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Superoxide dismutase and total peroxidase activities in relation to drought recovery performance of mycorrhizal shrub seedlings grown in an amended semiarid soil

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Summary

We studied the effect of inoculation with a mixture of three arbuscular mycorrhizal (AM) fungi (*Glomus intraradices* Schenck & Smith, *Glomus deserticola* (Trappe, Bloss. & Menge) and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe) and addition of a composted organic residue on plant growth, nutrient uptake, mycorrhizal colonisation and superoxide dismutase (SOD, EC 1.15.1.1) and total peroxidase (POX, EC 1.11.1.7) activities in shoots of *Juniperus oxycedrus* seedlings after well-watered, drought and recovery periods. The mycorrhizal inoculation and composted residue addition significantly increased the growth, foliar nutrients (N, P, K) and shoot water content of the plants, independent of the water regime. POX activity in control plants increased during drought (about 250% higher than under well-watered conditions) and returned to initial levels after re-watering. The seedlings inoculated with AM fungi showed the highest values of POX activity, followed by the plants grown in the amended soil, which varied little during the drought and recovery periods. Drought decreased the SOD activity in shoots of both *J. oxycedrus* seedlings inoculated with AM fungi and those grown with composted residue, but did not affect

Abbreviations: AOS, activated oxygen species; AM, arbuscular mycorrhizal; DW, dry weight; FW, fresh weight; LSD, least significant difference; POX, total peroxidase; SOD, superoxide dismutase

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that of control plants. After re-watering, the SOD activity in mycorrhizal or residue-amended plants increased, showing values similar to control plants.

Introduction

Soil degradation and semiarid climatic conditions make the re-establishment of a plant cover in Mediterranean areas, which have been affected by desertification processes, difficult. The climate exposes soil to frequent and severe desiccation interspersed with relatively rapid re-wetting events. Consequently, drought stress is considered to be one of the most important abiotic factors limiting growth and development of Mediterranean plants (Kramer and Boyer, 1997). Drought stress may cause damage to cells either directly or indirectly, through the formation of activated oxygen species (AOS) such as superoxide radicals and H_2O_2 (Mittler and Zilinskas, 1994). Superoxide is rapidly dismutated to H_2O_2 by the activation of superoxide dismutase (SOD) (Slooten et al., 1995). The H_2O_2 generated can be eliminated via peroxidases, which are found throughout the cell and have high affinity for H_2O_2 (Jiménez et al., 1997).

Desertification reduces the levels of soil organic matter and the diversity and abundance of key mutualistic microbial symbionts such as arbuscular mycorrhizal (AM) fungi (Azcón-Aguilar et al., 2003), which can protect host plants against the detrimental effects of drought stress. Drought tolerance mechanisms such as enhanced osmotic adjustment and leaf hydration, reduced oxidative damage or improved nutritional status have been proposed to explain the contribution of AM symbiosis to the drought resistance of host plants (Augé, 2001; Alguacil et al., 2003; Querejeta et al., 2003; Porcel and Ruiz-Lozano, 2004). The use of mycorrhizal fungi inoculation technology is an effective method for carrying out successful reforestation programmes in semiarid Mediterranean areas (Caravaca et al., 2002). While drought responses in mycorrhizal Mediterranean plant species have received considerable attention (Goicoechea et al., 2005), including the mycorrhizal shrub species *Juniperus oxycedrus* L. (Alguacil et al., 2006), the physiological responses during drought recovery are still poorly studied.

The quality and productivity of degraded soils can be improved by the addition of organic matter to the soil in the form of organic residues (Roldán et al., 1996). The addition of organic residues has been reported to enhance plant survival and growth under semiarid conditions (Querejeta et al., 2001). The beneficial role of organic matter has been attributed to indirect mechanisms related to improvement of the soil's physical properties, such as increased soil permeability, water-holding capacity and aggregate stability. However, little information regarding the influence of organic materials on the performance of plants under induced drought stress is available (Alguacil et al., 2006). Moreover, nothing is known about the interaction of organic materials and AM fungi and the physiological response of plants during the progression of drought stress and following recovery from drought.

The objective of this study was to evaluate the changes in antioxidant enzyme activities (SOD and total peroxidase (POX) activities) in shoots of *J. oxycedrus* seedlings in response to drought stress and following recovery, inoculation with a mixture of AM fungi and addition of a composted residue, and their consequences for the growth of the plant.

