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Arbuscular mycorrhizal fungi alleviate oxidative stress in pomegranate plants growing under different irrigation conditions

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Abstract: Drought greatly affects the growth and development of plants. This stressful condition can trigger an increase in reactive oxygen species (ROS) production that, in turn, can induce cellular, anatomical, and morphological changes that improve drought tolerance. A strain of arbuscular mycorrhizal fungi (AMF) is considered efficient when it colonizes roots quickly and extensively, absorbs and transfers nutrients to the plant host, promotes soil aggregation, and protects the host against disease. We evaluated the effect of inoculation of two strains of the AMF *Rhizophagus intraradices* (N.C. Schenck & G.S. Smith) C. Walker & A. Schüßler (GA5 and GC2) on pomegranate plants (*Punica granatum* L.) under two irrigation conditions. The response to oxidative stress depended on many factors, including the organism tissue and the degree of stress. Our study showed that, in most cases, mycorrhizal plants increased antioxidant defenses, such as the ROS-scavenging enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) in shoots under both irrigation levels, whereas the response for roots was ambiguous. AMF inoculation maintained the levels of malondialdehyde (MDA), probably by rapidly increasing antioxidant defenses and preventing lipid damage. We show that early AMF inoculation (particularly with the GC2 strain) in pomegranate propagation protects plants against abiotic stress.

Key words: arbuscular mycorrhizal fungi, pomegranate, catalase, ascorbate peroxidase, glutathione reductase, superoxide dismutase.

Résumé : La sécheresse affecte grandement la croissance et le développement des plantes. Cette condition de stress peut déclencher un accroissement de la production d'espèces réactives d'oxygène (ERO) qui, par la suite, peuvent induire des changements cellulaires, anatomiques et morphologiques qui améliorent la tolérance à la sécheresse. Une souche de champignon mycorhizien à arbuscule (CMA) est considérée efficace lorsqu'elle colonise les racines rapidement et largement, qu'elle absorbe et transfère les nutriments à la plante hôte, favorise l'agrégation du sol et protège l'hôte de la maladie. Nous avons évalué l'effet de l'inoculation de souches du CMA *Rhizophagus intraradices* (N.C. Schenck & G.S. Smith) C. Walker & A. Schüßler (GA5 et GC2) sur des plants de grenadier (*Punica granatum* L.) sous deux conditions d'irrigation. La réponse au stress oxydant dépendait de plusieurs facteurs, dont le tissu et le degré de stress. Notre étude a montré que dans la plupart des cas, sous les deux niveaux d'irrigation, les plants mycorhiziens accroissaient les défenses oxydantes dans les pousses telles la superoxyde dismutase (SOD), la catalase (CAT) et l'ascorbate peroxydase (APX), des enzymes qui piègent les ERO, alors que la réponse des racines était ambiguë. L'inoculation de CMA maintenait les niveaux de malondialdéhyde (MDA), probablement en augmentant rapidement des défenses anti-oxydantes et en prévenant le dommage lipidique. Nous montrons que l'inoculation précoce de CMA (particulièrement la souche GC2) du grenadier protège les plantes du stress abiotique. [Traduit par la Rédaction]

Mots-clés : champignon mycorhizien à arbuscule, grenadier, catalase, ascorbate peroxydase, glutathion réductase, superoxyde dismutase.

Introduction

Most (80%–90%) of the fresh mass of herbaceous plants and more than half of the fresh mass of woody plants consists of water. Drought greatly affects the growth and development of plants; therefore, different adaptations are required for survival (Nilsen and Orcutt 1996; Oelmüller et al. 2009). Drought stress can increase production of reactive oxygen species (ROS). These toxic molecules can cause oxidative damage to proteins, DNA, and lipids (Miller et al. 2010). The plant stress response involves several coordinated mechanisms, including morphologic, anatomic, and cellular changes that alleviate or improve stress tolerance (Dumas-Gaudot et al. 2000). Abiotic stress (including drought) causes a disruption of cellular homeostasis and increases the production of ROS from pathways such as respiration and photorespiration (Causin et al. 2009; Roqueiro et al. 2012).

