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REGULAR ARTICLE

Importance of native arbuscular mycorrhizal inoculation in the halophyte *Asteriscus maritimus* for successful establishment and growth under saline conditions

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Abstract
Background and aims The biological restoration of saline habitats could be achieved by using halophyte plant species together with adapted arbuscular mycorrhizal fungi (AMF). An interesting plant to be used in restoration of saline environments, *Asteriscus maritimus*, is highly mycotrophic. The aim of this study was to assess the effectiveness of native and allochthonous AMF to enhance the establishment and growth of the halophyte *A. maritimus* under saline conditions.
Methods We studied the symbiotic effectiveness of four AMF strains (three native fungal isolates from a saline soil and one allochthonous, from collection) in *A. maritimus* subjected to increasing salinity stress. We measured plant physiological parameters by which AMF may ameliorate the detrimental effects of salinity stress.
Results A. maritimus plants showed a high mycorrhizal dependency, even in absence of salt stress. Plants inoculated with native AMF had higher shoot dry weight, efficiency of photosystem II, stomatal conductance and accumulation of glutathione than those inoculated with the collection AMF at the highest level of salinity. Moreover, at this salt level, only 30 % of *A. maritimus* plants inoculated with the collection AMF survived, while with the three native AMF, the rate of survival was 100 %.
Conclusions Results points out the importance of native AMF inoculation in the establishment, survival and growth of *A. maritimus* plants. Inoculation with these native AMF enhanced *A. maritimus* salt tolerance by increasing efficiency of photosystem II, stomatal conductance and glutathione content and by reducing oxidative damage. Thus, the use of adequate native AMF inocula could be a critical issue for success in recovering saline degraded areas.

Keywords Native arbuscular mycorrhiza fungi · Mediterranean ecosystems · Halophyte · Restoration · Salinity

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Introduction

Mediterranean regions are characterized by very limited rainfall, high light irradiance and maximum air temperatures largely above 30 °C in summer, which difficult plant growth (Brito et al. 2011). The latter may provoke the loss of natural plant communities and accelerate the processes of soil degradation and environmental changes (Alguacil et al. 2011). These conditions make

Mediterranean ecosystems fragile and susceptible to degradation and desertification (Ferrol et al. 2004). Thus in Mediterranean areas, global change will involve not only increased in aridity but also significant changes in land use (Valladares 2004). This multiple stress situation is promoting desertification of large areas in Southeast Spain and an alarming increase in salinity. Salinization of soils is a major problem, not only agronomical but also ecological, in particular in arid and semiarid ecosystems, where water is scarce and salts cannot be properly dissolved and they accumulate in the soil (Evelin et al. 2009). High salinity induces ionic toxicity, osmotic stress and leads to secondary oxidative stress in plants (Ding et al. 2010). Indeed, under salinity plant stomata close and the availability of atmospheric CO_2 and consumption of NADPH by the Calvin cycle are restricted. When ferrodoxine is over-reduced during photosynthetic electron transfer, electrons may be transferred from photosystem I to oxygen to form superoxide radicals by the process known as Mehler reaction, which initiates chain reactions that produce more harmful reactive oxygen species (ROS) such as singlet oxygen, hydrogen peroxide and hydroxyl radicals (Gill and Tuteja 2010). The excess of ROS production can damage the structures of enzymes and other macromolecules in plant cells (Mittler 2002). Thus, they can seriously harm the metabolism through denaturation of proteins, mutagenesis of DNA and lipid peroxidation (Miller et al. 2010). However, plants have evolved appropriate detoxification systems, both enzymatic and non-enzymatic, to allow the removal of ROS. The tolerance of plants to salt stress has been correlated to the antioxidant capacity in several plant species (Türkan and Demiral 2009). One of the most important tasks to restore the productivity of saline lands is to improve soil conditions, reduce desertification and raise the fertility of soils (Tawfik et al. 2010). Hence in restoration of Mediterranean ecosystems there is a need to look for ecophysiological features that enhance plant performance under conditions that will be exacerbated by climate change, such as salinity (Vallejo et al. 2005).

The below-ground microbial communities, particularly arbuscular mycorrhizal fungi (AMF), are well known to improve soil structure and benefit plant performance by helping in the establishment, enhancing the resistance to environmental stresses and increasing plant nutrient and water uptake (Jeffries and Barea 2012). Mycorrhizal symbiosis is universally distributed among the majority of plants and forms a network of extra-radical mycelium that provides a direct physical link between the plant root and the soil (Smith and Read 2008). Moreover, they affect the diversity and productivity of plants helping in the stability and sustainability of ecosystems (van der Heijden et al. 1998). Although it has been reported that excess of salts in the soil inhibits spore germination and growth of AMF (Juniper and Abbott 2006), several studies found a high diversity of those fungi in saline soils (Yamato et al. 2008; Wilde et al. 2009). Even more, in arid and semiarid Mediterranean regions, AMF play a key ecological role in the functioning of ecosystems (Requena et al. 1996).

