66.9^{a}	66%	44.3^{bc}	109.6^{a}	62%	67.9^{c}	127.3^{a}	33%	42.2^{c}	150.6^{b}	132%	198.8^{a}
G. etunicatum	15.6^{ab}	63.4^{a}	70%	44.8^{bc}	85.2^{b}	79%	67.4^{c}	87.3^{bc}	29%	25.4^{ef}	94.4^{cde}	97%	91.9^{cde}
G. intraradices	14.8^{ab}	43.3^{bcd}	104%	45.1^{bc}	66.0^{cd}	67%	44.2^{f}	95.5^{b}	63%	59.8^{d}	97.9^{cde}	126%	123.3^{bcd}
G. fasciculatum	14.0^{b}	44.0^{bc}	89%	39.2^{bcde}	55.1^{def}	98%	53.8^{def}	79.5^{c}	52%	41.2^{e}	97.9^{cde}	106%	104.2^{cde}
G. mosseae	13.6^{b}	47.6^{b}	78%	37.1^{cde}	87.8^{b}	66%	57.5^{cde}	89.0^{bc}	22%	19.3^{fg}	125.2^{bc}	71%	89.4^{cde}
G. caledonium	9.1^{c}	22.9^{f}	87%	19.9^{f}	29.4^{g}	96%	28.2^{g}	33.8^{ef}	53%	17.9^{fg}	36.4^{f}	179%	65.3^{ef}
G. occultum	10.0^{c}	35.9^{de}	26%	9.3^{g}	50.2^{ef}	51%	25.7^{g}	66.9^{cd}	12%	7.9^{g}	65.0^{def}	109%	70.8^{ef}
Control	—			—	—	—	—	—	—	—	45.3^{f}	89%	40.1^{f}

Within each weekly evaluation, means followed by the same letters are not significantly different ($P < 0.05$) using Duncan's multiple range test.

Table 3
Evolution in transpiration rate (μmol $H_2O \cdot m^{-2} \cdot s^{-1}$) during 5 weeks under well-watered conditions (−0.04 MPa) or under progressive water stress periods (−0.06, −0.10 and −0.17 MPa) and after rewatering in plants colonized by seven *Glomus* species or control

Treatment	Week 6	Week 7			Week 8			Week 9			Week 11		
	−0.04 MPa	−0.04 MPa	−0.06 vs. −0.04	−0.06 MPa	−0.04 MPa	−0.10 vs. −0.04	−0.10 MPa	−0.04 MPa	−0.17 vs. −0.04	−0.17 MPa	−0.04 MPa	Rewatered vs. −0.04	Rewatered −0.04 MPa
G. deserticola	11.9^{a}	31.9^{ab}	103%	33.0^{ab}	35.3^{a}	78%	27.4^{def}	42.9^{a}	81%	34.8^{c}	54.3^{a}	100%	54.1^{a}
G. etunicatum	11.5^{a}	26.7^{bc}	75%	20.1^{def}	32.4^{b}	77%	25.1^{ef}	37.9^{b}	83%	31.5^{d}	51.7^{a}	98%	50.5^{a}
G. intraradices	11.6^{a}	22.8^{cd}	90%	20.5^{cde}	30.2^{bcd}	90%	27.2^{def}	39.0^{b}	73%	28.5^{ef}	43.8^{b}	93%	40.8^{bc}
G. fasciculatum	7.8^{b}	14.6^{efg}	164%	23.9^{cd}	28.3^{cde}	94%	26.7^{ef}	30.4^{c}	91%	27.6^{f}	43.2^{b}	100%	43.5^{b}
G. mosseae	8.9^{b}	32.8^{ab}	106%	34.9^{a}	31.0^{bc}	80%	24.9^{f}	32.5^{d}	79%	25.6^{g}	32.8^{d}	119%	39.0^{c}
G. caledonium	6.7^{b}	16.3^{cde}	60%	9.7^{gh}	18.5^{g}	79%	14.6^{h}	21.6^{i}	82%	17.8^{j}	29.1^{de}	95%	27.8^{e}
G. ocultum	6.9^{b}	13.4^{fg}	30%	4.0^{h}	18.5^{g}	18%	3.4^{i}	23.4^{h}	32%	7.6^{k}	21.9^{f}	53%	11.5^{g}
Control	—	—	—	—	—	–	—	—	—	–	13.5^{g}	73%	9.9^{g}

Within each weekly evaluation, means followed by the same letters are not significantly different ($P < 0.05$) using Duncan's multiple range test.

