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Physiological responses of halophytic C_4 grass *Aeluropus littoralis* to salinity and arbuscular mycorrhizal fungi colonization

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Abstract

The halophytic C_4 grass, *Aeluropus littoralis*, was cultivated under low (50 mM) and high (200 mM) NaCl salinity and inoculated with the arbuscular mycorrhizal fungi (AMF) *Claroideoglomus etunicatum* in a sand culture medium for 20 weeks. Shoot and root dry mass increased under salinity conditions up to 24 and 86%, respectively. Although the root colonization rate significantly decreased in the presence of salt, AMF-colonized (+AMF) plants had higher biomass compared with plants without AMF colonization (–AMF) only under saline conditions. Net CO_2 assimilation rate increased significantly by both salinity levels despite stable stomatal opening. In contrast, AMF-mediated elevation of the net CO_2 assimilation rate was associated with a higher stomatal conductance. Unexpectedly, leaf activity of phospho*enol*pyruvate carboxylase decreased by salinity and AMF colonization. Transpiration rate was not affected by treatments resulting in higher water-use efficiency under salinity and AMF conditions. Concentrations of soluble sugars and free α-amino acids increased by both salinity and AMF treatments in the shoot but not in the roots. Proline concentration in the leaves was higher in the salt-treated plants, but AMF colonization did not affect it significantly. Leaf activity of nitrate reductase increased by both salinity and AMF treatments. Mycorrhizal plants had significantly higher Na^+ and K^+ uptake, while Ca^{2+} uptake was not affected by salt or AMF colonization. The ratio of K^+/Na^+ increased by AMF in the shoot while it decreased in the roots. Leaf osmotic potential was lowered under salinity in both +AMF and –AMF plants. Our results indicated that higher dry matter production in the presence of salt and AMF could be attributed to higher CO_2 and nitrate assimilation rates in the leaves. Higher leaf accumulation of soluble sugars and α-amino acids but not proline and elevated water-use efficiency were associated with the improved growth of *A. littoralis* inoculated with AMF.

Additional key words: carotenoids; chlorophyll content; compatible solute; gas exchange; growth parameters; water relations.

Introduction

Soil salinity is one of the most important environmental stresses in both natural and agroecosystems worldwide. Salt-affected soils occupy more than 7% of the Earth land surface (Ashraf and Harris 2004). Halophytes are highly salt-tolerant species and occupy naturally saline habitats; they can complete their lifecycle in salinity equivalent to 200 mM NaCl or higher (Flowers and Colmer 2008, Shabala and Mackay 2011, Rozema and Schat 2013). The important criterion is the growth stimulation of halophytes in the presence of NaCl (100 mM and higher) in the soil solution (Flowers and Colmer 2008).

Enzymes from halophytes are as sensitive to salinity as those from glycophytes and are not compatible with high concentrations of NaCl. Accordingly, salinity tolerance in halophytes is coupled with compartmentation of excessive salt ions required for osmotic adjustment in vacuoles (Flowers and Colmer 2008). Under these conditions, maintenance of osmotic equilibrium across the tonoplast requires accumulation of compatible solutes in the cytoplasm. Possible compatible solutes, which have been found to accumulate in halophytic plants, include proline, glycinebetaine, and polyols (Flowers and Colmer 2008).

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Abbreviations: AMF – arbuscular mycorrhizal fungi; C_a – ambient CO_2 concentration; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DM – dry mass; E – transpiration rate; FM – fresh mass; g_s – stomatal conductance; NR – nitrate reductase; PEPC – phospho*enol*pyruvate carboxylase; P_N – net CO_2 assimilation rate; TM – turgid mass; WUE – water-use efficiency.
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Higher requirements for carbon (C) and nitrogen (N) metabolites as osmosolutes under saline conditions contrast with reduction of CO_2 assimilation because of stomatal limitations (Chaves *et al.* 2009, Caldwell 2012) and reduction of nitrate uptake and assimilation (Hussin *et al*. 2013) under saline conditions. Thus, maintenance of C and N metabolism under saline conditions may be of fundamental importance under salinity not only for glycophytes but also for halophytes. Salinity resistance of halophytic species has been associated with efficient N allocation towards synthesis of organic compounds that in turn is linked with processes for C assimilation into biomass in both C_3 (Geissler *et al*. 2009) and C_4 halophytes (Hussin *et al*. 2013).