Plants have developed efficient systems for ROS removal, including enzymatic and nonenzymatic molecules (Ruíz-Lozano et al. 2012). ROS-scavenging enzymes, such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR), have been found in almost all cellular compartments

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Table 1. Mycorrhizal colonization, arbuscules, vesicles, and survival percentages in pomegranate plants.

				Survival%[b]	
Treatment	MI%[a]	A%[a]	V%[a]	FC	½FC
C	n.d.	n.d.	n.d.	96.7±0.001a	96.7±0.007a
GA5	75.32±2.94a	43.98±7.35b	64.26±3.31a	93.5±0.9b	91.3±0.05d
GC2	56.37±6.05b	42.15±6.11b	38.8±5.02b	95.8±0.1b	95.8±0.02b
GA5+GC2	81.69±2.62a	71.08±2.89a	59.3±4.60a	100±0.4c	100±0.01c
ANOVA					
AMF	***	***	***	*	*
FC	—	—	—	n.s.	n.s.
AMF x FC	—	—	—	n.s.	n.s.

Note: Treatments: C, control without inoculation; GA5, *Rhizophagus intraradices* GA5 strain inoculation; GC2, *Rhizophagus intraradices* GC2 strain inoculation; GA5+GC2, mixture of GA5 and GC2 strains (1:1); AMF, arbuscular mycorrizal fungi. MI%, percent mycorrhiza; A%, percent arbuscules; V%, percent vesicles. n.d., not detectable; FC, field capacity; ½ FC, half field capacity. Different letters following the variables indicate statistically significant differences ($P < 0.05$). *, $P < 0.05$; ***, $P < 0.001$; n.s., not significant. Data represent the means of six replicates ± SE.

[a]Analyzed with one-way analysis of variance (ANOVA).
[b]Analyzed with factorial ANOVA.

(Miller et al. 2010). Therefore, testing the activities of these enzymes may improve our understanding of plant response against drought stress.

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that can colonize a wide range of plants species. They are nonspecific because the same strain can colonize different hosts. However, the ability of species to promote plant growth is variable (Trappe 1986; Alguacil et al. 2003; Wu et al. 2007, 2008). A strain is considered efficient when, under a wide range of environmental conditions, it colonizes roots quickly and extensively, competes with other microorganisms for the same sites of colonization and nutrient absorption, rapidly forms an extraradical hyphal net, absorbs and transfers nutrients to the plant, promotes nonnutritional benefits to the host, such as soil aggregation and stabilization, and protects the plant against disease (Saggin-Júnior and da Silva Ribeiro 2005). Several reports have paid special attention to the importance of AMF in alleviating oxidative stress; they found that the protection against oxidative damage was due to an increase in enzymatic antioxidant levels (Alguacil et al. 2003; Porcel and Ruíz-Lozano 2004; Wu et al. 2006; Bressano et al. 2010).

Pomegranate plants (*Punica granatum* L.) are propagated by cuttings, seeds, or grafts. They are used as ornamental plants and edible fruit, and are cultivated in the same area as olives and oranges. Pomegranate plants are typically from South Asia and the Mediterranean area, but their cultivation has recently expanded to China, the United States, Chile, and Argentina (Franck 2009). Pomegranate growing requirements include well-drained soil (up to 50%–60% water), proper fertilization, and sunshine throughout the year. Pomegranates are drought-tolerant, but they require normal watering to produce good fruit crops (Parodi 1978; Chopade et al. 2001; Khattab et al. 2011) and to avoid dehydration during the hottest season (particularly after transplant). In this study, pomegranate plants were chosen as a model to study the importance of the application of AMF to the production of semi-ligneous cuttings under nursery conditions.

There is a growing need to improve the resistance of cultivated plants in several regions of the world that are suffering from drought because of global climate change. AMF inoculation could be a useful tool for improving plant survival and growth by increasing resistance to drought stress (Waterer and Coltman 1989; Stahl et al. 1998; Oelmüller et al. 2009). The aim of this study was to evaluate the effect of the inoculation and co-inoculation of two strains of AMF with different colonization strategies on pomegranate plants under two levels of water irrigation.