Table 4
Evolution in stomatal conductance (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) during 5 weeks under well-watered conditions (−0.04 MPa) or under progressive water stress periods (−0.06, −0.10 and −0.17 MPa) and after rewatering in plants colonized by seven *Glomus* species or control

Treatment	Week 6	Week 7			Week 8			Week 9			Week 11		
	−0.04 MPa	−0.04 MPa	−0.06 vs. −0.04	−0.06 MPa	−0.04 MPa	−0.10 vs. −0.04	−0.10 MPa	−0.04 MPa	−0.17 vs. −0.04	−0.17 MPa	−0.04 MPa	Rewatered vs. −0.04	Rewatered −0.04 MPa
G. deserticola	1.85^{a}	1.53^{bcd}	112%	1.71^{bc}	3.47^{a}	31%	1.06^{gh}	4.50^{a}	78%	3.49^{d}	5.22^{a}	96%	5.00^{ab}
G. etunicatum	1.86^{a}	1.88^{b}	61%	1.14^{bcdef}	3.14^{b}	79%	2.48^{d}	3.68^{c}	86%	3.17^{e}	4.92^{ab}	95%	4.68^{bc}
G. intraradices	0.79^{c}	1.00^{cdefg}	83%	0.83^{defg}	2.96^{bc}	42%	1.25^{fg}	4.16^{b}	69%	2.85^{f}	4.49^{cd}	85%	3.80^{ef}
G. fasciculatum	1.23^{bc}	2.70^{a}	48%	1.29^{bcde}	3.03^{bc}	87%	2.64^{cd}	3.28^{e}	83%	2.72^{f}	4.12^{de}	97%	3.99^{ef}
G. mosseae	1.55^{ab}	1.95^{b}	36%	0.71^{defg}	2.40^{d}	36%	0.86^{h}	3.42^{d}	72%	2.45^{g}	3.11^{g}	117%	3.65^{f}
G. caledonium	1.22^{bc}	1.73^{bc}	22%	0.38^{fg}	1.76^{e}	81%	1.42^{f}	2.15^{h}	80%	1.72^{i}	2.72^{gh}	82%	2.56^{h}
G. occultum	0.14^{d}	0.58^{efg}	29%	0.17^{g}	1.81^{c}	18%	0.33^{i}	2.47^{g}	30%	0.75^{j}	2.11^{i}	50%	1.06^{j}
Control	—	—	—	—	—	—	—	—	—	—	1.70^{i}	88%	1.50^{j}

Within each weekly evaluation, means followed by the same letters are not significantly different ($P < 0.05$) using Duncan's multiple range test.

Table 5
Evolution in water use efficiency (WUE) (mmol CO_2/mol $H_2O \times 10^5$) during 5 weeks under well-watered conditions (−0.04 MPa) or under progressive water stress periods (−0.06, −0.10 and −0.17 MPa) and after rewatering in plants colonized by seven *Glomus* species or control

