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Involvement of antioxidant enzyme and nitrate reductase activities during water stress and recovery of mycorrhizal *Myrtus communis* and *Phillyrea angustifolia* plants

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Abstract

In a greenhouse experiment, we studied the effect of inoculation with an arbuscular mycorrhizal (AM) fungus (*Glomus intraradices* Schenck and Smith) or with a mixture of three AM fungi (*G. intraradices* Schenck and Smith, *Glomus deserticola* (Trappe, Bloss. and Menge) and *Glomus mosseae* (Nicol and Gerd.) Gerd. and Trappe) on root and shoot nitrate reductase (NR, EC 1.6.6.1.) activity, mycorrhizal colonisation, plant growth, nutrient uptake and superoxide dismutase (SOD) and peroxidase (POX) activities in shoots of *Myrtus communis* and *Phillyrea angustifolia* seedlings after well-watered, drought and recovery periods. The mycorrhizal inoculation treatments increased significantly the growth and foliar nutrients (N and P) of both species independent of the water regime. Drought reduced NR activity in roots of both species and in shoots of *M. communis*, although both species inoculated with the mixture of AM fungi reached values higher than non-inoculated plants and plants inoculated with *G. intraradices*. The mycorrhizal inoculation treatments decreased significantly POX and SOD activities in shoots of both species. POX and SOD activities in inoculated *P. angustifolia* seedlings hardly varied during the drought and recovery periods. SOD activity was enhanced by drought in non-AM plants of both species and in inoculated *M. communis*, but to a lesser extent than in control plants.

Keywords: Arbuscular mycorrhizal fungi; Mediterranean shrubs; Nitrate reductase; Peroxidase; Superoxide dismutase; Water stress

1. Introduction

Water stress is one of the most important environmental factors that regulate plant growth and development in Mediterranean environments. Among the diverse consequences of a drought effect on plant development in these ecosystems, restricted nutrient and water acquisition are commonly recognised [1]. In this context, and because arbuscular mycorrhizal (AM) fungi are known to enhance the ability of plants to establish and cope with stress situations (nutrient deficiency, drought, etc.), the use of these fungi, as plant inoculants, is being investigated to help plants to thrive in degraded arid/semiarid areas [2]. While drought responses in mycorrhizal Mediterranean plant species have received considerable attention [3–5], physiological responses during drought recovery are still poorly studied.

Increasing evidence suggests that drought induces oxidative stress in various plants, in which reactive oxygen species, such as superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) and alkoxyl radical ($RO^{\bullet}$), are produced [6]. The toxic superoxide radical has a half-life of less than 1 s and is usually rapidly dismutated by superoxide dismutase (SOD) to H_2O_2, a product which is relatively stable and can be detoxified by catalase and peroxidases. These metalloenzymes constitute an important primary defence of cells against superoxide free radicals generated under stress conditions. In this way, increased

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SOD activity is known to confer oxidative stress tolerance [7]. Ruiz-Lozano et al. [8] found increased SOD activity in mycorrhizal soybean plants and this correlated with enhanced plant production and drought resistance. To date, however, there is little information on SODs in AM symbiosis, particularly in shoots from shrub species, like *Myrtus communis* L. and *Phillyrea angustifolia* L., adapted to arid conditions.

Drought stress is also known to affect many biochemical activities, such as nitrate reductase (NR) activity, involved in assimilation of N in plants. NR (EC 1.6.6.1.) is the first enzyme in the nitrate assimilation pathway and represents the rate-limiting step in this process. This enzyme is inducible by its substrate, i.e. nitrate ions [9]. Nitrate reductase appears to be responsive to the metabolic and physiological status of plants and can be used as a biomarker of plant stress, such as drought. It has been shown that NR activity decreases in plants exposed to water limitation because of a lower flux of nitrate from the soil to the root [10]. In addition, drought-stressed plants suffer from a reduction in photosynthesis, which appears to limit the supply of reductants and energy for nitrate reduction [11]. The presence of such enzymatic activity in AM fungi [12] and the increase of NR activity in the AM symbiosis have also been shown [13,14]. Under induced water deficit, positive effects of mycorrhizal inoculation on nitrate acquisition and assimilation have been reported in herbaceous plants with high water requirements [15] and in semiarid Mediterranean shrubs [14], although these effects depended on the associated mycorrhizal fungus and the host plant species.