On the other hand, halophytes, which constitute about 1 % of the world flora, are specialised plants that can tolerate and complete their life cycles under high levels of salts in the soil (Munns and Tester 2008). They are physiologically and biochemically adapted to grow in saline soils (Tawfik et al. 2010). Most of the halophytes in saline sites belong to the *Chenopodiaceae*, *Juncaceae*, *Cyperaceae* or *Brassicaceae* families which are non -or weakly mycotrophic plants. However, associations between AMF and halophytes are also widely formed, including *Aster tripolium*, *Inula crithmoides*, *Plantago maritima*, *Salsola soda* and *Suaeda maritima* (Evelin et al. 2009; Sonjak et al. 2009). For the present study we selected a native halophyte of lands surrounding the Mediterranean Sea, especially Spain, *Asteriscus maritimus* (L.) (Lendínez et al. 2011). Rodríguez et al. (2005) considered this plant as an useful species in revegetation programmes in Mediterranean areas affected by salinity. *A. maritimus* is a member of the Asteraceae family and known since long time to be highly mycotrophic (Mason 1928). In fact, native plants thrive in various abiotically stressed ecosystems thanks to AMF that have co-evolved and are essential for their adaptation to stressed conditions (Rodriguez and Redman 2008). Nevertheless, in arid and semiarid ecosystems there is a low density of AM propagules making difficult the successful reestablishment of native plants (Jeffries et al. 2002). Thus, it is very important the ecophysiological study of autochthonous AMF isolates and the knowledge of their mechanisms of salinity stress adaptation and tolerance (Enkhtuya et al. 2000) in order to select the most adapted and efficient species/strains of AMF to serve as inocula in revegetation programs (Ferrol et al. 2004). Both halophytes and indigenous AMF isolates from saline habitats have developed a variety of modifications to survive in saline environments, such as regulating ionic homeostasis and detoxifying ROS (Zhu 2001; Ruiz-Lozano et

al. 2012). The biological restoration of saline ecosystems could be achieved by using halophytes together with inoculation of adapted AMF. However the biochemical mechanisms by which AM-colonized halophytic plants tolerate or reduce salt stress are poorly understood.

In the present work, we studied the symbiotic effectiveness of four AMF strains (three native isolates from a saline soil and one allochthonous, belonging to the EEZ collection) in *Asteriscus maritimus* subjected to salinity stress. The native strains of AMF were successfully isolated from the rizhosphere of *A. maritimus* at Cabo de Gata Natural Park (Almería, SE Spain), which is the most arid ecosystem in Europe (Geiger 1973) with important salinity problems as well. The aim was to assess the effectiveness of AM association among different AM isolates under saline conditions, to enhance the establishment and growth of the halophyte *A. maritimus* for revegetation in salt affected areas. In addition, we tried to elucidate the physiological and biochemical basis of the mechanisms involved in the variability of the symbiotic performance.

Materials and methods

Identification of the mycorrhizal strains isolated from Cabo de Gata Natural Park

AMF spores were separated from the soil samples by a wet sieving process (Sieverding 1991). The morphological spore characteristics and their subcellular structures were studied as described by Estrada et al. (2013a). In addition to the morphological identification, a molecular identification based on rDNA sequencing was also carried out as described by Estrada et al. (2013a). Thus, on the basis of both morphological and molecular analyses, the AM fungal strains were identified at the species level (Krüger et al. 2012) as *Rhizophagus intraradices* (Schenk and Smith), *Claroideoglomus etunicatum* (Becker and Gerdemann) and *Septoglomus constrictum* (Trappe). The AM fungal strains have been incorporated to the collection of Zaidin Experimental Station, Granada, Spain, under accession numbers EEZ 195, EEZ 196 and EEZ 163, respectively.