Materials and methods

Materials

The soil was collected from Los Cuadros in the Province of Murcia (SE Spain) (coordinates: 1°05′W and 38°10′N). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2 °C; the potential evapo-transpiration reaches 1000 mm yr^{-1}. The main characteristics of the soil used were: pH (1:5), 8.89; electrical conductivity, 0.18 dS m^{-1}; total organic carbon, 1.80%; total N, 2.01 g kg^{-1}; available P, 70 μg g^{-1}; extractable K, 440 μg g^{-1}; cationic exchange capacity, 15 cmol kg^{-1}.

The composted organic residue used was the organic fraction of a municipal solid waste obtained from a municipal waste treatment plant in Murcia. The composted residue was mechanically produced by fast fermentation (60 d), mixing the waste heap daily under aerobic conditions. The main characteristics of the composted residue used were: pH (1:10), 6.7; total organic C, 276.0 g kg^{-1}; water soluble C, 1950 μg g^{-1}; water soluble carbohydrates, 76 μg g^{-1}; total N, 14.5 g kg^{-1}; total P, 3.8 g kg^{-1}.

Plants and mycorrhizal treatments

The plant used for the experiment, *J. oxycedrus*, is a low-growing tree reaching a height of 3–4 m, although it often grows as a shrub. This shrub has a typical Mediterranean distribution and is well adapted to drought

conditions; it can thrive with mean annual rainfall of less than 230 mm and a summer drought period which can extend for 4 months. Seeds were grown for 6 months in peat substrate under nursery conditions, without any fertilisation treatment.

The mycorrhizal fungi used in the experiment were a mixture of *Glomus intraradices* Schenck & Smith (EEZ 1), *Glomus deserticola* (Trappe, Bloss. & Menge) (EEZ 45) and *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe (EEZ 43) and 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.

Water stress and recovery treatments

In early February 2004, the experimental soil was placed in 1500-mL (13-cm diameter, 11.3-cm height) capacity pots. Composted residue was mixed manually with the soil at a rate of 5% (v/v) into half of the pots. The AM inoculum was applied at a rate of 5% (v/v). The same amount of the autoclaved inoculum was added to control plants, supplemented with a filtrate (Whatman no. 1 paper) of the culture to provide the microbial populations accompanying the mycorrhizal fungi. *J. oxycedrus* seedlings were transplanted to pots (one per pot). Sixteen replicates per treatment were set up, making a total of 64 seedlings. Plants were grown and watered regularly with decalcified water over 14 months, maintaining soil moisture adjusted to 70% of water-holding capacity (corresponding with a soil matric potential of −0.2 MPa). The irrigation was then interrupted and the soil was allowed to dry for 12 d, until gravimetric water content reached approximately 5% (corresponding with a soil matric potential of −1.2 MPa). After the soil drying period, plants were re-watered (corresponding with a soil matric potential of −0.2 MPa) and kept at this potential for a week. The soil water content of each pot was adjusted daily with decalcified water, which was added by spraying onto the soil surface and by capillary action from the bottom. 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 averaged 260 $\mu E\, m^{-2}\, s^{-1}$.

Soil water potential

Soil water potential was determined by a pressure plate apparatus, and soil water content was measured by weighing the soil before and after drying at 110 °C for 24 h (Richards, 1941). A characteristic soil moisture curve was constructed and used to correlate soil water content and soil water potential (Ψ) by gravimetric measurement of soil water content in the pots.

Plant analyses

In the beginning of the water stress period, and at times corresponding to the middle (corresponding with a soil matric potential of −0.6 MPa) and end of water stress and recovery periods, five plants per treatment were harvested and the basal stem diameters were measured with callipers. The roots were washed free from soil under a stream of cold tap water and fresh and dry (105 °C, 5 h) weights of shoots and roots were recorded. Shoot water content was calculated as $((FW-DW)/FW)\times 100$ where FW stands for fresh weight and DW for dry weight. Plant tissues were ground before chemical analysis. The foliar contents of phosphorus were determined, after digestion in nitric–perchloric acid (5:3) for 6 h, by colorimetry (Murphy and Riley, 1962) and the plant K was estimated by flame photometry. The N concentration was determined by colorimetry after the Kjeldahl digestion.

The percentage of root length colonised by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Phillips and Hayman, 1970).