The aim of this study was to compare the mechanisms developed by *M. communis* and *P. angustifolia* seedlings, inoculated with an AM fungus or with a mixture of three AM fungi, to cope with drought. For this, growth, foliar nutrients, nitrate reductase activity in roots and shoots, SOD and peroxidase activities in shoots and colonisation were measured during water-stress and recovery periods.

2. Materials and methods

2.1. Plants and mycorrhizal treatments

The plants used, *M. communis* and *P. angustifolia*, are two representative shrub species from semiarid shrublands in Southeast Spain. They are also well adapted to water-stress conditions, and therefore, potentially could be used in the revegetation of semiarid disturbed lands. Seeds of both plant species were grown for 6 months in peat substrate under nursery conditions, without any fertilization treatment.

The mycorrhizal fungi used were either *Glomus intraradices* Schenck and Smith (EEZ 1), or a mixture of *G. intraradices*, *Glomus deserticola* (Trappe, Bloss. and Menge) (EEZ 45) and *Glomus mosseae* (Nicol and Gerd.) Gerd. and Trappe (EEZ 43), which were obtained from the collection of the experimental field station of Zaidín, Granada. The acronym EEZ refers to Estación Experimental del Zaidín.

AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum* sp.) containing spores, hyphae and mycorrhizal root fragments.

2.2. Water-stress and recovery treatments

In early February 2004, the *M. communis* or *P. angustifolia* seedlings were transplanted into the growth substrate, consisting of peat and cocopeat (1:1, v/v) sterilized by autoclaving at 120 °C for 60 min. The corresponding arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v) in 600-mL containers. The same amount of an autoclaved mixture of the inocula was added to control plants, supplemented with a filtrate (<20 μm) of the culture to provide the microbial populations accompanying the mycorrhizal fungi. Fifteen replicates per treatment were set up, making a total of 45 seedlings per plant species. Inoculated and non-inoculated seedlings were grown for 4 months under nursery conditions without any fertiliser treatment and were watered with decalcified water until the initiation of the drought treatment. Soil water shortage was imposed for 1 week (from 14 June to 21 June). The experiment was conducted in a greenhouse, located in the Campus of Espinardo (Murcia, Spain). During the experiment, the temperature ranged from 11 to 34 °C, and the relative humidity was between 40 and 80%. Midday photosynthetically active radiation (PAR) averaged 260 $\mu E\, m^{-2}\, s^{-1}$. Stressed plants (inoculated and non-inoculated) were maintained at a substrate water potential close to −0.60 MPa. After the water-stress period, plants were rewatered for another week. Water was supplied daily to maintain constant soil water potential equivalent to field capacity (−0.03 MPa). Soil moisture was monitored gravimetrically before each watering. Water content in the substrate, calculated as a percentage of dry weight and corresponding with substrate water potential at field capacity and at permanent wilting, was determined according to the method of Richards [16].

2.3. Plant analyses

In the beginning of water-stress period, and at times corresponding to the end of water-stress and recovery periods, five plants per treatment were harvested and basal stem diameters and heights of plants were measured with callipers and rulers, respectively. Plants were harvested and the roots were washed free from soil under a stream of cold tap water. Fresh and dry (105 °C, 5 h) weights of shoots and roots were recorded. Plant tissues were ground before chemical analysis. The foliar concentrations of phosphorus were determined, after digestion in nitric–perchloric acid (5:3) for 6 h, by colorimetry [17] and the N concentration was determined by the Kjeldahl method.

The percentage of root length colonised by arbuscular mycorrhizal fungi was calculated by the gridline intersect method [18] after staining with trypan blue [19].