Experimental design

The experiment consisted of a randomized complete block design with five inoculation treatments: (1) non-mycorrhizal control plants, (2) plants inoculated with the model AM fungus *Rh. intraradices* (Ri collect) reproduced at the collection of the Zaidin Experimental Station (isolate EEZ 58), (3) plants inoculated with the AM fungal strain *Rh. intraradices* isolated from Cabo de Gata Natural Park (Ri CdG), (4) plants inoculated with the AM fungal strain *Se. constrictum* isolated from CdG (Sc CdG) and (5) plants inoculated with the AM fungal strain *Cl. etunicatum* isolated from CdG (Ce CdG). There were 30 replicates of each inoculation treatment, totalling 150 pots (one plant per pot), so that ten of each microbial treatment were grown under nonsaline conditions throughout the entire experiment (only the salinity provided by the soil/sand mixture used), while ten pots per treatment were subjected to 100 mM of NaCl and the remaining ten pots per treatment were subjected to 175 mM of NaCl.

Soil and biological materials

Loamy soil was collected from Cabo de Gata Natural Park (Almería province) (Spain, 36º45′24″N 02º13′17″W), sieved (5 mm), diluted with quartz-sand (<2 mm) (1:1, soil:sand, v/v) and sterilized by steaming (100 °C for 1 h on 3 consecutive days). The original soil had a pH of 8.7 [measured in water 1:5 (w/v)]; 0.26 % organic matter, nutrient concentrations ($g kg^{-1}$): N, 0.3; available P, 47.0 and soil electrical conductivity 3.95 dS m^{-1}.

Seeds of *Asteriscus maritimus* L. were sown on a vermiculite:sand mixture (1:1, v/v) for germination. Two weeks after germination, four seedlings of *A. maritimus* were transplanted per pot containing 900 g of the same soil/sand mixture as described above and thinned to one plant per pot after establishment was successfully done (plants were selected with uniform size for each treatment).

Inoculation treatments

Mycorrhizal inoculum consisted of soil, spores, mycelia and infected root fragments. The AMF species used were three strains isolated from Cabo de Gata Natural Park (Almería, Spain): *Rhizophagus intraradices* (previously named *Glomus intraradices*), *Septoglomus constrictum* and *Claroideoglomus etunicatum* and a *Rhizophagus intraradices* strain from our culture collection. Appropriate amounts of each inoculum containing about 700 infective propagules (according to

the most probable number test), were added to the corresponding pots at transplanting time just below *A. maritimus* plantlets. The AM inoculum containing the highest number of propagules was diluted with sterile sand in order to equalize the amount of AM inoculum used for each AM fungus. Thus, 20 g of inoculum (about 35 propagules per gram) were used for each fungal strain. Non-mycorrhizal control plants received the same amount of autoclaved mycorrhizal inocula together with a 10 ml aliquot of a filtrate (<20 μm) of the four AM inocula in order to provide a general microbial population free of AM propagules.

Growth conditions

The experiment was carried out under glasshouse conditions with temperatures ranging from 19 to 25 °C, 16/8 light/dark period, and a relative humidity of 50–60 %. The experiment was conducted between January 2010 and June 2010. A photosynthetic photon flux density of 800 μE $m^{-2}s^{-1}$ was measured with a light meter (LICOR, Lincoln, NE, USA, model LI-188B). Water was supplied daily to the entire period of plant growth to avoid any drought effect. Plants were established for 6 weeks prior to salinization to allow adequate plant growth and symbiotic establishment. Three concentrations (0, 100, and 175 mM NaCl) of saline solution were reached in the soil substrate by adding appropriate dilutions of a stock 2 M saline solution. The concentration of NaCl in the soil was increased gradually on alternative days to avoid an osmotic shock. It took 6 weeks, to reach the desired 100 and 175 mM NaCl levels. The electrical conductivities in the soil:sand mixture used as growing substrate were 2.2, 8.9 and 13.5 dS m^{-1} for the salt levels of 0, 100, and 175 mM NaCl, respectively. Plants were maintained under these conditions for additional 8 weeks.

Symbiotic development

The percentage of mycorrhizal root infection in *A. maritimus* plants was estimated by visual observation of fungal colonization after clearing washed roots in 10 % KOH and staining with 0.05 % trypan blue in lactic acid (v/v), as described by Phillips and Hayman (1970). The extent of mycorrhizal colonization was calculated according to the gridline intersect method Giovannetti and Mosse (1980).

Biomass production

At harvest (5 months after planting), the shoot and root system were separated and the shoot dry weight (SDW) and root dry weight (RDW) were measured after drying in a forced hot-air oven at 70 °C for 2 days. Ten plants per treatment were used (except for plants inoculated with Ri collect and subjected to 175 mM NaCl, where only three plants survived).