Materials and methods

Plants

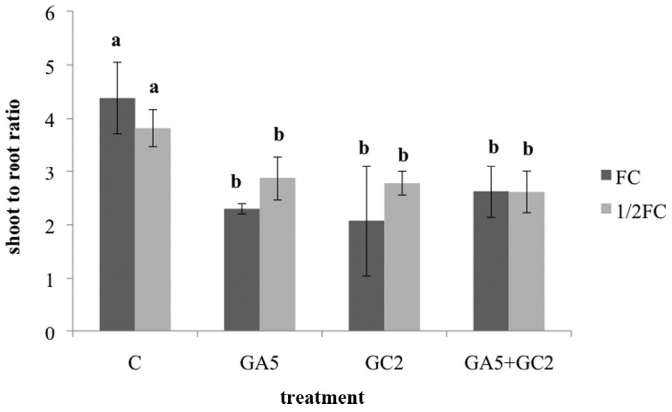
A vigorous and youthful mother plant of *Punica granatum* L. cv. Plena Voss was chosen from the Departamento de Producción Vegetal (Facultad de Agronomía, FAUBA, Argentina). A total of 110 young branch cuttings of 7 cm in length and two shoots from the same pomegranate plant were used, to eliminate genetic variability. Indolebutyric acid (concentration of 2500 ppm) was added to a wound made at the bottom of each cutting. Rooting was performed on a raised table with perlite at a density of 1000 cuttings·m^{-2} with intermittent irrigation over 40 days under nursery conditions. A 90% rooting success was calculated to obtain sufficient plants for this experiment.

Mycorrhizal inoculation

Two strains (GA5 and GC2) of *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler (formerly *Glomus intraradices*) were provided by the Banco de Glomeromycota In Vitro (BGIV) (http://www.bgiv.com.ar/strains/Rhizophagus-intraradices/gc2; http://www.bgiv.com.ar/strains/Rhizophagus-intraradices/ga5), which have different strategies of colonization. The GC2 strain has a high density of external mycelium, is slow growing at the beginning of the in vitro culture, increases with the proportion of mycelium ramification, and possesses relatively few larger spores as compared with the GA5 strain (mycelium density, 160.5 ± 19.8 cm^2; spore diameter, 87.4 ± 0.4 μm) (Silvani 2011). The spores and mycelium are limited to the vicinity of the roots where the infection takes place. In contrast, GA5 have little external mycelium at the beginning of the in vitro culture, which then increases in density and forms a mycelium with few ramifications. This strain has a higher growth rate, and its spores are smaller and more abundant than the GC2 strain (mycelium density, 293.4 ± 81.8 cm^2; spore diameter, 70.8 ± 0.5 μm) (Silvani 2011). The strains were propagated on *Trifolium repens* L. as the host plant in 1.5 L pots with a tindalized perlite–soil mixture (3:1, perlite to soil). The soil characteristics were: pH of 7.1, 12.08 g·kg^{-1} total C, 1.1 g·kg^{-1} N, 34.2 mg·kg^{-1} P, 0.9 cmol·kg^{-1} K, 7.5 cmol·kg^{-1} Ca, 1.7 cmol·kg^{-1} Mg, and 0.2 cmol·kg^{-1} Na. All plants were maintained for 4 months under greenhouse conditions (450 μmol photons·m^{-2}·s^{-2}, 400–700 nm; 16 h light – 8 h dark; 25 °C day and 18 °C night temperatures; 60%–70% relative humidity). Plants were watered with Hewitt (1952) solution without phosphorous every 15 days and unwatered thereafter until dry to obtain dry mycorrhizal inoculum.

Cuttings were placed on a raised rooting table, which was divided into four parts separated with plastic panels to prevent the advance of mycorrhizae and roots. After 30 days of rooting, they were inoculated with the GC2 and GA5 strains of *R. intraradices*.

Fig. 1. Shoot-to-root ratio in pomegranate plants. Treatments: C, control without inoculation; GA5, *Rhizophagus intraradices* GA5 strain inoculation; GC2, *Rhizophagus intraradices* GC2 strain inoculation; GA5+GC2, 1:1 mixture of GA5 and GC2 strains. FC, field capacity; 1/2FC, half field capacity. Different letters over the columns indicate statistically significant differences ($P < 0.05$). Data were analyzed with a factorial analysis of variance (ANOVA). Data represent the means of six replicates ±SE.