NR activity was assayed in vivo by measuring NO_2^- production in tissue that had been vacuum-infiltrated with buffered NO_3^- solutions [20]. The leaves and roots from the stressed and rewatered seedlings were collected in the morning, between 8:30 and 11:00 h solar time. Leaves and roots of *M. communis* or *P. angustifolia* were cut into 5-mm sections. Approximately 300 mg of leaf punches and 300 mg of roots were placed in tubes containing 2 mL of an incubation medium consisting of 0.05 M Tris–HCl, pH 7.8, and 0.25 M KNO_3. The tubes were sealed and kept in the dark at 30 °C for 1 h. The nitrite released into the medium was determined after incubation by treating 1-mL aliquots with 1 mL of 1% sulphanilamide in 1 M HCl and 1 mL of 0.01% *N*-1-napthyl-ethylenediamine hydrochloride. After 15 min, the optical density was measured at 540 nm with a Beckman spectrophotometer.

2.4. Leaf enzyme extraction

All operations were performed at 4 °C. Shoots (2 g) were homogenised with a mortar and pestle in 4 mL of ice-cold 50 mM Tris–acetate buffer, pH 6.0, containing 0.1 mM EDTA, 5 mM cysteine, 2% (w/v) PVP, 0.1 mM PMSF and 0.2% (v/v) Triton X-100. The homogenate was centrifuged at 14,000 × *g* for 20 min and the supernatant fraction was filtered through Sephadex G-25 NAP columns (Pharmacia Biotech AB, Uppsala, Sweden), equilibrated with the same buffer used for the homogenisation.

2.5. Assays performed

Total SOD activity was assayed by the ferricytochrome *c* method using xanthine/xanthine oxidase as the source of $O_2^{\bullet -}$ radicals [21]. Total peroxidase was analysed according to Ros-Barceló [22].

2.6. Statistical analysis

Data were log-transformed to achieve normality. Mycorrhizal inoculation, water regime and their interactions effects on measured variables were tested by a two-way analysis of variance and comparisons among means were made using the least significant difference (LSD) test calculated at $P < 0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

3. Results

3.1. Growth, nutrient assimilation and mycorrhizal colonisation

The mycorrhizal inoculation treatments increased significantly shoot and root dry weights, height and basal diameter in *M. communis* and *P. angustifolia* seedlings with respect to the non-inoculated plants (Table 1). Before water

Table 1
Growth parameters of *M. communis* and *P. angustifolia* seedlings in response to mycorrhizal inoculation treatments during drought and recovery periods ($n = 5$)

Parameter	*M. communis*			*P. angustifolia*		
	Initial stress	Final stress	Recovery	Initial stress	Final stress	Recovery
Shoot (g dw)						
C	6.44a	7.35a	6.53a	2.06a	2.36a	2.38a
G	8.63bc	9.42b	8.67bc	4.45bc	5.15b	4.84bc
M	11.25c	10.37b	10.14c	5.68c	5.05b	5.37c
Root (g dw)						
C	3.62a	3.63a	3.80a	1.81a	1.64a	1.90a
G	4.83ab	5.18bc	5.10bc	3.69b	3.64b	3.57b
M	6.49b	6.91c	6.55c	3.20b	3.16b	3.31b
Root/shoot						
C	0.56a	0.49a	0.58a	0.88c	0.69b	0.80c
G	0.56a	0.55b	0.59a	0.83bc	0.71b	0.74bc
M	0.58a	0.67c	0.65b	0.56a	0.63a	0.62a
Height (cm)						
C	31.3a	28.3a	28.5a	19.2a	19.0a	19.5a
G	33.3ab	35.3b	33.2b	32.1c	32.8b	30.2b
M	34.0b	34.7b	34.0b	26.5bc	29.5b	27.4b
Basal diameter (mm)						
C	4.9a	5.2a	5.5a	3.5a	3.3a	3.3a
G	6.7bc	6.6b	6.3b	4.0b	4.3b	4.4b
M	8.3c	7.8b	8.0b	4.8b	4.5b	4.9b

C, plants non-inoculated; G, plants inoculated with *G. intraradices*; M, plants inoculated with a mixture of three arbuscular mycorrhizal fungi. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the LSD test.

stress, the mixture of AM fungi was more effective than *G. intraradices* in increasing plant growth, particularly in *P. angustifolia* seedlings (about 176% greater than control plants). One week of water shortage did not have significant effects on the growth parameters determined in both shrub species (Table 2). The increases produced by the mycorrhizal inoculation treatments at the end of the drought period were similar to those observed in plants cultivated under well-watered conditions. Inoculation of plants with the mixture of AM fungi decreased the root/shoot ratio for *P. angustifolia*, in comparison with control plants and *G. intraradices*-colonised plants. However, the root/shoot ratio of inoculated *M. communis* seedlings was higher than that of non-inoculated plants, under water-stress conditions. After 1 week of rewatering, the inoculated plants were significantly greater than non-inoculated plants but there were no differences in growth with respect to inoculated plants after drought.