Photosynthetic efficiency

The efficiency of photosystem II was measured with FluorPen FP100 (Photon Systems Instruments, Brno, Czech Republic), which allows a non-invasive assessment of plant photosynthetic performance by measuring chlorophyll a fluorescence. FluorPen quantifies the quantum yield of photosystem II as the ratio between the actual fluorescence yield in the light-adapted state (FV‘) and the maximum fluorescence yield in the light-adapted state (FM‘), according to Oxborough and Baker (1997). Measurements were taken in the third youngest leaf of ten different plants of each treatment.

Stomatal conductance

Stomatal conductance was measured 2 h after light turned on by using a porometer system (Porometer AP4, Delta-T Devices Ltd, Cambridge, UK) following the user manual instructions. Stomatal conductance measurements were taken in the third youngest leaf from five different plants from each treatment.

Proline content

Free proline was extracted from 0.5 g of fresh leaves and roots (Bligh and Dyer 1959) in five plants per treatment. The methanolic phase was used for quantification of proline content. Proline was estimated by spectrophotometric analysis at 530 nm of the ninhydrin reaction according to Bates et al. (1973).

Glutathione content

Glutathione content was measured as described by (Smith 1985). Five hundred milligrams of roots and leaves of five plants from each treatment were homogenized in a cold mortar with 5 ml 5 % (w/v) sulfosalicylic acid and the homogenate was filtered and

centrifuged at 10,000 × g for 10 min. 0.75 ml of supernatant was neutralized by 1.125 ml 0.5 MK-phosphate buffer (pH7.5). The standard incubation medium was a mixture of: 0.5 ml 0.1 M sodium phosphate buffer (pH7.5) containing 5 mM EDTA, 0.2 ml 6 mM 5,5′- dithiobis-(2-nitrobenzoic acid), 0.1 ml 2 mM NADPH, and 0.1 ml (1 unit) glutathione reductase. The reaction was initiated by the addition of 0.1 ml of extract or glutathione. The change in absorbance at 412 nm was recorded for 9 min.

Ascorbate content

Ascorbate was assayed photometrically by the reduction of 2,6-dichlorophenolindophenol (DCPIP) as described by Leipner et al. (1997). Two hundred mg of roots and leaves of five plants from each treatment were homogenized in 5 ml ice-cold 2 % (w/v) metaphosphoric acid in the presence of 1 g NaCl. The homogenate was filtered through a filter paper. An aliquot of 30 μl of the extract was mixed with 20 μl 45 % (w/v) K_2HPO_4. After 15 min incubation at 25 °C, 100 μl 2 M citrate-phosphate buffer (pH2.3) and 100 μl 0.003 % (w/v) DCPIP were added. The absorbance at 524 nm was measured immediately. The content of ascorbate was calculated by reference to a standard curve made of ascorbate.

Oxidative damage to lipids

Lipid peroxides were extracted by grinding 500 mg of leaves and roots of five plants from each treatment with an ice-cold mortar and 6 ml of 100 mM potassium phosphate buffer (pH7). Homogenates were filtered through one Miracloth layer and centrifuged at 15,000 × g for 20 min. The chromogen was formed by mixing 200 μl of supernatants with 1 ml of a reaction mixture containing 15 % (w/v), trichloroacetic acid, 0.375 % (w/v) 2-thiobarbituric acid (TBA), 0.1 % (w/v) butyl hydroxytoluene, 0.25 N HCl and then incubating the mixture at 100 °C for 30 min (Minotti and Aust 1987). After cooling at room temperature, tubes were centrifuged at 800 × g for 5 min and the supernatant was used for spectrophotometric reading at 532 nm. Lipid peroxidation was estimated as the content of 2-thiobarbituric acid-reactive substances (TBARS) and expressed as equivalents of malondialdehyde (MDA) according to Halliwell and Gutteridge (1989). The calibration curve was made using MDA in the range of 0.1–10 nmol. A blank for all samples was prepared by replacing the sample with extraction medium, and controls for each sample were prepared by replacing TBA with 0.25 N HCl. In all cases, 0.1 % (w/v) butyl hydroxytoluene was included in the reaction mixtures to prevent artifactual formation of TBARS during the acid-heating step of the assay.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 statistical program (SPSS Inc., Chicago, IL, USA) performing first a one-way ANOVA followed by the Tukey test with $P<0.05$ as the significance cut-off.

Results

Symbiotic development

All the fungi exhibited similar colonization rates at all salinity levels (Fig. 1). The highest rate of AM root colonization was achieved in plants inoculated with Ri collect (up to 82 %). High levels of root colonization were also found in plants inoculated with Ri CdG and Sc CdG (Fig. 1). The lowest root colonization was always found in plants colonized by Ce CdG (about 25 %).