We performed the following treatments: control (C); inoculation with *R. intraradices* strain GC2; inoculation with *R. intraradices* strain GA5; and inoculation with a 1:1 mixture of both strains (GA5+GC2). For the inoculations, we made 3-cm-deep furrows between groups of cuttings, to which we added 10 g of dry inoculum of the strain used in each treatment (1161 ± 13 spores and 851 ± 5 spores per 100 g dry soil for GA5 and GC2, respectively). The control treatment received 10 g of autoclaved inoculum supplemented with a filtrate (<20 μm) of mycorrhizal inoculum (to provide microbial populations). The experiment was arranged using a completely randomized design with equal replications of six pots for each treatment.

Experimental design

After 30 days of inoculation on a raised rooting table, 96 plants were transplanted to 1 L pots with a tindalized perlite:vermiculite: soil mixture (2:1:1 by volume; see above for soil characteristics). They were maintained for 30 days at 100% field capacity (FC) under nursery conditions. Thereafter, half of the plants (48 plants) were maintained at FC and the other half at 50% FC (1/2 FC) for 60 days under nursery conditions. Irrigation levels were previously tested in a preliminary experiment (unpublished results). All plants were irrigated once, 30 days after the start of the experiment, with nutritive solution without added phosphorous (Hewitt 1952).

Growing parameters

Mycorrhization was tested 30 days after inoculation. A representative portion of root was stained for each treatment (Phillips and Hayman 1970). The proportions of mycorrhizae (MI%), arbuscules (A%), and vesicles (V%) in each stained sample were determined separately (Giovanetti and Mosse 1980). Plant survival and the fresh and dry mass (at 70 °C until constant mass) of shoots and roots were quantified. Water content was calculated as the difference between fresh and dry mass. The shoot to root biomass ratio was evaluated. The microbial inoculation effect (MIE%) was measured as the average mycorrhizal plant biomass divided by the average nonmycorrhizal plant biomass (Menge et al. 1978).

Enzyme extraction

For enzymatic measurements, fresh plant materials (shoots and roots) were weighed, and 1 g of shoots and of roots from each plant was immediately frozen in liquid nitrogen to maintain the integrity of the tissue. Each sample was pulverized in a mortar, with polyvinylpolypyrrolidone (PVPP, 0.06 g per 6 mL extraction buffer) and 5 mL of extraction buffer (KH_2PO_4–K_2HPO_4 50 m, pH 7.8, plus 0.1 mmol·L^{-1}) EDTA). The resulting mixture was filtered through a nylon membrane to remove cell debris and centrifuged at 20 000 *g* for 20 min. Supernatants were aliquoted and maintained at –70 °C until use (Gogorcena et al. 1995). For APX determination, ascorbic acid (4 mmol·L^{-1}) was added to aliquots to preserve enzymatic activity (Moran et al. 1994). For GR determination, β-mercaptoethanol (10 mmol·L^{-1}) was added to maintain the reducing environment (Moran et al. 1994).

Enzymatic measurements and lipid peroxidation

The intracellular activities of the following enzymes were measured: CAT (EC 1.16.1.6), APX (EC 1.11.1.11), SOD (EC 1.15.1.1), and GR (EC 1.6.4.2). Measurement of CAT was based on the decrease in absorbance at 240 nm caused by the disappearance of H_2O_2 (Aebi 1984). APX was measured as the absorbance at 290 nm while ascorbic acid was oxidized (Hossain and Asada 1984). SOD was measured at 560 nm according to its capacity to inhibit the photochemical reduction of nitro blue tetrazolium in the presence of riboflavin (Beyer and Fridovich 1987). GR was estimated as the oxidation rate of nicotinamide adenine dinucleotide phosphate (NADPH) as glutathione is converted from its oxidized to its reduced form (Carlberg and Mannervik 1985). Enzymatic extracts from the shoots and roots were used to measure the malondialdehyde (MDA) content after reaction with a solution of trichloroacetic acid and thiobarbituric acid (Hodges et al. 1999). Enzyme activities and MDA content measurements were standardized by total protein (PROT) determination (Bradford 1976).

