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Effect of drought stress on growth and water relations of the mycorrhizal association *Helianthemum almeriense-Terfezia claveryi*

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Abstract Plants of *Helianthemum almeriense* were micropropagated on MS medium and inoculated *in vitro* with *Terfezia claveryi* mycelium on MH medium and vermiculite. Mycorrhizal (M) and non-mycorrhizal (NM) plants were subjected to a drought stress period of 3 weeks in greenhouse conditions with the soil matric potential maintained at –0.5 MPa. Drought stress did not affect the amount of mycorrhizal colonization. The survival rate of M plants at the end of the drought stress period was higher than that of NM plants. The water potential was higher in M plants than in NM plants by 14% in well-watered and 26% in drought-stressed plants. Transpiration, stomatal conductance and net photosynthesis were higher in M plants than in NM plants. Transpiration was 92% higher in M plants than in NM plants under drought-stress conditions and 40% when irrigated. Stomatal conductance was 45% and 14% higher and net photosynthesis 88% and 54% higher, respectively, in M than in NM plants. Drought-stressed M plants accumulated more N, P and K than drought-stressed NM plants. Reduced negative effects of drought stress on *H. almeriense* by the desert truffle *T. claveryi* could be ascribed to specific physiological and nutritional mechanisms, suggesting that this mycorrhizal symbiosis aids adaptation to arid climates.

Keywords Desert truffle · Drought stress · *Helianthemum* · Mycorrhiza · *Terfezia*

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Introduction

Mycorrhizal fungi modify water relations in host plants (Nelsen 1987). Stomatal conductance, transpiration rate and leaf water potential are often higher in mycorrhizal (M) plants under drought conditions due to a higher water uptake (Augé et al. 1987; Duan et al. 1996; Subramanian et al. 1995), which allows such plants to maintain higher rates of photosynthesis and higher water contents than non-mycorrhizal (NM) plants. However, the mechanism by which the fungus modifies host-plant water relations remains unknown. Different hypotheses have been tested with inconclusive results. The relevant hypotheses are (1) an indirect effect of improved P nutrition in M plants (Augé et al. 1986; Fitter 1988), (2) improvement of water uptake in M root systems either by the extraradical phase (Ruíz-Lozano and Azcón 1995), by increasing effective root hydraulic conductivity (Safir et al. 1971), or by modifying root architecture (Kothari et al. 1990), (3) biochemical modification of water regulation in the host plant through changes in hormonal signalling (Duan et al. 1996; Goicoechea et al. 1995), (4) induction of osmoregulatory responses in M plants (Augé et al. 1986) or changes in soil water-retention properties (RM Augé, personal communication).

Such mechanisms have been studied mostly in arbuscular mycorrhizal (AM) symbioses and little information exists for other types of mycorrhiza. The objective of this present work was to study the responses to water stress of *Helianthemum almeriense* Pau, a Mediterranean shrub which is well adapted to the semiarid conditions of the southeastern Iberian peninsula, in symbiosis with the desert truffle *Terfezia claveryi* Chatin, an edible fungus frequent in marl with gypsum soils of the semiarid western Mediterranean area. Both fungus and host plant respond to marked xerophytic conditions. This symbiotic association is an endo-, ectendo- or ectomycorrhiza according to the fertility of the substrate (Dexheimar et al. 1985; Fortas and Chevalier

1992). The potential applications of the results of this study are obvious, since desert truffles are in demand in Mediterranean areas of Europe, Asia and Africa but their production is limited. It is well known among producers that reduced water availability severely limits sporocarp production.

Materials and methods

Plant material

H. almeriense plants were micropropagated following the method described by Morte and Honrubia (1992, 1997). Vitroplants were rooted on Murashige and Skoog (MS) medium (1962) with salts diluted to 25% and without plant growth regulators.

Fungal inoculum

T. claveryi mycelium (ref. T.c. V) was isolated from sporocarps collected in the region of Murcia (Spain) and cultured on MMN medium at pH 7.0 (Marx 1969).