Percentage of survival and plant biomass production

Non-mycorrhizal *A. maritimus* plants did not survive under the conditions of this experiment, even in the absence of salinity. At the highest salinity level, only 30 % of plants inoculated with Ri collect survived. The treatments inoculated with the three native AMF had 100 % of survival rate (Table 1).

The increase of salt application affected negatively the shoot biomass production in all treatments except in plants inoculated with Ri CdG (Table 1). In plants inoculated with Ri collect, Sc CdG and Ce CdG the reduction was only significant when 175 mM NaCl was applied (Table 1). A similar trend was observed for root dry weight (Table 1), although it was unaffected by salinity in plants inoculated with Ce CdG. Root dry weight was reduced to a half in plants inoculated with Sc CdG at 100 mM NaCl and it was not changed further at 175 mM NaCl (Table 1).

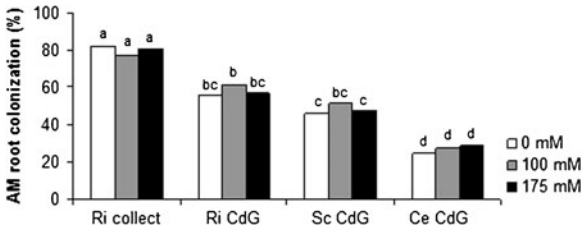

Fig. 1 Percentage of mycorrhizal root length in *Asteriscus maritimus* plants. *White bars* represent plants subjected to 0 mM NaCl; *grey bars*, plants subjected to 100 mM NaCl and *black bars*, plants subjected to 175 mM NaCl. The inoculation treatments were: collection *Rhizophagus intraradices* strain (Ri collect); native *Rh. intraradices* CdG strain (Ri CdG); native *Septoglomus claroideum* CdG strain (Sc CdG) and native *Claroideoglomus etunicatum* CdG strain (Ce CdG). Different letters indicate significant differences ($p<0.05$)

Photosystem II efficiency

Salinity application did not cause any significant descent in photosynthetic efficiency in *A. maritimus* plants inoculated with the three native AMF from Cabo de Gata. Only plants inoculated with Ri collect had a significant decrease (by 40 %) in the efficiency of photosystem II when they were subjected to 175 mM NaCl (data not shown).

Stomatal conductance

Under non saline conditions no significant differences in stomatal conductance were observed among inoculation treatments (Fig. 2). Salinity application decreased stomatal conductance in all the treatments. However, at 175 mM NaCl, plants inoculated with Ri collect showed significantly lower stomatal conductance than those inoculated with the three native AMF from Cabo de Gata (Fig. 2).

Accumulation of proline

The accumulation of proline was more pronounced in shoot than in root tissues (Fig. 3a, b). Proline content in leaves increased in all treatments after exposure to salinity in the growth medium. Differences among treatments were only found at 100 mM NaCl, where plants inoculated by the two Ri strains had the highest values (Fig. 3a). In roots, plants inoculated with Ri CdG and Ce CdG increased their accumulation of proline with increasing salinity from 100 to 175 mM NaCl, while plants inoculated with Ri collect and Se CdG did not change the accumulation of proline (Fig. 3b).

Table 1 Percentage of survival, shoot and root dry weights (g.plant^{-1}) in *Asteriscus maritimus* plants. NM represents non-mycorrhizal control plants; Ri collect, plants inoculated with the collection *Rhizophagus intraradices* strain; Ri CdG, plants inoculated with the native *Rh. intraradices* CdG strain; Sc CdG, plants inoculated with the native *Septoglomus claroideum* CdG strain and Ce CdG plants inoculated with the native *Claroideoglomus etunicatum* CdG strain. Plants were subjected to 0, 100 or 175 mM NaCl. Different letters indicate significant differences ($p<0.05$)

AMF treatment	NaCl (mM)	% survival	Shoot dry weight	Root dry weight
NM	0	0	0	0
	100	0	0	0
	175	0	0	0
Ri collect	0	100	0.41 bc	0.37 bc
	100	100	0.36 bcd	0.21 cde
	175	30	0.22 d	0.11 e
Ri CdG	0	100	0.36 bcd	0.43 ab
	100	100	0.33 cd	0.28 bcde
	175	100	0.31 cd	0.15 de
Sc CdG	0	100	0.58 a	0.61 a
	100	100	0.42 abc	0.35 bc
	175	100	0.33 cd	0.28 bcde
Ce CdG	0	100	0.50 ab	0.33 bcd
	100	100	0.39 bcd	0.23 cde
	175	100	0.32 cd	0.24 bcde