It has been stated that the C_4 pathway is an adaptation to compensate for water and C deficiencies and high rates of photorespiration. Hence, high temperatures, drought, and soil salinity are the most obvious factors that could potentially select for traits leading to the C_4 metabolism (Sage 2001). C_4 species occur with a higher frequency and larger soil area under conditions of drought and high soil salinity when compared with C_3 plants within the same area (Feldman *et al.* 2008).

Arbuscular mycorrhizal fungi (AMF), the most widespread root fungi symbionts, are associated with roots of majority of higher plants (Smith and Read 2008). Beside improving nutrients uptake, AMF also enable plants to cope with abiotic stresses, such as drought, toxicity of heavy metals, and salinity. AMF ameliorates effects of salt stress in plants by an improved plant nutrition and nutrient balance, reduction of Na^+ uptake and/or an improved K^+/Na^+ ratio, higher ability for extraction of water from soil, activation of antioxidant defense, greater photosynthesis because of elevated stomatal conductance, and accumulation of organic osmolytes that improve plants ability for water uptake and protect cell structures against damages caused by excess of salt ions (Evelin *et al.* 2009, Porcel *et al.* 2012, Ruiz-Lozano *et al.* 2012, Hajiboland 2013). AMF exist in highly saline soils (Aliasgharzadeh *et al.* 2001) and colonize various halophytic species in their natural habitats (Füzy *et al.* 2008, Hajiboland 2013). However, effect of AMF colonization on salt resistance of these species is still poorly understood.

Aeluropus littoralis (Gouan) Parl. is a C_4 perennial halophytic grass species that inhabits saline soils of Europe, north and west Africa, the Caucasus, the Middle East, India, China, and Mongolia (Clayton *et al.* 2014). This species occurs in Iran in many parts of the country, particularly in the northwest and central parts (Akhani 2006). In northwest Iran, *A. littoralis* is found as natural vegetation around Lake Urmia with a soil salinity of about 7 to 92 dS m^{-1}. Some detailed studies have been undertaken on leaf anatomy of this salt-excreting halophyte (Barhoumi *et al.* 2007), although physiological mechanisms for a high salt resistance in this species have attracted much less attention. Apart from one report on the occurrence of mycorrhizal associations with *A. littoralis* in its natural habitats (Wang *et al.* 2004), there is no published study on the effect of AMF on the salt tolerance of *A. littoralis* or other species of the genus *Aeluropus*.

Expansion of salt-affected soils in northwest Iran around Lake Urmia has become a serious environmental threat and economic problem in recent years in this region (Eimanifar and Mohebbi 2007). The objective of this work was to study the role of inoculation with AMF (*Claroideoglomus etunicatum*) for the improvement of salinity tolerance in halophytic grass, *A. littoralis*. In addition to growth, carbon and nitrogen metabolism, water-related parameters and ion homeostasis were studied in this species under low (50 mM, LS) and high (200 mM, HS) salt concentrations.

Materials and methods

Plant culture, inoculation and treatments: The AMF species *Claroideoglomus etunicatum* (previously named *Glomus etunicatum* Becker & Gerdemann) isolated from saline soils of the Tabriz Plain (northwest Iran) with electrical conductivity of 40 dS m^{-1} (Aliasgharzadeh *et al.* 2001) was used in this study. The fungus was propagated on maize plants under greenhouse conditions. After four months, top plants were cut off and pot material containing soil, mycorrhizal roots, hyphae, and spores were thoroughly mixed and used as fungal inoculum.