Statistical analysis

All data were analyzed using factorial analysis of variance (ANOVA). Assumptions of homogeneity of variance and normality were checked. Comparisons of mean values among different treatments were made using Tukey's honestly significant difference (HSD) test using a significance level of $P < 0.05$ (Clewer and Scarisbrick 2001). Statistical procedures were carried out using the software STATISTICA 6.0 for Windows XP.

Results

Plants colonized by the GC2 strain of *R. intraradices* had significantly lower proportions of mycorrhizae than plants colonized by the GA5 strain or the co-inoculated plants (Table 1). Proportions of arbuscules were similar for plants with single inoculations; however, co-inoculated plants had higher proportions of arbuscules. The GC2 strain resulted in fewer vesicles compared with the GA5 strain and the combination of both strains.

There was no interaction between inoculation and the level of irrigation applied on plant survival and shoot-to-root ratios. All co-inoculated plants survived; the next highest survival rate was for the noninoculated plants (Table 1). Regardless of the irrigation level, the assayed shoot-to-root ratios were significantly lower in inoculated compared with noninoculated treatments (Fig. 1). However, there were no significant differences among treatments for shoot and root fresh and dry mass, water content, or microbial inoculation effect (data not shown).

There was an interaction between inoculation treatments and irrigation levels in SOD activity in pomegranate shoots. Inoculated plants had significantly increased SOD enzyme activity compared with control plants at both irrigation levels. Moreover, the GC2-inoculated plants had significantly increased SOD activity at 1/2FC compared with FC (Fig. 2*a*). The CAT, APX, and GR activities were significantly higher in GC2-inoculated plants, regardless of the irrigation level (Figs. 2*c*, 2*e*, 2*g*). At both irrigation levels, the CAT enzyme activity was significantly greater for GA5-inoculated plants compared with control plants (Fig. 2*c*). No significant differences among treatments were observed for the MDA content

Fig. 2. Enzyme specific activities in pomegranate shoots (*a*, *c*, *e*, *g*) and roots (*b*, *d*, *f*, *h*). SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; PROT, total protein. Treatments: C, control without inoculation; GA5, *Rhizophagus intraradices* GA5 strain inoculation; GC2, *Rhizophagus intraradices* GC2 strain inoculation; GA5+GC2, 1:1 mixture of GA5 and GC2 strains. FC, field capacity; 1/2FC, half field capacity. Different letters indicate significant differences at $P < 0.05$. Data were analyzed with factorial analysis of variance (ANOVA). Data represent the means of six replicates ±SE.