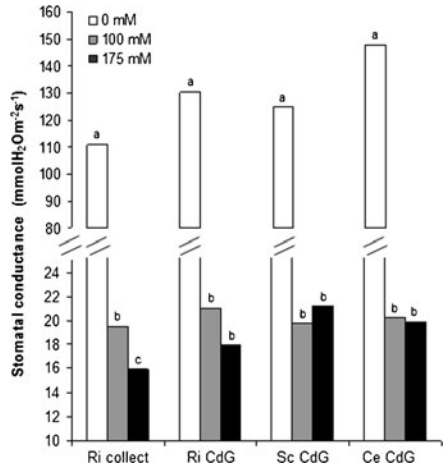

Fig. 2 Stomatal conductance in *A. maritimus* plants. See legend for Fig. 1

Glutathione content

The content of glutathione in leaves of plants inoculated with the three native AMF from Cabo de Gata increased at the highest level of salinity, as compared to the non-saline treatment (Fig. 4a). In contrast, *A. maritimus* plants inoculated with Ri collect had a significant reduction in the accumulation of glutathione at 175 mM NaCl, having the lowest glutathione content. In roots, only plants inoculated with Sc CdG increased the content of glutathione at 175 mM NaCl (Fig. 4b).

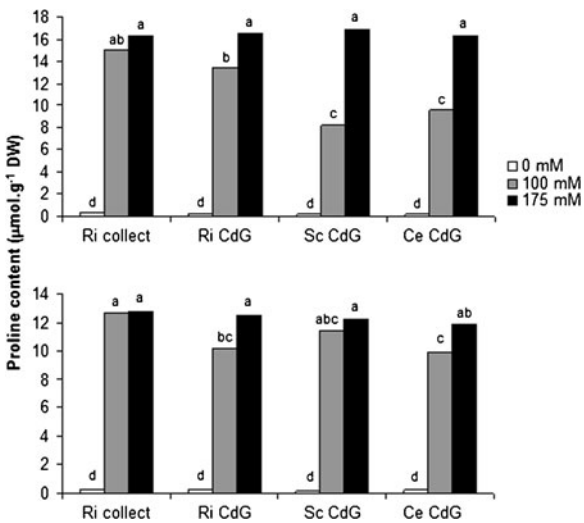

Fig. 3 Shoot (**a**) and root (**b**) proline content in *A. maritimus* plants. See legend for Fig. 1

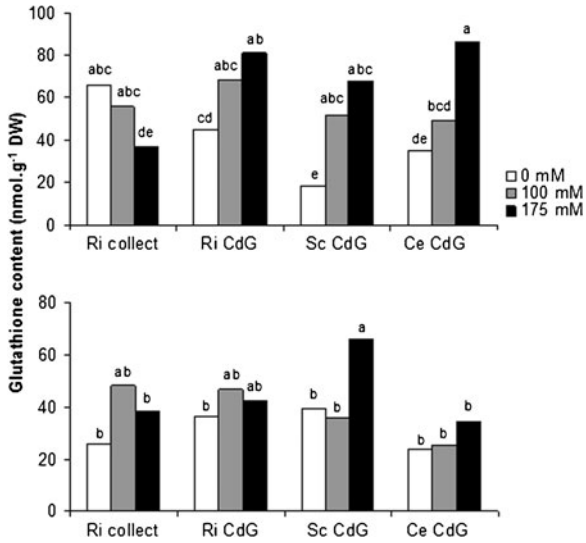

Fig. 4 Shoot (**a**) and root (**b**) glutathione content in *A. maritimus* plants. See legend for Fig. 1

Ascorbate content

In leaves, the content of ascorbate only rose in plants colonized by Ri CdG when subjected to 100 mM NaCl (data not shown). In roots, exposure to 100 or 175 mM NaCl did not significantly affect the ascorbate content in *A. maritimus* plants (data not shown).

Oxidative damage to lipids

The application of 100 mM NaCl in the growth medium increased lipid peroxidation in leaves of plants inoculated with Ri collect or Ce CdG (Fig. 5a). When plants were grown at 175 mM NaCl, no further increase in lipid peroxidation was observed for any treatment as compared to 100 mM NaCl. At both saline levels, plants inoculated with Sc CdG showed the lowest values of lipid peroxidation in their leaves as compared to the other treatments. In roots, the application of salinity did not cause a significant increase of lipid peroxidation in any treatment, except in plants inoculated with Sc CdG (Fig. 5b). However, these plants also showed the lowest values of lipid peroxidation in roots (Fig. 5b).