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 ethylenediaminetetraacetic acid, 5 mM cysteine, 2% (w/v) polyvinylpyrrolidone, 0.1 mM phenylmethylsulphonyl fluoride 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 columns (NAP, Pharmacia Biotech AB, Uppsala, Sweden), equilibrated with the same buffer used for the homogenisation.

Assays performed

Total SOD activity was assayed by the ferricytochrome *c* method using xanthine/xanthine oxidase as the source of $O_2^{\bullet -}$ radicals (McCord and Fridovich, 1969). POX was analysed according to Ros-Barcelo (1998).

Statistical analysis

Data were log-transformed to achieve normality. The effects of composted residue addition, inoculation with mycorrhizal fungi and soil drying and their interactions on measured variables were tested by a three-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.

Results

Growth, nutrient assimilation and mycorrhizal colonisation

Both the inoculation with exotic mycorrhizal fungi and addition of composted residue significantly increased shoot and root dry biomass and the basal diameter of seedlings with respect to the control plants (Tables 1 and 2). Before water stress, the mixture of AM fungi and composted residue produced similar increases in plant growth (about 150% greater than control plants). Twelve days of water shortage did not have a significant effect on the growth parameters (Table 1). The increases produced by the mycorrhizal inoculation and composted residue at the end of the drought period were similar to those observed for plants cultivated under well-watered conditions. This effect of the assayed treatments on plant growth was also observed after 1 week of re-watering. The greatest increases in the shoot dry biomass of *J. oxycedrus* plants were recorded in the combined treatment of composted residue addition and mycorrhizal inoculation (about 3.4-fold greater than control plants), regardless of the water regime. Both mycorrhizal inoculation and residue addition positively affected the water status of *J. oxycedrus* (Table 1). The increases in shoot water content produced by the treatments persisted during the progression of drought stress and through drought recovery.

As observed for growth parameters, the highest foliar concentrations of nitrogen, phosphorus and potassium were seen in the plants inoculated with the mixture of three exotic AM fungi and in the plants grown in the soil with composted residue, under both well-watered and drought-stressed conditions (Table 3). Likewise, the drought did not have an effect on foliar nutrient concentrations in shoots of *J. oxycedrus* seedlings (Table 2). The increases produced in the foliar N and P concentrations by the assayed treatments were maintained after re-watering, whereas the foliar K concentration was not affected by either treatment in the re-watered plants.

Only the mycorrhizal inoculation treatment had a significant effect on the level of colonisation in roots of *J. oxycedrus* ($P<0.001$, Table 2). Before and at the end of the drought period, the plants inoculated with the mixture of exotic *Glomus* species presented a similar level of root colonisation (Table 3). Naturally colonised seedlings grown in the soil with or without composted residue addition showed about 15% colonisation of the root length. The re-watering did not significantly affect mycorrhizal colonisation of the inoculated or non-inoculated plants.

Table 1. Growth parameters and shoot water content of *J. oxycedrus* seedlings in response to mycorrhizal inoculation and composted residue addition during drought-recovery cycle ($n = 5$)

	Initial stress	Middle stress	Final stress	Recovery
Shoot dry biomass (*g dw*)				
C	5.7±0.8	6.0±0.4	5.9±0.3	6.4±0.6
M	13.5±0.5	13.5±0.5	13.0±0.4	14.0±0.5
R	15.0±0.6	16.6±1.4	15.6±0.7	16.0±0.8
M+R	19.3±1.8	22.0±0.3	20.1±1.3	22.5±1.3
Root dry biomass (*g dw*)				
C	6.2±0.3	6.6±0.2	6.1±0.4	6.1±0.2
M	11.6±0.6	11.7±0.6	10.9±0.6	12.3±0.4
R	9.7±0.7	10.2±0.4	10.0±0.7	10.2±0.4
M+R	10.3±0.7	12.6±0.2	12.6±0.9	11.2±0.5
Basal diameter (*mm*)				
C	3.38±0.18	4.39±0.14	3.85±0.15	4.24±0.17
M	6.07±0.42	6.10±0.23	5.69±0.13	6.57±0.03
R	7.71±0.15	6.77±0.33	6.21±0.11	6.28±0.22
M+R	6.90±0.45	6.47±0.08	6.80±0.12	6.99±0.20
Shoot water content (*%*)				
C	48.2±0.9	50.3±0.9	47.7±0.1	51.4±0.2
M	51.8±0.2	53.9±0.3	52.5±0.2	51.2±0.2
R	52.5±0.4	55.2±0.5	54.6±0.8	52.3±0.6
M+R	55.5±0.3	53.8±0.4	54.5±0.8	51.2±0.5

C = control; M = inoculation with a mixture of three AM fungi; R = addition of a composted residue; M+R = addition of a composted residue and inoculation with a mixture of three AM fungi.