Seeds of *Aeluropus littoralis* collected from the Khour-Khour village (northwest Iran) were surface sterilized for 1 min using sodium hypochlorite (0.85%). Sterilized seeds were germinated in 2-L pots filled with washed, sterilized, and sieved (4 mm) sand. The pots had one removable drain plug that was kept closed during plant culture. After germination, seedlings were thinned to 20 per pot. Before germination, half of the pots received 50 g of soil inoculum (+AMF treatment). Noninoculated pots received the same amount of autoclaved soil inoculum (–AMF treatment). Pots were daily irrigated to field capacity with Hoagland nutrient solution after weighing. Water was used as intervals if needed. The volume of nutrient solution used for irrigation started with 150 ml per week and reached 400 ml per week at the end of the growth period. Four weeks after sowing, three salinity treatments including control (without salt addition, Ct), 50 mM (low salinity, LS), and 200 mM (high salinity, HS) NaCl were applied gradually within the following three weeks along with the addition of nutrient solution. In order to avoid salt accumulation, pots were washed thoroughly with sterilized and distilled water every four weeks by removing drain plugs. Subsequently, salt was added again, but in a single step.

Plants were grown for 20 weeks (16 weeks after the beginning of salt treatments) under controlled conditions with a temperature regime of 25/18°C day/night, 14/10 h

light/dark period, a relative humidity of 70/80%, and at a photon flux density of about 350 μmol m^{-2} s^{-1}.

Harvest and evaluation of colonization: Shoots were separated from roots at the base and washed thoroughly with distilled water in order to remove the excreted salts from the leaf surfaces. Roots were placed in a tray filled with distilled water for removing adhering sand particles and then rinsed shortly with distilled water. After blotting dry and determining fresh mass (FM), subsamples were dried at 70°C for determination of dry mass (DM) and ion analyses. For biochemical analysis, subsamples with known FM were stored at –80°C until analysis.

For Na^+, K^+, and Ca^{2+} determination, oven-dried samples were weighed and ashed in a muffle furnace at 550°C for 8 h, resolved in HCl, and made up to desired volume by distilled water. Concentrations of Na^+, K^+, and Ca^{2+} were determined by flame photometry (*PFP7*, *Jenway*, Essex, UK).

For evaluation of AMF colonization, fine roots (1 g FM) were cleared in 10% (m/v) KOH (*Merck*, Darmstadt, Germany) and stained with 0.05% (m/v) Trypan blue (*Merck*, Darmstadt, Germany) in lacto-glycerin (lactic acid:glycerin:distilled water, 1:1:1, v/v/v) (*Merck*, Darmstadt, Germany). The colonization rate of roots was determined by the gridline intersect method (Giovanetti and Mosse 1980). Mycorrhizal responsiveness was calculated according to the following formula: [DM of +AMF plants – DM of –AMF plants]/[DM of –AMF plants] × 100 (Sawers *et al.* 2008).

Gas-exchange parameters including the net CO_2 assimilation rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were recorded using a calibrated portable gas-exchange system (*LCA-4*, *ADC Bioscientific Ltd.*, Great Amwell, UK) during the light period between 9:00 and 13:00 h under a photon flux density of about 350 μmol m^{-2} s^{-1}. Gas-exchange parameters were calculated by the instrument as described in the operating manual using the values of CO_2 (380 ±2 0 μmol mol^{-1}) and H_2O (2 ± 0.5 mmol mol^{-1}) inside the leaf chamber measured by the infrared gas analyzer of the photosynthesis system and at a temperature of 27°C controlled by a cooling system attached to the leaf chamber. An average of four records from different parts of each individual leaf was considered for each replicate. Water-use efficiency (WUE) was calculated as the ratio of P_N/E.

Leaf pigments, organic solutes, osmotic potential of leaf and root, and relative water content (RWC): Leaf concentration of chlorophyll (Chl) *a*, *b*, and carotenoids (Car) were determined after extraction of pigments in 80% cold acetone in the dark at 4°C (Lichtenthaler and Wellburn 1985). After 24 h, the absorbance of samples was determined at 663 (Chl *a*), 646 (Chl *b*), and 470 (Car) nm using spectrophotometer *(Specord 200*, *Analytik Jena*, Jena, Germany). For determination of carbohydrates, samples were homogenized in 100 mM phosphate buffer (pH 7.5) at 4°C, after centrifugation at 12,000 × *g* for 15 min, the supernatant was used for determination of total soluble sugars, whereas the pellets were kept for starch analysis (Yemm and Willis 1954). The content of total free α-amino acids was assayed using a ninhydrin colorimetric method. Glycine (*Merck*, Darmstadt, Germany) was used for production of a standard curve (Yemm and Cocking 1955). Proline was extracted in 3% sulfosalicylic acid (*Merck*, Darmstadt, Germany). After centrifugation at 6,000 × *g*, the supernatant was treated with acetic acid and acid ninhydrin (1:1, v/v) (*Merck*, Darmstadt, Germany) and boiled for 1 h. The absorbance of samples was determined at 520 nm and compared with standard curve created for proline (*Sigma*, St. Louis, USA) (Bates *et al.* 1973).