Mycorrhizal inoculation

Two-month-old micropropagated and rooted plants were inoculated in vitro with cultured mycelium and grown on vermiculite watered with MH liquid medium at pH 7.0 (Morte and Honrubia 1994, 1995). Control plants were grown under the same conditions but were not inoculated. At inoculation, the shoots of rooted plantlets were about 3 cm and had 4–5 nodal segments with two leaves each. The average number of roots per plantlet was three, with lengths ranging from 1.5 to 2 cm. Culture conditions throughout the process were 25 ± 2 °C, 40 μmol m^{-2} s^{-1} Growlux fluorescent light and a 16-h photoperiod. After 2 months, plants were transferred to greenhouse conditions in 1-l pots containing soil: peat: vermiculite (4:1:1, v/v/v). This substrate was sterilized by autoclaving at 100 °C for 60 min on three alternate days. Plantlets were acclimatized in greenhouse conditions by reducing relative humidity during a period of 2–3 weeks.

Water-stress conditions

After 4 months of acclimation in nursery conditions, both M and NM plants had developed a good aerial branch system. Sixteen plants, eight M and eight NM, were used for the water-stress experiment. Within each group of plants, four were watered to container capacity (substrate matric potential = –0.03 MPa) and four were drought-stressed by providing 70% less water, the substrate matric potential being maintained at –0.5 MPa by low irrigation for 3 weeks. Water content in the substrate was gravimetrically determined every 3 days and related to substrate matric potential (Lovisolo and Schubert 1998); a water retention curve was previously established by pressure plate measurements (Richards 1965).

Fungal colonization assessment

Fungal colonization was assessed on cleared and stained root samples (Phillips and Hayman 1970) after the inoculation process and before the water-stress treatment to check the presence or absence of mycorrhiza. The percentage of fungal root colonization was estimated according to the gridline intersect method (Giovannetti and Mosse 1980) under a stereomicroscope.

Measurements

Growth

Harvested plants were weighed and the percentage of mycorrhizal colonization was determined before drying for 48 h at 100 °C to determine the relative water content.

Gas exchange

During the water stress period leaf gas exchange was measured every 3 days at maximal light intensity (>1000 μmol m^{-2} s^{-1} photon flux density). Gas exchange was measured on a portion of the whole plant using an ADC LCA3 infra-red gas analyzer equipped with a Parkinson Leaf Chamber (Analytical Development Company, Hoddesdon, UK). The apex of a stem including five leaves was inserted into the chamber. Measurements were taken three times for each plant at about midday.

Water potential

At the end of the water-stress period, the water potential of plants was measured in three 10-cm-long plant apices per plant using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, Calif, USA). Water potential (Ψ) measurements were made at the time of maximal stress between 12:00 and 15:00 h.

Chlorophyll content

Plants were harvested, weighed and stored at –20 °C. Chlorophyll was extracted in acetone and the absorbance of the extract assessed with a spectrophotometer. The chlorophyll concentration was calculated using the equations of Inskeep and Bloom (1985).

Mineral analyses

The concentrations of N, P, K, and Na were determined in dried shoot and root tissue (M.A.P.A. 1981).

Plant hydraulic conductance

Plant hydraulic conductance (g_{plant}) was calculated for Ψ soil of –0.03 or –0.5 MPa according to the different treatments using the equation

$$g_{plant} = \frac{E}{\Psi soil - \Psi leaf}$$

where E = transpiration measured as gas exchange.

Statistical analyses

The effects of treatments were assessed by an analysis of variance and treatment means were compared by least significant difference ($P < 0.05$) using Student's *t*-test.