Antioxidant enzyme activities

POX activity in shoots of *J. oxycedrus* was significantly affected by mycorrhization and residue (Table 2). Before water stress, the seedlings inoculated with exotic AM fungi showed the highest values of shoot POX activity followed by the plants grown in the amended soil (Table 4). The increases produced by the assayed treatments for this antioxidant enzyme remained nearly constant during the water stress period. POX activity in shoots of the control plants was enhanced by drought stress (about 250% higher than under well-watered conditions). After the re-watering, POX activity in the control plants returned nearly to the levels observed before water stress.

Neither mycorrhizal inoculation nor composted residue addition had significant effects on SOD activity in shoots of *J. oxycedrus* (Table 2). With the exception of control plants, water stress decreased

Table 2. Three-factor ANOVA (mycorrhizal inoculation, composted residue addition and drying) for all parameters studied

Source of variation	Mycorrhiza (M)	Composted residue (R)	Drought stress (D)	Interactions			
				M × R	M × D	R × D	M × R × D
Shoot dry biomass	<0.001*	<0.001	0.776	<0.001	0.939	0.896	0.900
Root dry biomass	<0.001	0.001	0.504	0.006	0.818	0.446	0.603
Basal diameter	<0.001	<0.001	0.559	<0.001	0.830	0.059	0.057
Foliar N	0.001	<0.001	0.815	<0.001	0.773	0.939	0.547
Foliar P	<0.001	<0.001	0.265	<0.001	0.058	0.325	0.073
Foliar K	<0.001	0.003	0.502	<0.001	0.258	0.188	0.224
Colonisation	<0.001	0.064	0.334	0.569	0.087	0.245	0.182
POX	<0.001	0.016	0.176	0.601	0.141	0.261	0.781
SOD	0.358	0.353	<0.001	0.001	0.001	0.187	0.023

*P significance values.

Table 3. Foliar nutrients and root infection of *J. oxycedrus* seedlings in response to mycorrhizal inoculation and composted residue addition during drought-recovery cycle ($n = 5$)

	Initial stress	Middle stress	Final stress	Recovery
Nitrogen ($g\,kg^{-1}$)				
C	6.5±0.2	6.9±0.2	6.9±0.1	7.3±0.1
M	9.2±0.3	9.3±0.1	9.2±0.2	9.2±0.1
R	10.3±0.1	10.6±0.3	9.9±0.2	9.4±0.2
M+R	9.5±0.4	9.6±0.2	9.9±0.4	10.3±0.3
Phosphorus ($mg\,kg^{-1}$)				
C	314±26	330±5	359±3	334±17
M	952±32	983±14	998±18	980±17
R	886±7	952±40	998±4	1007±45
M+R	1001±43	1051±10	898±8	956±16
Potassium ($g\,kg^{-1}$)				
C	6.1±0.5	5.8±0.1	5.8±0.3	8.1±0.1
M	9.2±0.2	9.1±0.3	11.1±0.3	8.1±0.1
R	8.6±0.4	8.5±0.1	8.3±0.2	8.4±0.1
M+R	9.2±0.7	9.0±0.2	8.8±0.2	8.2±0.3
Colonisation (%)				
C	14.0±2.0	13.5±0.7	12.5±1.2	15.3±0.6
M	67.3±4.7	72.0±1.6	70.3±1.3	74.5±1.8
R	19.3±2.3	14.3±1.7	13.8±1.1	15.8±2.1
M+R	72.0±1.8	80.3±2.2	79.5±1.2	81.8±1.5

C = control; M = inoculation with a mixture of three AM fungi; R = addition of a composted residue; M+R = addition of a composted residue and inoculation with a mixture of three AM fungi.