The inoculation with *G. intraradices* or with a mixture of three AM fungi increased foliar nutrients with respect to the control plants, in both water regimes (Table 3), the inoculation with the mixture of three AM fungi being the most effective for increasing foliar N and P contents. As observed for the growth parameters, the water regime did not have an effect on foliar nutrients in shoots of *M. communis* or *P. angustifolia* seedlings (Table 2). Likewise, the contents of foliar nutrients in rewatered plants were similar to those recorded in the inoculated and non-inoculated plants before and after drought.

Only the mycorrhizal inoculation treatments had effect on the level of colonisation in roots of both shrub species (Table 2). Before water stress, both inoculation treatments produced a similar level (about 25 and 48%) of root colonisation in *M. communis* and *P. angustifolia*, respectively. However, at the end of the water-stress period, the mixture of AM fungi was more effective for increasing root colonisation for both species. Naturally colonised seedlings showed <6% colonisation of the root length in both shrub species.

Table 2
P significance values from two-way analysis of variance of the growth parameters, foliar nutrients, colonisation, NR, POX, and SOD activities of *M. communis* and *P. angustifolia* as affected by mycorrhizal inoculation treatments and water regime

	Mycorrhiza (M)	Water regime (WR)	Interaction (M × WR)
M. communis			
Shoot dry biomass	0.008	0.666	0.632
Root dry biomass	0.003	0.771	0.774
Height	0.042	0.671	0.304
Basal diameter	0.014	0.966	0.600
Foliar N	0.048	0.980	0.984
Foliar P	0.024	0.895	0.905
Colonisation	<0.001	0.370	0.334
Shoot NR activity	0.526	<0.001	0.983
Root NR activity	0.023	<0.001	0.078
SOD	<0.001	<0.001	0.025
P. angustifolia			
Shoot dry biomass	<0.001	0.702	0.764
Root dry biomass	<0.001	0.716	0.887
Height	<0.001	0.706	0.643
Basal diameter	<0.001	0.634	0.703
Foliar N	0.002	0.984	0.978
Foliar P	0.001	0.899	0.845
Colonisation	<0.001	0.166	0.484
Shoot NR activity	<0.001	0.012	0.430
Root NR activity	<0.001	<0.001	0.015
POX	<0.001	0.001	<0.001
SOD	<0.001	0.184	0.004

3.2. Nitrate reductase activity

The NR activity, except in shoots of *M. communis*, was affected by drought or mycorrhizal treatments (Table 2). The mycorrhizal treatments increased NR activity in roots more markedly than in shoots under well-watered

Table 3
Foliar nutrients and root infection of *M. communis* and *P. angustifolia* seedlings in response to mycorrhizal inoculation treatments during drought and recovery periods ($n = 5$)

Parameter	*M. communis*			*P. angustifolia*		
	Initial stress	Final stress	Recovery	Initial stress	Final stress	Recovery
Nitrogen (mg per plant)						
C	80a	76a	81a	15a	12a	18a
G	91b	95b	90b	34b	37b	40b
M	140c	136c	138c	84c	90c	94c
Phosphorus (mg per plant)						
C	3.53a	3.42a	3.75a	1.24a	1.30a	1.36a
G	4.76b	4.91b	5.00b	2.80b	2.73b	2.90b
M	7.25c	7.60c	7.30c	4.29c	4.02c	4.50c
Colonisation (%)						
C	0.7a	0.3a	0.0a	1.5a	5.1a	2.4a
G	20.0b	25.8b	38.0b	52.0b	50.5b	43.0b
M	30.7b	42.0c	46.3b	44.0b	67.0c	56.1b

C, plants non-inoculated; G, plants inoculated with *G. intraradices*; M, plants inoculated with a mixture of three arbuscular mycorrhizal fungi. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the LSD test.