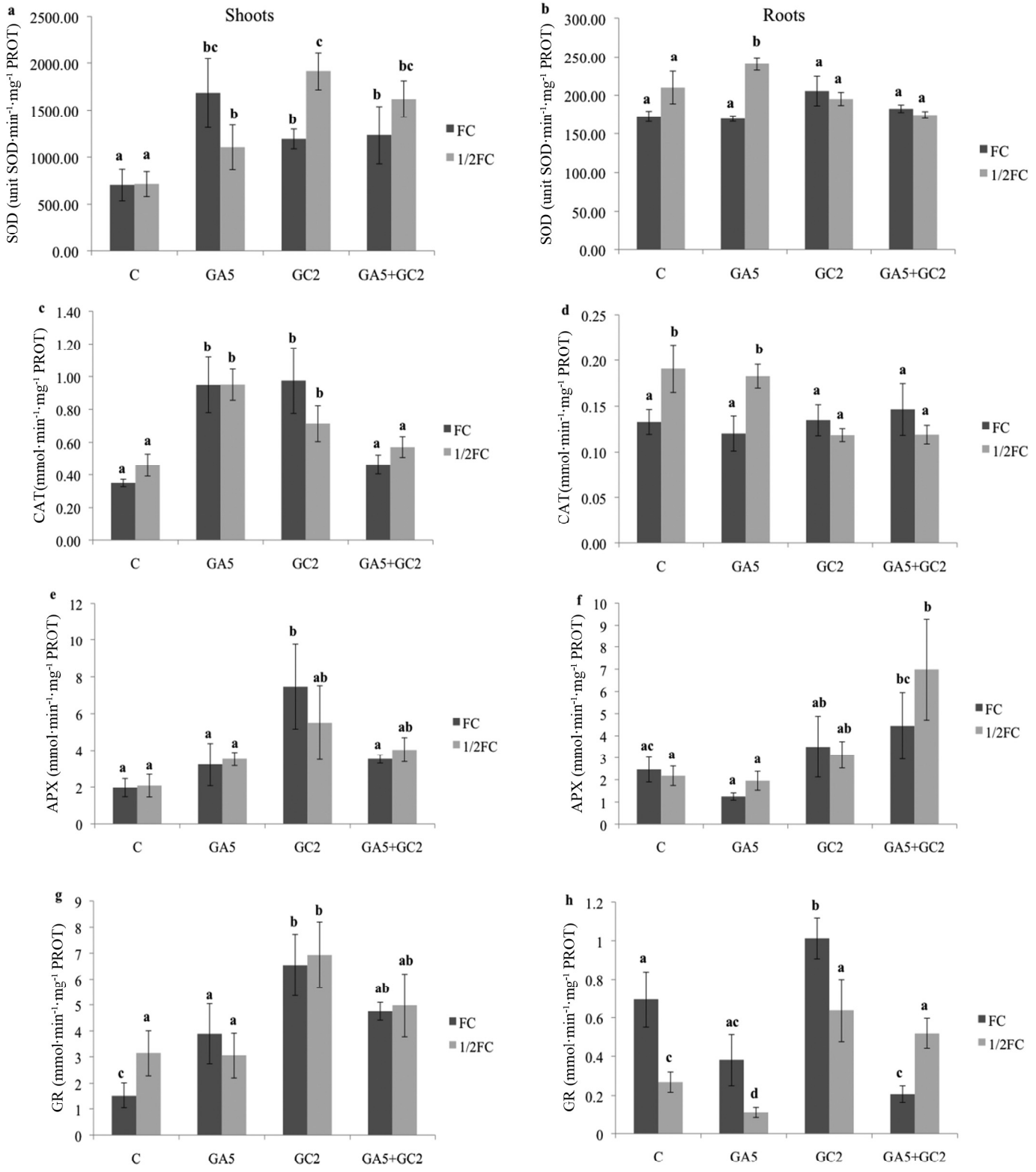
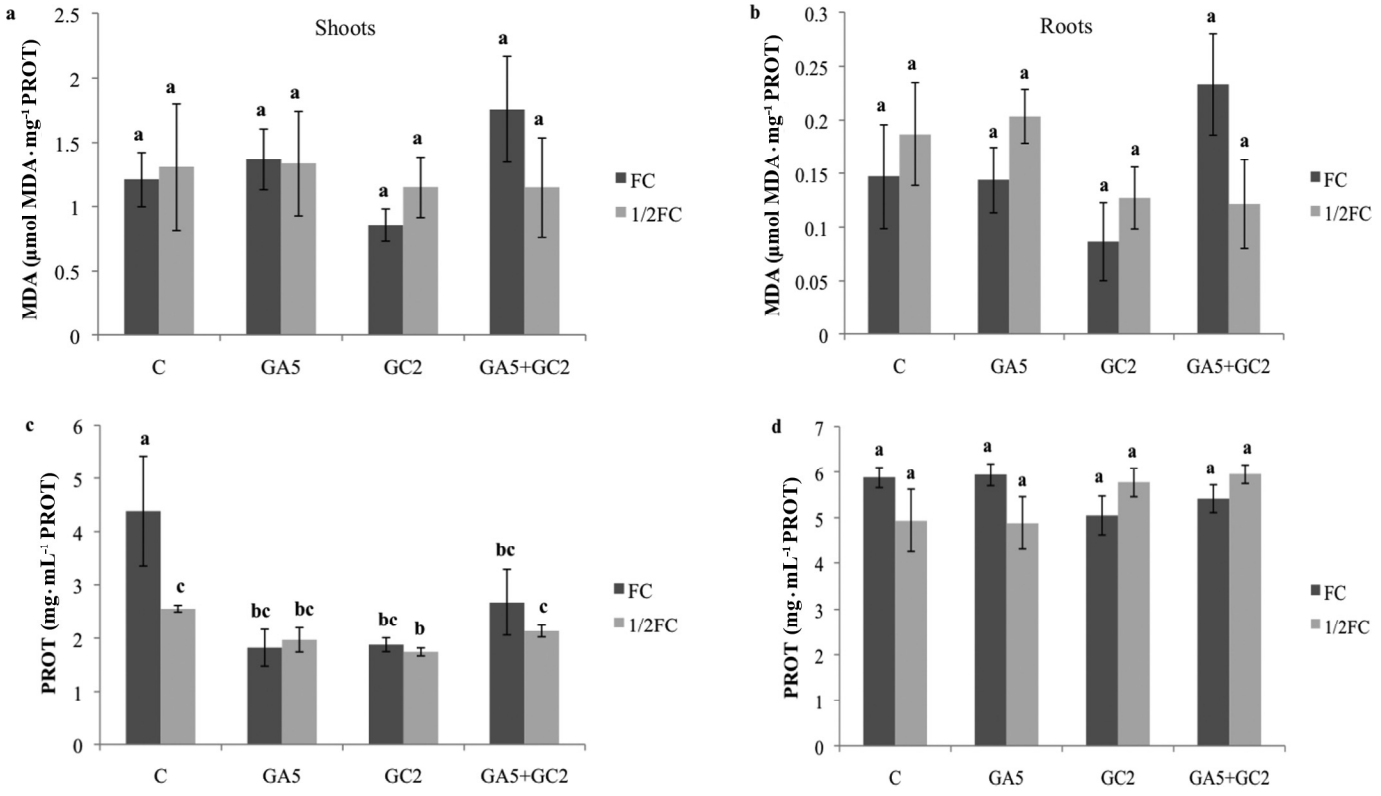