Osmotic potential was determined in the leaf and root samples after homogenization and centrifugation at 4,000 × *g* for 20 min by an osmometer (*Micro-Osmometer*, *Heman Roebling Messtechnik*, Berlin, Germany). RWC was calculated according to the formula: [FM – DM]/ [TM – DM] × 100. Leaf disks (5 mm diameter) were prepared and after determination of FM they were submerged for 5 h in distilled water, blotted dry gently on a paper towel, and weighed (TM). Finally, the leaf disks were oven-dried for 24 h at 80°C for determination of dry mass (DM).

Nitrate reductase and phospho*enol*pyruvate carboxylase activities: *In vivo* nitrate reductase (NR, EC 1.6.6.1) activity was determined using the method described by Jaworski (1971). Leaf blades and root samples were cut into 5 mm sections and placed in the incubation buffer (100 mg of tissue for 10 mL of buffer) containing 25 mM potassium phosphate buffer (pH 7.2), 25 mM KNO_3, and 1% Triton X-100 (*Sigma*, St. Louis, USA). The samples were infiltrated using vacuum (80 kPa). After 1 h (roots) or 5 h (leaves), the vacuum was released and the samples were incubated at 30°C in darkness for 1 h, then placed in a boiling water bath to stop the NR activity. The resulting nitrite was determined spectrophotometerically at 540 nm in a reaction mixture containing sulfanilamide and naphthylethylenediamine dihydrochloride (*N-NEDA*, *Sigma*, St. Louis, USA). Activity of NR was calculated from a standard curve established with $NaNO_2$ (*Merck*, Darmstadt, Germany) and expressed in produced pmol(NO_2^-) mg^{-1}(protein) s^{-1}.

In vitro activity of phospho*enol*pyruvate carboxylase (PEPC, EC 4.1.1.31) was determined according to the method described by Ashton *et al.* (1990). Homogenized samples were extracted with 25 mM cold Tris-HCl (*Merck*, Darmstadt, Germany) buffer (pH 8.0). After centrifugation at 10,000 × *g* for 10 min, the supernatant was used to determine activity of enzyme. Assay buffer contained 25 mM Tris-HCl, 5 mM $MgCl_2$ (*Merck*, Darmstadt, Germany), 2 mM dithiotreitol (DTT, *Sigma*, St. Louis, USA), and 1 mM $KHCO_3$ (*Merck*, Darmstadt, Germany). Activity was monitored by disappearance of NADH (*Sigma*, St. Louis, USA) (0.2 mM) in a coupled reaction with

malate dehydrogenase (MDH, EC 1.1.1.37) at 340 mm over 3 min and expressed as nmol(NADH) mg^{-1}(protein) s^{-1}.

Soluble proteins were determined using a commercial reagent (Bradford reagent, *Sigma*, St. Louis, USA) and bovine albumin serum (*Merck*, Darmstadt, Germany) as a standard.

Nitrate and nitrite: Nitrate was determined according to the procedure developed by Cataldo *et al.* (1975). The standard curve was created using KNO_3 (*Merck*, Darmstadt, Germany) in the range of 0–10 mM. Nitrite was extracted from fresh materials using 25 mM potassium phosphate buffer (pH 7.5). After centrifugation and proper dilution, nitrite concentration was assayed after formation of a red-violet colored, water-soluble azo dye as used for determination of nitrite produced by the activity of NR described above.

Statistical analysis: An experiment was undertaken using randomized block design with three salinity and two inoculation levels and four independent pots as four replications. Analysis of data was carried out using *Sigma Stat 3.5* software (*Systat Software Inc.*, San José, California) with *Tukey*'s test (p<0.05).