Results

Root colonization by *T. claveryi* apparently enhanced the shoot and root weights of *H. almeriense* plants slightly but not significantly under both normal irrigation and drought-stress conditions (Table 1). However, the survival rate of M plants was 50% higher than NM

Table 1 Shoot and root fresh weight and dry weight and mycorrhizal colonization (%) of *Helianthemum almeriense* plants inoculated with *Terfezia claveryi*. Non-inoculated (control) and inoculated (*T claveryi*) plants grown under normal irrigation or drought-stress conditions. Values ± SE followed by the same letter are not significantly different ($P<0.05$) according to Student's *t*-test

Plants	Treatment	Shoot fresh wt. (g)	Root fresh wt. (g)	Shoot dry wt. (g)	Root dry wt. (g)	Mycorrhization (%)
Well-watered	Control	8.98 ± 1.8 ab	8.97 ± 0.4 ab	5.28 ± 1.0 a	3.95 ± 0.2 ab	–
	T. claveryi	10.86 ± 0.4 b	10.61 ± 1.1 b	5.29 ± 0.3 a	4.96 ± 0.63 b	13.46 ± 1.24 a
Drought-stressed	Control	5.40 ± 1.7 a	5.25 ± 1.8 a	3.82 ± 0.9 a	2.98 ± 0.2 a	–
	T. claveryi	5.94 ± 1.3 a	6.42 ± 0.6 ab	4.53 ± 1.2 a	3.58 ± 0.05ab	13.44 ± 0.61 a

plants at the end of the drought-stress period. Drought stress did not affect the degree of mycorrhizal colonization (about 13% for both well-irrigated and water-stressed plants).

Water potential was less negative in M plants than in NM plants, both for well-watered and drought-stressed plants. The water potential in M plants was 14% higher than in NM plants under well-watered conditions and 26% for drought-stressed plants (Table 2).

Transpiration, stomatal conductance and net photosynthesis were higher in M plants than in the NM controls. Differences in water content and CO_2 exchange rates were greater under drought-stress than under well-watered conditions. Compared with NM plants, transpiration increased by 92% in M plants under drought-stress conditions, and by 40% in irrigated conditions. Stomatal conductance increased in M plants by 45% and 14% and net photosynthesis by 88% and 54% under drought-stress and irrigated conditions, respectively (Table 2). Plant hydraulic conductance increased by 65% in well-watered M plants compared with well-watered NM plants. The increase was 195% in drought-stress conditions (Table 2).

Mycorrhizal infection also affected the rates at which transpiration, stomatal conductance and net photosynthesis decreased as leaf water potential was reduced (Fig. 1). The decreases were greater in M than in NM plants (Fig. 1).

Chlorophyll content per leaf fresh weight and leaf area was higher in M plants than NM plants but differences were only significant under drought-stress conditions (Fig. 2).

Drought-stressed M plants accumulated more N and P in shoots than either drought-stressed NM plants or irrigated M and NM plants. However, drought-stressed NM plants accumulated more P in roots than the plants of the other three treatments (Table 3). The percentages of N in roots and Na in shoots and roots were not significantly different between M and NM plants with either normal irrigation or drought-stress conditions. However, M plants accumulated more K in shoots and roots than NM plants under both normal irrigation and drought-stress conditions (Table 3).

Discussion

Mycorrhizal fungi can influence water uptake ability and water use efficiency in host plants (Allen 1982). In our experiment, water relations (measured as gas exchange) were significantly affected by *T. claveryi* inoculation in drought-stressed *H. almeriense* plants, while they were little modified under well-watered conditions. Thus suggests an adaptive effect of mycorrhizal symbiosis in arid climates. Under well-watered conditions, the leaf water potential was quite low (–1.94 MPa) in M plants, indicating that the symbiosis adapted well to dry conditions. Moreover, at high drought stress levels (3 weeks at a soil water potential of –0.5 MPa), the leaf water potential of M plants did not decrease strongly. The increase in plant hydraulic conductance was more evident for M plants under drought-stress conditions than under well-watered conditions. Consequently, mycorrhization could increase water uptake by increasing effective root hydraulic conductivity. These results agree with those obtained by Safir et al. (1971). As soil dried, both water potential and stomatal conductance of *H. almeriense* declined,