Fig. 3. Malondialdehyde (MDA) and total protein (PROT) contents in pomegranate shoots (*a*, *c*) and roots (*b*, *d*). Treatments: C, control without inoculation; GA5, *Rhizophagus intraradices* GA5 strain inoculation; GC2, *Rhizophagus intraradices* GC2 strain inoculation; GA5+GC2, 1:1 mixture of GA5 and GC2 strains. FC, field capacity; 1/2FC, half field capacity. Different letters indicate significant differences at $P < 0.05$. Data were analyzed with factorial analysis of variance (ANOVA). Data represent the means of six replicates ±SE.



(Fig. 3*a*), but the PROT content was significantly lower in inoculated plants compared with the FC (Fig. 3*c*).

Irrigation level and inoculation treatment affected enzyme activities in pomegranate roots. The GA5-inoculated plants had greater SOD and lower GR activities under the 1/2FC condition compared with the FC condition. The GC2-inoculated plants had greater GR activity under the FC condition compared with control plants. Under the 1/2FC condition, GR enzyme activity decreased but was significantly higher in the GC2-inoculated plants than in control plants for the same condition (Figs. 2*b*, 2*h*). Co-inoculation resulted in a significant increase in APX and GR activities of roots under the 1/2FC condition compared with the control plants (Figs. 2*f*, 2*h*). The MDA and PROT were not affected by any treatment (Figs. 3*b*, 3*d*).

Discussion

Although inoculation with the GC2 strain of *R. intraradices* decreased the plant growth rate, co-inoculation with another strain may have had a synergistic effect that resulted in an increase in the proportion of arbuscules but not mycorrhizae or vesicles. Our results of greater plant survival rates with co-inoculation are in accordance with Alguacil et al. (2003) who found high survival rates in *Olea europaea* L., *Retama sphaerocarpa* Boiss., and *Rhamnus lycioides* L. plants inoculated with a mixture of native AMF. The lack of significance in the difference in growth parameters among different treatments in our experiments was also observed by Wu et al. (2006) on citrus seeds inoculated with *Glomus versiforme* (P. Karst.) S.M. Berch.

We found a denser root system in small mycorrhizal plants, which is in agreement with several reports (Davies et al. 2002; Alguacil et al. 2003; Franco et al. 2011). Therefore, a lower shoot-to-root ratio in inoculated plants compared with noninoculated plants could be because of low mycorrhizal activity in relation to biomass production (Alguacil et al. 2003). Plant biomass was not affected by these strains. The shoot-to-root ratio was smaller in inoculated plants, but these plants were more resistant than control plants to the antioxidant enzyme responses.