Table 4. Shoot total peroxidase (POX) and superoxide dismutase (SOD) activities of *J. oxycedrus* seedlings in response to mycorrhizal inoculation and composted residue addition during drought-recovery cycle ($n = 5$)

	Initial stress	Middle stress	Final stress	Recovery
POX ($Units\,g^{-1}\,dw$)				
C	108.1±4.4	386.5±43.3	388.9±6.4	133.2±7.1
M	582.0±38.4	421.2±23.7	513.3±8.8	590.9±12.0
R	482.6±7.5	453.3±12.2	415.1±16.6	520.1±14.6
M+R	544.8±7.7	474.3±9.9	459.4±30.7	409.8±12.2
SOD ($Units\,g^{-1}\,dw$)				
C	26.0±3.0	30.1±3.2	26.0±2.5	32.3±4.4
M	30.5±1.2	26.8±1.6	10.7±1.6	39.6±2.3
R	26.6±1.9	27.3±1.1	14.0±1.2	25.5±2.2
M+R	32.4±1.5	36.0±2.4	12.1±1.2	22.1±1.5

C = control; M = inoculation with a mixture of three AM fungi; R = addition of a composted residue; M+R = addition of a composted residue and inoculation with a mixture of three AM fungi.

shoot SOD activity, particularly in the inoculated plants and those grown in the amended soil (Table 4). There was a significant negative interaction between mycorrhization, residue addition and water regime. The SOD activity in the shoots of control plants varied little from the beginning of water stress to the end of recovery. In contrast, a marked increase was found in mycorrhizal or residue-amended plants recovering from drought.

Discussion

Effect of the mycorrhizal fungi and composted residue on the growth of *J. oxycedrus*

The results of this study demonstrate that the inoculation of seedlings with a mixture of three exotic AM fungi and the addition of composted residue to soil stimulated the growth of

J. oxycedrus, irrespective of the water regime. The extent of mycorrhizal infection is of importance when studying the influence of AM fungi on the host plant. Large differences in AM percentage colonisation between non-inoculated seedlings and those inoculated with exotic AM fungi persisted throughout the drought recovery experiment, as the local indigenous AM fungi from the soil and/or composted residue showed little capacity to colonise shrub roots. The community of local AM fungi from the experimental soil was much less effective than the added *Glomus* inoculum with regard to stimulating host plant growth. The high level of infectivity of the mixture of AM fungi, despite the low soil water content, suggests that the added endophytes were the best adapted and/or most aggressive coloniser under drought conditions. Remarkably, *Juniperus* shrubs inoculated with the exotic AM fungi species were of comparable size to those treated with composted residue. This could be due to an improvement in the available nutrient supply in the soil arising from the composted residue. Cox et al. (2001) showed that the use of soil amendments can improve soil productivity, increasing the soil nutrient status for some limiting nutrients such as N and P. Thus, we report here that plants grown in the amended soil had higher N, P and K concentrations in their tissues than plants grown in the non-amended soil. Likewise, differential improvement of host plant nutrient status by the inoculum of exotic AM fungi, compared with native AM fungi from the soil, was also recorded before and after drought. In particular, the protection of mycorrhizal plants against water stress was related to the effect that the added endophytes had on increasing K uptake. The concentration of foliar K in mycorrhizal plants was particularly increased under drought conditions. Potassium plays a key role in plant water stress and has been found to be the cationic solute responsible for stomatal movements in response to changes in bulk leaf water status (Ruiz-Lozano et al., 1995).

Many drought-adapted species from arid environments have a highly developed root system and the root/shoot ratio is therefore high, which may be considered a mechanism of drought tolerance. Mycorrhizal symbiosis is known to decrease the root/shoot ratio (Smith and Read, 1997). The lack of roots is then compensated for by the extension of the mycorrhizal fungus extra-radical mycelium. The lower root/shoot ratio observed in stressed seedlings colonised by the mixture of AM fungi, as compared to control plants, may indicate partitioning of carbon to the fungus at the expense of root production (Wright et al., 1998). In this case, the extra-radical mycelium may have contributed to a more effective uptake of nutrients and water by plants submitted to water deficit (Augé, 2001). The increased shoot biomass of inoculated seedlings could be partly related to the increase in water uptake that a high level of root mycorrhizal infection provides under water deficit conditions. The higher shoot water content in plants inoculated with AM fungi supports that possibility. In general, it is worth noting that the water regime had no effect on growth and nutrient uptake of *J. oxycedrus* seedlings, likely due to the short duration of the drought treatment and because these plants are well adapted to drought conditions.