Table 2 Water potential (Ψ), transpiration (E), stomatal conductance (g_s), net photosynthesis (Pn) and plant hydraulic conductance (g_{plant}) in *H. almeriense* plants non-inoculated (control) and inoculated with *T. claveryi* grown under normal irrigation or drought-stress conditions. Values ± SE followed by the same letter are not significantly different ($P<0.05$) according to Student's *t*-test

Plants	Treatment	Ψ (MPa)	E ($mmol \times m^{-2} \times s^{-1}$)	g_s ($mmol \times m^{-2} \times s^{-1}$)	Pn ($\mu mol \times m^{-2} \times s^{-1}$)	(g_{plant}) (g per plant)
Well-watered	Control	–1.22 ± 0.03 c	3.11 ± 0.57 b	144.23 ± 9.38 b	8.51 ± 1.78 b	2.61 ± 0.48
	T. claveryi	–1.04 ± 0.04 d	4.36 ± 0.67 c	164.66 ± 8.83 d	13.1 ± 1.65 c	4.31 ± 0.66
Drought-stressed	Control	–1.94 ± 0.16 a	1.47 ± 0.35 a	78.67 ± 8.13 a	4.79 ± 1.07 a	1.02 ± 0.24
	T. claveryi	–1.44 ± 0.15 b	2.83 ± 0.51 b	114 ± 15.47 c	9.01 ± 1.83 b	3.01 ± 0.54

Table 3 Effect of *T. claveryi* and drought acclimation treatments on N, P, K and Na contents of *H. almeriense* plants. Values followed by the same letter are not significantly different according to Student's *t*-test

Plants	Treatment	%N		%P		%K		%Na	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Well-watered	Control	1.03 a	0.75 a	0.13 a	0.16 a	0.67 a	1.71 a	0.07 a	0.12 a
	T. claveryi	1.09 a	0.74 a	0.14 a	0.13 a	0.83 b	2.27 b	0.04 a	0.12 a
Drought-stressed	Control	0.99 a	0.87 a	0.15 a	0.18 b	0.71 a	1.59 a	0.05 a	0.12 a
	T. claveryi	1.32 b	0.78 a	0.18 b	0.13 a	0.92 b	2.46 b	0.06 a	0.14 a