Table 4
Shoot and root nitrate reductase (NR), shoot total peroxidase total (POX) and superoxide dismutase (SOD) activities of *M. communis* and *P. angustifolia* seedlings in response to mycorrhizal inoculation treatments during drought and recovery periods ($n = 5$)

Parameter	*M. communis*			*P. angustifolia*		
	Initial stress	Final stress	Recovery	Initial stress	Final stress	Recovery
Shoot NR activity (nmol NO_2^- g^{-1} FW h^{-1})						
C	23.5bc	0.6a	3.3a	6.2a	0.2a	5.2a
G	29.9c	0.8a	36.1c	15.3b	14.4c	87.1c
M	20.0ab	3.6b	26.6b	12.5b	6.9b	27.8b
Root NR activity (nmol NO_2^- g^{-1} FW h^{-1})						
C	16.2a	0.9a	5.1a	6.1a	1.1a	4.9a
G	17.2a	0.9a	7.0a	22.4b	2.8a	16.6b
M	23.1b	4.0b	5.0a	23.3b	8.1b	19.6b
POX (units mg^{-1} protein)						
C	–	–	–	16.7c	28.1c	21.7b
G	–	–	–	12.1b	6.2a	7.0a
M	–	–	–	7.0a	10.7b	6.3a
SOD (units mg^{-1} protein)						
C	100c	241c	262b	164b	215b	144b
G	42a	85a	83a	103a	90a	89a
M	82b	140b	72a	118a	89a	95a

C, plants non-inoculated; G, plants inoculated with *G. intraradices*; M, plants inoculated with a mixture of three arbuscular mycorrhizal fungi. Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the LSD test.

conditions (Table 4). Before water stress, the mixture of AM fungi increased NR activity in roots of *M. communis* and *P. angustifolia*, whilst the inoculation with *G. intraradices* was only effective for *P. angustifolia* (Table 3). Drought significantly reduced NR activity in roots of both species and in shoots of *M. communis*, although both species inoculated with the mixture of AM fungi reached values higher than non-inoculated plants and plants inoculated with *G. intraradices*. At the end of the recovery period, NR activity in recovered inoculated and non-inoculated plants of both species was increased compared to the enzyme activity recorded after drought. The positive effect of mycorrhizal treatments on NR activity in recovered plants was more pronounced in shoots than in roots of both species. The inoculation with *G. intraradices* increased NR activity in shoots of recovered *M. communis* and *P. angustifolia* plants by 11 and 72 times, respectively.

3.3. Antioxidant enzyme activities

POX activity was not detected in the shoots of *M. communis* seedlings (Table 3).

Mycorrhizal inoculation and water regime had significant effects on POX activity in shoots of *P. angustifolia* (Table 2). For shoot POX activity, there was a significant negative interaction between mycorrhization and water regime. At the beginning of water stress, the mycorrhizal inoculation treatments significantly reduced the POX activity in the shoots of *P. angustifolia*, particularly in plants inoculated with the mixture of AM fungi. At the end of the drought and recovery periods, only the non-inoculated plants had increased POX activity. The POX activity in the shoots of inoculated plants hardly varied from the beginning of water stress to the end of recovery.

SOD activity was significantly affected by mycorrhization treatments in shoots of *M. communis* and *P. angustifolia*, but the water regime only had effect in *M. communis* plants. SOD activity was higher in non-inoculated plants than in inoculated plants. SOD activity was enhanced by drought stress in shoots of non-AM plants of both species and in shoots of inoculated *M. communis*. However, the difference was more evident in non-inoculated plants, where the increase in SOD activity reached 141% in *M. communis* and 31% in *P. angustifolia*. SOD activity hardly changed in shoots of inoculated *P. angustifolia* plants during the drought and recovery periods.