Treatment	Week 6	Week 7			Week 8			Week 9			Week 11		
	−0.04 MPa	−0.04 MPa	−0.06 vs. −0.04	−0.06 MPa	−0.04 MPa	−0.10 vs. −0.04	−0.10 MPa	−0.04 MPa	−0.17 vs. −0.04	−0.17 MPa	−0.04 MPa	Rewatered vs. −0.04	Rewatered −0.04 MPa
G. deserticola	83.3^{a}	139.1^{a}	56%	78.5^{cde}	228.8^{a}	69%	157.9^{bcd}	230.1^{a}	37%	84.1^{f}	248.6^{ab}	116%	287.5^{a}
G. etunicatum	77.7^{a}	159.5^{a}	82%	131.2^{ab}	178.9^{bc}	99%	176.8^{bc}	169.0^{bc}	29%	49.6^{g}	147.9^{cde}	86%	127.9^{def}
G. intraradices	60.7^{ab}	138.3^{a}	79%	108.8^{bc}	138.1^{cde}	63%	87.2^{fg}	164.7^{bc}	72%	119.3^{de}	172.7^{cde}	110%	189.8^{bcd}
G. fasciculatum	76.9^{a}	141.5^{a}	56%	78.9^{cde}	121.2^{def}	57%	110.3^{ef}	184.6^{b}	45%	82.8^{f}	116.6^{def}	137%	160.3^{cde}
G. mosseae	65.4^{ab}	72.9^{de}	63%	45.9^{ef}	193.5^{ab}	47%	90.0^{fg}	148.1^{cd}	25%	37.6^{gh}	245.0^{ab}	53%	129.8^{def}
G. caledonium	44.4^{b}	56.4^{ef}	80%	45.4^{ef}	66.1^{g}	96%	63.4^{g}	45.4^{gh}	80%	36.1^{gh}	58.9^{f}	244%	144.3^{de}
G. occultum	43.4^{b}	91.3^{cd}	32%	29.1^{f}	114.8^{def}	93%	106.5^{efg}	110.9^{e}	18%	20.4^{h}	100.3^{ef}	108%	108.4^{ef}
Control	—	—	—	—	—	—	—	—	—	—	43.1^{fg}	88%	37.9^{g}

Within each weekly evaluation, means followed by the same letters are not significantly different ($P < 0.05$) using Duncan's multiple range test.

ed by the inoculation of *G. deserticola* regardless water treatments. At harvest, AM plants, stressed and non-stressed were significantly different in foliar area, root length and AM colonization according to endophyte involved (Table 1).

Comparison of *Glomus* isolates' efficiency under progressive stress and well-watered conditions showed that leaf area was reduced in *G. deserticola*-plants by 9% due to drought, while in *G. occultum*-plants the decline in foliar biomass was 70%. Except for *G. mosseae*, drought stress also affected root development for the other endophytes assayed (Table 1). Regarding the total root length infected by *Glomus* isolates, the most invasive fungus was *G. deserticola* and the least *G. caledonium*. In general, the *Glomus* species assayed did not differ in their ability to colonize plant root as a consequence of drought.

Photosynthetic activity, WUE, stomatal conductance and transpiration significantly increased through plant growth. The effects of symbiotic association on physiological plant parameters were clearly expressed from the seventh week after plant inoculation, probably when AM colonization ought to be fully spreading and functioning. Regarding these parameters, *Glomus deserticola*-plants showed the highest and *G. caledonium*- and *G. occultum*-plants the lowest values. The effect of AM fungi on these physiological parameters increased with time (Tables 2–5).

In 6-week-old plants, prior to the drought periods, photosynthetic activity, WUE and transpiration were little affected by AM colonization. However, 1 week later, the fungal effect on these parameters was evident. In general, the progressive drought stress negatively affected the physiological parameters, particularly CO_2 assimilation and WUE. *G. occultum*-colonized plants showed the lowest photosynthetic values and the strongest reduction as a consequence of whatever level of stress applied. *G. deserticola* was the most efficient mycorrhizal endophyte increasing these parameters under stressed and unstressed conditions. The most detrimental effect of drought on CER and WUE was evident at week 9, under the most severe water limitation. In contrast, transpiration and conductance rates were more depressed at week 8, a moderate water stress (−0.1 MPa).

The improvement of host water relations and growth responses is a consistent effect of AM colonization [4,14,25]. The nature of the stress (severity and duration) as well as the functional compatibility of the fungal association may specifically affect mycorrhizal effect under water stress conditions (Tables 2–5). In this sense, selected endophytes such as *G. deserticola, G. etunicatum, G. mosseae,* and *G. occultum* showed a high sensitivity for decreasing CO_2 assimilation even to light stress (−0.06 MPa), while *G. fasciculatum*, for instance, maintained this parameter, under light or even moderate stress (−0.06 and −0.10 MPa), close to values reached under well-watered conditions. Plants colonized with *G. etunicatum* did not reduce WUE until severe water stress (−0.17 MPa). In this mycorrhizal treatment, the effect of water limitation on stomatal conductance was less than in plants colonized by the other *Glomus* spp. assayed. These results may explain part of the strategies by which AM fungi specifically alter plant water relations with relevant consequences for drought tolerance, acclimatisation and recovery from water deficit. As discussed elsewhere [10,17] stomata may have evolved as regulatory organs functioning to minimize water loss for a given amount of carbon gained.