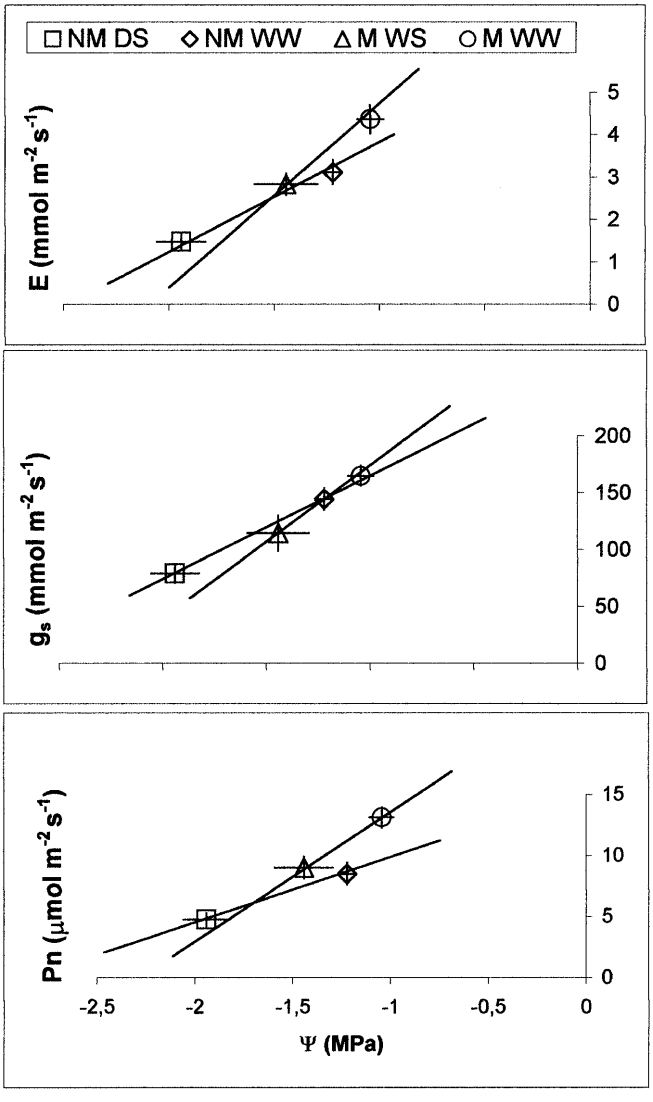

Fig. 1 Transpiration rate (E), stomatal conductance (g_s) and net photosynthesis (Pn) plotted versus leaf water potential (Ψ) in *Helianthemum almeriense* plants inoculated (M) and not inoculated (NM) with *Terfezia claveryi*. Plants were either subjected to a drought-stress period (DS) or well watered (WW)

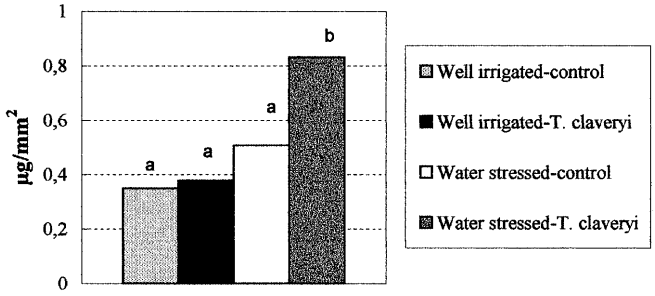

Fig. 2 Chlorophyll content per leaf area unit of *H. almeriense* plants inoculated with *T. claveryi.* Values followed by the same letter are not significantly different ($P<0.05$) according to Student's *t*-test

which slowed transpiration and photosynthesis (stomatal regulation of photosynthesis) in both M and NM plants. However, transpiration rate, stomatal conductance and net photosynthesis of M plants decreased more than the corresponding values in NM plants for the same decrease in leaf water potential. Drying out of soil may be accompanied by an amplified stress signal, such as an increase in concentration or amount of abscisic acid (Duan et al. 1996) in M roots. Abscisic acid when transferred to the stomata would induce faster closing before excessively low values of leaf water potential are reached. This mechanism could explain the lower mortality of M plants than of NM plants in drought, but further studies are necessary to test this hypothesis.

The improvement of plant water relations did not affect plant growth, especially in terms of shoot development, while root weight tended to increase in M plants. Greater root development might account for the higher P accumulation observed in M than NM plants grown under stress conditions in this study. The protection of M plants against drought stress was related to *T. claveryi*-induced increases in leaf conductance and transpiration as well as P, N and K uptake. Potassium plays a key role in plant water stress and is the cationic solute responsible for stomatal movement in response to changes in bulk leaf water status (Ruíz-Lozano et al. 1995). The response of *T. claveryi*-infected plants to drought stress and the K content were closely related.

Drought stress did not affect the mycorrhizal colonization percentage. This colonization was mostly intracellular and external mycelium around the roots was very scarce. Previous studies have shown that the percentage of roots colonized by AM fungi is not affected by water stress (Bethlenfalvay et al. 1988; Bryla and

Duniway 1997; Davies et al. 1992; Nelsen and Safir 1982).

In conclusion, mitigation of the negative effects of drought stress by the desert truffle *T. claveryi* can be attributed to specific physiological (transpiration, water use efficiency) and nutritional (P, N and K) alterations in *H. almeriense*. Furthermore, the introduction of desert truffles into dry environments may be a useful way to exploit lands which until now have been regarded as unproductive.

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