4. Discussion

Nitrate reductase activity can be used as a stress index for plants grown in soils where nitrate is the main form of N available to plants [14]. The presence of AM fungi in the roots positively affected NR activity in the roots and shoots of both shrub species. The increased NR activity found in AM plants is an indication of the mycorrhizal ability to promote plant adaptation to drought [23]. According to the results from the present study, the increase of this activity as a consequence of mycorrhizal inoculation treatments under non-stressed conditions was considerably higher in roots than in shoots of both species. These results could be due to the AM fungi located in the roots possessing this enzymatic activity per se, as pointed out also by Kaldorf et al. [12]. In contrast, the effect of mycorrhizal inoculation treatments on NR activity was greater in shoots than in roots of both shrub

species under water-stress conditions. This agrees with the findings of Azcón and Tobar [23] who detected higher NR in shoots of stressed, mycorrhizal *Allium cepa*. The assimilation sites of N forms may be affected by mycorrhizal colonisation and such aspects are known to affect physiological responses by plants [11]. In mycorrhizal roots, the large carbohydrate requirement of nitrate reduction may be a factor limiting the ability of roots to perform this enzymatic process due to the fact that mycorrhizal roots require an extra amount of C for fungal development [24]. In general, the mixture of AM fungi was more effective for increasing NR activity and foliar N content of both shrub species when grown under stress conditions. Some authors have indicated that the increase in NR activity of mycorrhizal plants with respect to non-mycorrhizal ones can be related to the phosphate requirements of this enzyme [25]. In this sense, the mycorrhizal effect could be interpreted as an indirect response to the improved nutrient status, particularly of phosphorus. The fact that the higher foliar P contents in shoots of both shrub species occurred in plants inoculated with the mixture of AM fungi could explain how these plants had higher values of NR activity. On the other hand, the increase in N-assimilating enzymes, such as nitrate reductase, may be attributed to the contribution of hyphal transport of N [26]. In fact, as water stress impedes the mobility of nitrate [10], mycorrhizal plants may have access, through the extraradical mycelium, to the forms of N which are usually unavailable to the non-inoculated plants. The rewatered, non-inoculated plants had significantly lower activities of NR than inoculated plants, these differences being more pronounced in shoots. The enhanced NR activity in shoots of AM plants suggests that AM association is an important factor in sustaining N assimilation until the full recovery of the host plant. These results suggest that mycorrhizae may be a crucial factor in normal and limited-water environments.

Many drought-adapted species from arid environments have a highly developed root system and thus the root/shoot ratio is high, which may be considered a mechanism of drought tolerance. Mycorrhizal symbiosis is known to decrease the root/shoot ratio [27]. The lack of roots is then compensated for by the extension of the mycorrhizal fungus extraradical mycelium. The lower root/shoot ratio observed in stressed and non-stressed *P. angustifolia* seedlings colonised by the mixture of three AM fungi, as compared to non-inoculated plants, may indicate partitioning of carbon to the fungus at the expense of root production [28]. In this case, the extraradical mycelium may have contributed to a more effective uptake of nutrients and water by plants submitted to water deficit [3]. As mentioned above, the mixture of AM fungi was more effective for increasing assimilation of nutrients (N and P) in *P. angustifolia* seedlings. There are also reports where mycorrhizal fungi have increased the root growth of infected plants [29]. This type of response was observed mainly in plants of *M. communis* inoculated with the mixture of AM fungi, at the end of the stress and recovery periods. It has been hypothesised that mycorrhizae can alter the morphology of the root system, yielding a more extensive absorbing area, which may be considered a mechanism of drought tolerance [30]. Likewise, mycorrhizae are known to increase the xylem pressure potential by increasing root biomass and therefore improving water uptake [3].