The AM symbiosis is able to enhance defense mechanisms of the host plant to stress as a result of changes in physiological host response. Drought stress decreases CO_2 exchange. Depending on the host-endophyte combination and on the degree of water limitation, mycorrhizal plants can affect photosynthetic rates differently during the drought periods [24]. In fact, at −0.17 MPa plants colonized by *G. mosseae* exhibited only 19.3 nmo $\cdot m^{-2} \cdot s^{-1}$ (one of the lowest CO_2-exchange rates tested here). Under the same water conditions, *G. intraradices*-inoculated plants were more protected against decreases in these parameters than the rest of the colonized plants. Associated processes, such as transpiration and leaf conductance are then also necessarily affected. The differences observed in the relationship between transpiration and leaf conductance in stressed versus non-stressed mycorrhizal plants indicate that there is an AM effect on water use efficiency as Nelsen [20] and Bethlenfalvay et al. [4] also

observed. In this study, plants colonized with *G. etunicatum* did not reduce WUE until −0.17 MPa (severe stress). Water use efficiency has been defined at the leaf level as the ratio of net CO_2 assimilation per unit water transpired [10,17] and is an important aspect of adaptation to drought.

A particular characteristic shown by *G. fasciculatum*-infected plants was the capacity to maintain unaltered photosynthesis and transpiration until a low soil water potential was reached (−0.17 MPa). In contrast, other treatments reduced such parameters when subjected to light and moderate stress (−0.06 and −0.01 MPa). Increased CO_2 assimilation has been considered to be a plant strategy for water stress tolerance [12]. Differential carbon drain by more or less extensive root infection and the dynamics of mycorrhizal development by different AM fungal isolates may have interacting effects on host carbon resources and carbon partitioning. On the other hand, AM fungi have the ability to enhance CO_2 assimilation. The fungi may also affect osmoregulation in leaf tissues to lower leaf water potential and thereby enhance water retention and turgor.

G. deserticola- and *G. etunicatum*-colonized plants showed the highest transpiration and conductance values under all experimental conditions assayed here. These values were at least twice as high in these treatments than in those of the plants colonized by the other endophytes. This result must be emphasized since plant water requirements are closely related to plant size. If plant growth was enhanced by a particular endophyte and increased leaf areas, plants must require more water per unit leaf surface area. In fact, larger plants may use more water, and consequently suffer more from stress than less developed ones. In this study, all the AM plants independent of size or leaf surface area had the same soil water availability. However, there were dissimilar drought stress effects between the more and the less developed plants. The inoculation with *G. occultum* was the least effective in preventing the negative effect from progressive drought stress on plant growth and physiological parameters.

Following re-irrigation, most of the plants exhibited a high capacity to recover physiological parameters from drought (CO_2 assimilation, WUE, conductance and transpiration). These parameters returned or increased over the levels observed in the non-stressed plants. *G. deserticola*-plants reached the highest values, particularly in the rewatered plants after the drought period.

During the first period of stress application (after 7 weeks of growth and at −0.06 MPa), stress was light and duration of the water limitation was short; nevertheless, photosynthesis and WUE were considerably affected in particular treatments. In fact, *G. etunicatum* and *G. intraradices* increased photosynthesis and WUE in plants at −0.10 MPa, while *G. mosseae* and *G. fasciculatum* decreased such parameters only under severe stress (−0.17 MPa).

Results from this study suggest that the ability of AM fungi to protect the host plant against progressive drought stress seems not to be linked with any specific physiological mechanism affected by the *Glomus* species. The observed effects on drought tolerance by mycorrhizal inoculation can be ascribed to particular physiological trends in the host according to the endophyte involved and environmental (light, moderate or severe stress) conditions as well as to the intrinsic capacity of the fungi to resist stress. Differences in leaf-gas exchange values among treatments reflected that symbiotic activity and efficiency was a function of plant growth stage, stress intensity and the endophyte involved.

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