Several authors have associated biomass and nutrient status with increased activities of antioxidant enzymes in mycorrhizal plants (Alguacil et al. 2003; Huang et al. 2008; Roldán et al. 2008; Franco et al. 2011). Dissipation of excess energy absorbed by the photosynthetic apparatus is a fundamental process that is essential for the survival of photosynthetic organisms. It prevents the photo-oxidative damage that occurs when excited chlorophyll molecules improperly transfer their higher energy state to oxygen or neighboring molecules and convert them into reactive molecules or toxic radicals (Rizhsky et al. 2003). Several authors have found changes in CAT and SOD activities with mycorrhizal inoculation under abiotic stress (Gogorcena et al. 1995; Porcel et al. 2003; Wu et al. 2006, 2010; Roldán et al. 2008; Wu and Zou 2009).

Although our results show that MDA content was not affected by treatments and irrigation levels, several other authors have found a differential behavior in MDA content. Wu et al. (2010) observed a reduction of lipid peroxidation in trifoliate orange inoculated with *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler and *G. versiforme* under salt stress. Moreover, Rahmaty and Khara (2011) observed lower levels of MDA at high concentrations of chromium in maize plants inoculated with *R. intraradices* in comparison with control plants. However, Zhu et al. (2011) found no changes in MDA shoot content in maize plants inoculated with *Glomus etunicatum* W.N. Becker & Gerd., regardless of water status.

In pomegranate plants inoculated with the GC2 strain, shoots were protected against oxidative stress. The PROT content in in-

oculated plants was not affected by the irrigation condition; however, similar to control plants at the low irrigation level, the PROT content in inoculated plants was reduced after stress compared with before stress. These results are contrary to those of Wu et al. (2010), who observed no changes in soluble protein content in trifoliate orange seedlings inoculated with *F. mosseae* under NaCl stress, even though the values were significantly higher than those in control plants. Wu et al. (2006) proposed that when plants are subjected to water stress, the equilibrium between ROS production and scavenging is broken, resulting in oxidative damage to proteins, DNA, and lipids. We found no change in MDA content (associated with the oxidative damage of lipids), indicating a lower accumulation of ROS in AMF shoots.

In roots, the GR and APX enzyme activities varied among treatments, but without observed changes in MDA content. This finding is in accordance with several authors, who found benefits of AMF through osmotic adjustment and an improvement of ROS metabolism (Porcel and Ruíz-Lozano 2004; Wu et al. 2006; Zhu et al. 2011; Rahmaty and Khara 2011). Porcel et al. (2003) observed that GR and APX are two of the main antioxidant defense systems in plants. However, Wu et al. (2006) and Wu and Zou (2009) observed a decrease in MDA content in inoculated plant roots under abiotic stress. This decrease was due to a low content of H_2O_2, which implies a lower accumulation of ROS in AM seedlings and, therefore, less membrane damage. Oelmüller et al. (2009) observed that mutualism is often accompanied by upregulation of ROS scavenging systems, which prevents ROS accumulation and oxidative burst.

The shoots of mycorrhizal plants generally showed increased levels of antioxidant defense factors, such as SOD, CAT, and APX, under both irrigation levels. However, roots showed an ambiguous response, perhaps because of an improvement in the osmotic regulation of roots by mycorrhizae. AMF inoculation maintained the MDA levels, probably by rapidly increasing antioxidant defense and preventing lipid damage.

Conclusion

We have shown that early AMF inoculation during pomegranate propagation protects plants against abiotic stress. The response to oxidative stress depends on many factors, including the tissue under analysis and the extent of the stress. Assuming that different AMF strains could activate different antioxidant responses, co-inoculation could favor a more pronounced response. However, plants inoculated with only one strain of AMF (particularly with the GC2 strain) responded to the water stress condition, alleviating lipid damage but at the expense of growth.

These findings support the benefits of mycorrhizae for pomegranate plants in terms of abiotic stress tolerance, which might be useful for expanding cultivation areas devoted to the pomegranate fruit industry. However, all treatments caused a decrease in protein content, suggesting that further study on mycorrhizal inoculation and fruit quality should be done to evaluate the risks or benefits of its use as a biofertilizer.

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