It is well known that the antioxidant systems of plants act as important tolerance mechanisms against drought stress. Enhanced SOD and POX activities have been associated with induced resistance of plants to drought stress [31]. Both activities are involved in superoxide radical and hydrogen peroxide scavenging. In previous works, where several antioxidant enzymes have been studied under the same stress conditions, differential responses in mycorrhizal plants have frequently been observed. Under induced water stress, SOD specific activity was higher in shoots and roots of inoculated plants than in P-fertilised, non-inoculated *Lactuca sativa* plants, showing increases of 99 and 150%, respectively [32]. An increase in several antioxidant enzymes has been observed also in shoots of mycorrhizal shrubs in semiarid Mediterranean conditions [4]. In our study, drought considerably enhanced oxidative damage in shoots of non-mycorrhizal plants of both species, which was confirmed by the increases in SOD and POX activities observed in such plants. The mycorrhizal inoculation treatments produced a decrease in POX activity in shoots of *M. communis* under both water regimes and in SOD activity in shoots of stressed and non-stressed *P. angustifolia* plants. A similar response was observed in mycorrhizal soybean plants that showed lower APX values relative to the corresponding non-mycorrhizal plants [33]. Similarly, in nodules, SOD, catalase and APX activities were lower in droughted, mycorrhizal plants than in non-mycorrhizal plants [33]. The decrease in antioxidant enzymes observed in mycorrhizal plants could be explained partially by the fact that these plants may be submitted to a lower oxidative stress under both control and drought-stress conditions. This could be attributed to primary drought-avoidance mechanisms, such as the active water transfer from AM fungi to the host [34] or increased water uptake related to mycorrhizal changes in root morphology [30]. The inoculated *M. communis* plants seemed to be less protected against oxidative stress than *P. angustifolia*, because SOD activity increased under water-shortage conditions, although to a lesser extent than in control plants. These observations agree with the proposal by Bartels [35] that both the prevention of oxidative stress and the elimination of reactive oxygen species are the most effective approaches used by plants to gain tolerance against several abiotic stresses, including drought.

In conclusion, the effectiveness of mycorrhizal inoculation with respect to the growth of *M. communis* and *P. angustifolia* seedlings was independent of the water regime. The capacity of AM fungi for increasing plant tolerance to

the drought stress imposed may have been related to nutrient uptake improvement and to an increase in N assimilation through NR activity, particularly in plants inoculated with the mixture of AM fungi. The mycorrhizal inoculation treatments produced a decrease in POX and SOD activities in shoots of both shrub species during drought and recovery periods. This might indicate that the plants inoculated with AM fungi also developed mechanisms to avoid oxidative damage produced under water-shortage conditions.