Discussion

A. maritimus, a native halophyte plant from Mediterranean areas, showed a high degree of mycotrophy since all non-

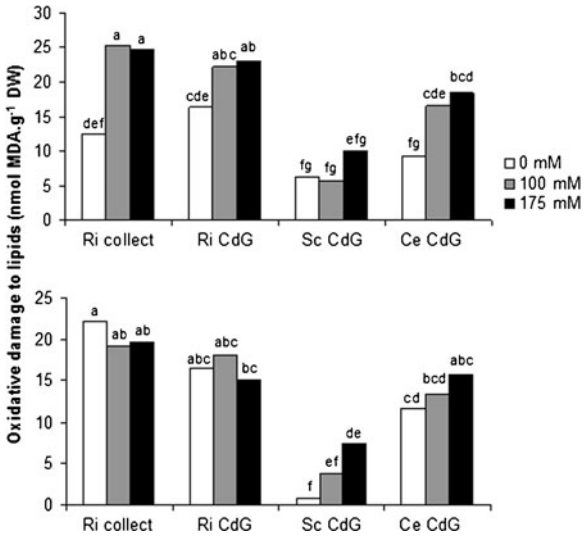

Fig. 5 Shoot (**a**) and root (**b**) oxidative damage to lipids in *A. maritimus* plants. See legend for Fig. 1

mycorrhizal plants did not survive, even in absence of an additional NaCl treatment. This evidences the importance of the rhizosphere microbiota, particularly the AMF, in the establishment and growth of this plant species. Herrera et al. (1993) showed that the restoration of a desertified area was most successful using native plant species inoculated with soil microbiota, including AMF. In fact, Klironomos (2003) concluded that for the establishment of a diverse plant community it is important to use locally adapted AMF community, as plant species benefited most from certain AMF species. In fact, at the highest salinity level, only 30 % of *A. maritimus* plants inoculated with the collection AMF survived, while with the three native AMF, the rate of survival was 100 %, pointing out the importance of inoculation with native-AMF in the survival of this plant species. Thus, any attempt of revegetation of degraded saline Mediterranean areas using *A. maritimus* should consider the mycorrhizal dependency of this plant and the use of adequate inocula. Although there are AMF species which seem to be globally distributed (Öpik et al. 2006), van der Heijden et al. (1998) pointed out that plant species benefited differently from the fungal strains regarding biomass production. Our results showed that plants inoculated with native AMF had higher shoot dry weight than those inoculated with the collection AMF at the highest level of salinity. This is in accordance to Requena et al. (2001) who found that native AMF species were more efficient in promoting plant growth of *Anthyllis cytisoides* than AMF derived from a different habitat. Similar results were obtained in *Pulsatilla* species (Moora et al. 2004), *Conyza bilbaoana* (Oliveira et al. 2005) and *Arnica montana* (Vergeer et al. 2006).

The nature of AMF hampers the progress in mycorrhiza research, especially in the field of applied ecology (Rosendahl 2008; Young 2008). Thus in this work we tried to elucidate differences in symbiotic efficiency among three AMF native from a saline Mediterranean area and a collection AM fungus when associated to *A. maritimus*. Mycorrhizal colonization was not affected by increasing salinity, as some other authors found (Yamato et al. 2008; Wu et al. 2010). Although Ri collect had the highest percentage of root colonization, the native AMF strains maintained a higher symbiotic efficiency with *A. maritimus,* as well as a 100 % of survival rate under 175 mM NaCl. In fact, the high percentage of root colonization by Ri collect could demand excessive carbohydrates from the plant (Klironomos 2003).

The salt stress induces a lowering of the photosynthetic rate which can lead to an overreduction of the reaction centres in photosystem II. If the plant is unable to dissipate the excess energy, this may result in damage the photosynthetic machinery. The damage, as well as the ability of the plant to dissipate the excess energy, can be quantified by measuring the fluorescence of chlorophyll a (Baker 2008). In this study, when plants of *A. maritimus* were subjected to high salinity stress (175 mM NaCl), plants inoculated with the three native-AMF exhibited better performance of photosystem II and higher stomatal conductance. Results on efficiency of photosystem II and stomatal conductance indicate that plants inoculated with native-AMF may improve the net assimilation rates by protecting the photosynthetic machinery and enhancing transpiration rates. Some other authors showed a similar tendency (Sheng et al. 2008; Hajiboland et al. 2010 and Estrada et al. 2013a) reported that the improvement in photosystem II was higher with native AMF than with collection fungi together with the enhancement of plant stomatal conductance, in agreement with Querejeta et al. (2006). These two effects may have accounted for the enhanced growth of *A. maritimus* during high salinity stress.