Effect of drought recovery on the physiological response of *J. oxycedrus*

Increasing evidence suggests that some 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 (Ruiz-Lozano, 2003). Both activities are involved in superoxide radical and hydrogen peroxide scavenging. In previous work, 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 (Ruiz-Lozano et al., 1996). Recently, Wu et al. (2006) observed that *G. versiforme* inoculation increased concentrations of antioxidant enzymes and non-enzymatic antioxidants in trifoliate orange seedlings, at least partly alleviating oxidative stress under water stress. An increase in several antioxidant enzymes has been observed also in shoots of mycorrhizal shrubs in semiarid Mediterranean conditions (Alguacil et al., 2003). In our study, drought enhanced POX activity only in shoots of non-mycorrhizal plants and grown in the soil non-amended. Peroxidases are involved in the defence against abiotic stresses (de Gara, 2004) by means of their role in the detoxification of H_2O_2 in the apoplast of lignifying tissues (Ros-Barcelo et al., 2006). Higher POX activity in inoculated plants and in plants grown in the soil amended, under both water regimes, could just reflect large size differences with respect to control plants. In particular, both the amended and mycorrhizal plants had greater basal diameter and shoot biomass than the control plants under well-watered

and stress conditions. It should be noted that POX are also responsible for cell wall lignification and other cell wall stiffening processes which conclude in the maturation of the cell wall (Passardi et al., 2004). The re-watering affected only the control plants, which reached levels of POX activity similar to that observed before water stress.

The mycorrhizal inoculation and composted residue produced a decrease in SOD activity in shoots of stressed *J. oxycedrus* plants compared to plants neither inoculated nor treated with composted residue. This decrease occurred only at the end of the drought period. Similarly, Porcel et al. (2003) found that in nodules, SOD, catalase and APX activities were lower in drought, mycorrhizal soybean plants than in the corresponding non-mycorrhizal plants. The decrease in SOD activity observed in mycorrhizal and amended plants has again provided evidence that these plants may be submitted to a lower oxidative stress under water shortage conditions. This could be attributed to primary drought avoidance mechanisms, such as the active water transfer from AM fungi to the host (Porcel and Ruiz-Lozano, 2004), or increased water uptake related to mycorrhizal changes in root morphology (Ibrahim et al., 1990) or to the better water retention properties of an amended soil (Caravaca et al., 2002). As mentioned above, the shoots of both mycorrhizal and amended plants were more hydrated than those of the control plants. These observations are in agreement with the proposal by Bartels (2001) that both the prevention of oxidative stress and the elimination of AOS are the most effective approaches used by plants to gain tolerance against several abiotic stresses, including drought. However, there are also several reports showing enhanced SOD activity in AM plants subjected to drought (Porcel and Ruiz-Lozano, 2004). These apparent contradictory results could be related not only to the host plant, but also to the fungal species involved in the association. Dissimilar behaviour of AM fungi in relation to several plant enzymatic activities has been often reported (Caravaca et al., 2005). The recovery seems to provoke oxidative stress because the levels of SOD activity increased in the shoots of mycorrhizal or residue-amended plants recovering from drought. In contrast, in a recent study, we found that SOD activity in shoots of mycorrhizal *Phillyrea angustifolia* seedlings did not change following drought recovery (Caravaca et al., 2005). Mittler and Zilinskas (1994) found that pea cytosolic Cu/Zn-SOD transcript levels increased during drought stress, but, surprisingly, were much higher during recovery. It has also been shown that the production of H_2O_2 can be induced by transferring suspension-cultured tobacco cells to a hypo-osmotic medium (Cazalé et al., 1998). Activation of an oxidative burst by osmotic stress may play a role in the regulation of cationic flux, such as K^+ flux. It is worth noting that all the re-watered plants reached similar concentrations of K in their shoots. The production of AOS, through modification of cell wall structure, could play a role in cell volume and turgor regulation after hypoosmotic stress.

In conclusion, drought decreased the SOD activity and did not affect the POX activity in shoots of both *J. oxycedrus* seedlings inoculated with exotic AM fungi and grown with composted residue, which may indicate that such plants developed mechanisms to avoid the oxidative damage produced under water shortage conditions. High constitutive POX levels in such plants could be correlated also with the avoidance mechanisms of the drought-induced oxidative stress. The capacity of the AM fungi for increasing plant tolerance to the drought stress imposed may have been related to the nutrient and water uptake improvement that a high level of root mycorrhizal infection provides. Increased levels of SOD activity in shoots of mycorrhizal or residue-amended plants during the drought recovery suggest that the re-watered plants suffered an oxidative stress.

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