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References

[1] C. Agnew, A. Warren, A framework for tackling drought and land degradation, J. Arid Environ. 33 (1996) 309–320.
[2] N. Requena, E. Pérez-Solís, C. Azcón-Aguilar, P. Jeffries, J.M. Barea, Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems, Appl. Environ. Microbiol. 67 (2001) 495–498.
[3] R.M. Augé, Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis, Mycorrhiza 11 (2001) 3–42.
[4] M.M. Alguacil, J.A. Hernández, F. Caravaca, B. Portillo, A. Roldán, Antioxidant enzyme activities in shoots from three mycorrhizal shrub species afforestated in a degraded semi-arid soil, Physiol. Plant. 118 (2003) 562–570.
[5] J.I. Querejeta, J.M. Barea, M.F. Allen, F. Caravaca, A. Roldán, Differential response of $\delta^{13}C$ and water use efficiency to arbuscular mycorrhizal infection in two aridland woody plant species, Oecologia 135 (2003) 510–515.
[6] S. Munné-Bosch, J. Peñuelas, Photo-and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants, Planta 217 (2003) 758–766.
[7] L. Slooten, K. Capiau, W. Van Camp, M. Van Montagu, C. Sybesma, D. Inze, Factors affecting the enhancement of oxidative stress tolerance in transgenic tobacco overexpressing manganese superoxide dismutase in the chloroplasts, Plant Physiol. 107 (1995) 373–380.
[8] J.M. Ruiz-Lozano, C. Collados, J.M. Barea, R. Azcón, Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants, New Phytol. 151 (2001) 493–502.
[9] A. Kandlbinder, H. Weiner, W.M. Kaiser, Nitrate reductase from leaves of Ricinus (*Ricinus communis* L.) and spinach (*Spinacia olerecea* L.) have different regulatory properties, J. Exp. Bot. 51 (2000) 1099–1105.
[10] R. Azcón, M. Gómez, R.M. Tobar, Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions, Biol. Fertil. Soils 22 (1996) 156–161.
[11] K.S. Subramanian, C. Charest, Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery, Physiol. Plant. 102 (1998) 285–296.
[12] M. Kaldorf, E. Schmelzer, H. Bothe, Expression of maize and fungal nitrate reductase genes in arbuscular mycorrhiza, Mol. Plant. Microbiol. Interact. 11 (1998) 439–448.
[13] J.M. Ruíz-Lozano, R. Azcón, Mycorrhizal colonization and drought stress as factors affecting nitrate reductase activity in lettuce plants, Agric. Ecosyst. Environ. 60 (1996) 175–181.
[14] F. Caravaca, M.M. Alguacil, G. Díaz, A. Roldán, Use of nitrate reductase activity for assessing the effectiveness of mycorrhizal symbiosis in *Dorycnium pentaphyllum* under induced water deficit, Comm. Soil Sci. Plant. Anal. 34 (2003) 2291–2302.
[15] R. Azcón, J.M. Ruiz-Lozano, R. Rodríguez, Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (^{15}N) under increasing N supply to the soil, Can. J. Bot. 79 (2001) 1175–1180.
[16] L.A. Richards, A pressure-membrane extraction apparatus for soil solution, Soil Sci. 51 (1941) 377–386.
[17] J. Murphy, J.P. Riley, A modified single solution method for determination of phosphate in natural waters, Anal. Chim. Acta 27 (1962) 31–36.
[18] M. Giovannetti, B. Mosse, An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots, New Phytol. 84 (1980) 489–499.
[19] J.M. Phillips, D.S. Hayman, Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection, Trans. Br. Mycol. Soc. 55 (1970) 158–161.
[20] M.R. Downs, K.J. Nadelhoffer, J.M. Melillo, J.D. Aber, Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability, Trees (Berl.) 7 (1993) 233–236.
[21] J.M. McCord, I. Fridovich, Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein), J. Biol. Chem. 244 (1969) 6049–6055.
[22] A. Ros-Barceló, The generation of H_2O_2 in the xylem of *Zinnia elegans* is mediated by an NADPH-oxidase-like enzyme, Planta 207 (1998) 207–216.
[23] R. Azcón, R.M. Tobar, Activity of nitrate reductase and glutamine synthetase in shoot and root of mycorrhizal *Allium cepa*. Effect of drought stress, Plant Sci. 133 (1998) 1–8.
[24] S.S.E. Smith, Mycorrhizas of autotrophic higher plants, Biol. Rev. 55 (1980) 475–510.
[25] R.H. Hageman, A.J. Reed, Nitrate reductase from higher plants, Methods Enzymol. 49 (1980) 270–280.
[26] K.S. Subramanian, C. Charest, Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions, Mycorrhiza 9 (1999) 69–75.
[27] S.E. Smith, D.J. Read, Mycorrhizal Symbiosis, Academic Press, San Diego, 1997, p. 605.
[28] D.P. Wright, J.D. Scholes, D.J. Read, Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L., Plant Cell Environ. 21 (1998) 209–216.
[29] A.R. Lansac, A. Martin, A. Roldán, Mycorrhizal colonization and drought interactions of Mediterranean shrubs under greenhouse conditions, Arid Soil Res. Rehabil. 9 (1995) 167–175.
[30] M.A. Ibrahim, W.F. Campbell, L.A. Rupp, E.B. Allen, Effects of mycorrhizae on sorghum growth, photosynthesis, and stomatal conductance under drought conditions, Arid Soil Res. Rehabil. 4 (1990) 99–107.
[31] J.M. Ruiz-Lozano, Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress: new perspectives for molecular studies, Mycorrhiza 13 (2003) 309–317.
[32] J.M. Ruiz-Lozano, R. Azcón, J.M. Palma, Superoxide dismutase activity in arbuscular mycorrhizal *Lactuca sativa* plants subjected to drought stress, New Phytol. 134 (1996) 327–333.
[33] R. Porcel, J.M. Barea, J.M. Ruiz-Lozano, Antioxidant activities in mycorrhizal soybean plants and their possible relationship to the process of nodule senescence, New Phytol. 157 (2003) 135–143.
[34] R. Porcel, J.M. Ruiz-Lozano, Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress, J. Exp. Bot. 55 (2004) 1743–1750.
[35] D. Bartels, Targeting detoxification pathways: an efficient approach to obtain plants with multiple stress tolerance, Trends Plant Sci. 6 (2001) 284–286.