In addition to the beneficial effect of native mycorrhizal colonization in *A. maritimus* salt tolerance by improving photosynthetic ability and stomatal conductance; water and nutrient uptake, ion balance and osmolite concentration among others may have also contributed to it (Ruiz-Lozano et al. 2012). It has been previously shown that a native AMF from Cabo de

Gata developed better under salinity than a collection fungal strain (Estrada et al. 2013b). Thus, the extensive hyphal network of native AMF can explore a larger soil volume, contributing to water and nutrient uptake (Evelin et al. 2012). The maintenance of proper ionic homoeostasis and osmotic adjustment may also prevent salt injury. The synthesis of organic osmolites, especially proline that is normally located in the cytosol, appears to be of importance in osmotic adjustment (Moghaieb et al. 2004). Our results did not show remarkable differences in proline content among fungal treatments, although proline accumulation increased in all treatments with the salinity stress.

Salinity stress leads to secondary oxidative damage, thus the improvement of stress tolerance is often related to enhancement of contents of antioxidant compounds in plants. Due to the toxicity of ROS, plants have developed appropriate detoxification systems to remove these molecules. These systems include, among others, non-enzymatic antioxidants soluble compounds, such as glutathione and ascorbate that are major plant metabolites that regulate essential cell functions and play a key role in antioxidant defence (Tunc-Ozdemir et al. 2009). Glutathione reacts with superoxide radicals, peroxy radicals and singlet oxygen and forms oxidized glutathione and other disulphides (Meyer 2007). The ascorbate is involved in the removal of H_2O_2 by ascorbate peroxidases, which use ascorbate as electron donor, and is closely related to glutathione in the ascorbate-glutathione cycle where glutathione participates in the reduction of oxidized ascorbate (Foyer and Noctor 2011). Our results showed that glutathione content had the most significant differences among fungal treatments. *A. maritimus* plants inoculated with native AMF increased it shoot glutathione content with increasing salinity. Plants inoculated with Ri collect reduced the content at 175 mM NaCl and at this salinity level, the content of glutathione in shoots was lower compared to native AMF-inoculated plants. In contrast, the ascorbate content did not show a significant trend. From these results we may propose that *A. maritimus* could be more dependent on glutathione than on ascorbate to cope with salt stress. The reason for that could be related to the additional physiological functions ascribed to glutathione, such as the induction of enzyme activities and its participation in sulphur metabolism and regulation of gene expression (Noctor et al. 2012).

Another major effect of salinity stress in plants is the loss of membrane integrity due to the oxidation of membrane lipids. Thus the prevention of lipid peroxidation is of crucial importance to maintain membrane integrity (Garg and Manchanda 2009). Our findings showed that in *A. maritimus,* MDA content (a specific product of lipid peroxidation induced by ROS) increased with salinity in shoots but not in roots, with exception of Sc CdG inoculated plants, which always increased the MDA content. Lipid peroxidation is considered an useful indicator of cellular oxidative damage (Li et al. 2012), however it might not be a good indicator in halophytes due to the particular characteristics of these plants. We cannot compare with some other works that observed a reduction of oxidative damage to lipids by AM symbiosis in plants subjected to salt stress (He et al. 2007; Hajiboland et al. 2010) because of the lack of non-mycorrhizal plants. In spite of that, we observed that, at each salt level, plants inoculated with Sc CdG always had the lowest MDA content.

Summarizing, the present study showed the elevated degree of mycotrophy of *A. maritimus* plants, especially regarding native AMF from its environment. In addition, we have observed several physiological mechanisms by which native AMF ameliorate the detrimental effects of salinity stress in *A. maritimus* better than an exotic AMF, such as better performance of photosynthetic machinery or higher levels of the antioxidant molecule glutathione. In degraded semiarid Mediterranean areas, Alguacil et al. (2011) demonstrated the important impact of inoculation with native AM fungi on the growth of shrub species. Moreover, reestablishment of plants species and restoration of specific ecosystems need careful considerations regarding the AMF inocula and the plant species used. Also the introduction of novel AMF species or isolates to the site could result in the out-competition of native fungal strains that could provoke the up-coming or survival of invasive plant species (Schwartz et al. 2006). Thus, inadequate inocula should be avoided due to possible negative effects on plant species and the plant community. Van der Heijden et al. (1998) showed that under particular environmental conditions, different AMF strains had negative or positive effect on the colonized plant, pointing out the importance of the interactions between AMF and plant species. The results in the present work remark the importance of native AMF inoculation in *A. maritimus* establishment, survival and growth for successful restoration of Mediterranean areas, particularly on sites with high salt levels in the soil.

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