Results

Fresh mass of shoots was not affected significantly by salt treatments (Fig. 1*A*). In contrast, shoot DM increased by applied salt, however, effect of two NaCl concentrations did not differ in this regard (Fig. 1*B*). Increase of biomass in the presence of salt was more pronounced in the roots. The highest root fresh and dry biomass was obtained at HS being about 1.9 fold higher than in the Ct plants (Fig. 1*C*,*D*). Colonization with AMF did not promote plant growth in the absence of salt; shoot and root biomass rather slightly decreased by AMF colonization in the Ct plants. In the presence of salt, however, the +AMF plants showed considerably higher FM and DM compared with the –AMF ones. Effect of AMF colonization in salt-treated plants was significant with the exception of root DM under HS (Fig. 1).

Root colonization rate [%] significantly decreased in the presence of both applied concentrations of NaCl (Fig. 2). However, the effect of two salt concentrations did not differ significantly.

Leaf concentration of Chl *a* was not affected significantly by salt treatments, while concentrations of Chl *b* was higher in the +AMF plants at HS treatment compared with other treatment combinations. Leaf Car concentration, in contrast, increased significantly by HS in the –AMF plants. In addition, in the absence of salt, the Car

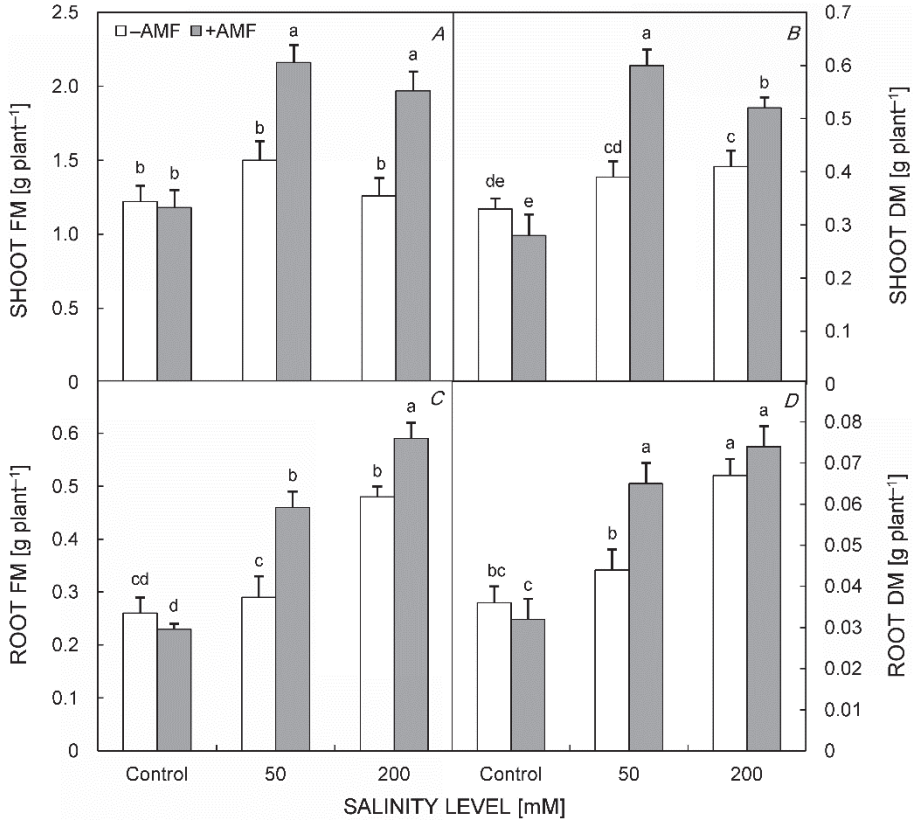

Fig. 1. Fresh mass (FM) and dry mass (DM) of shoots (*A*,*B*) and roots (*C*,*D*) in halophytic grass, *Aeluropus littoralis*, grown under three levels of NaCl salinity in the absence (–AMF) or presence (+AMF) of arbuscular mycorrhizal fungi. Values are means ± SD, n = 4. Bars with *the same letter* are not significantly different (p<0.05).

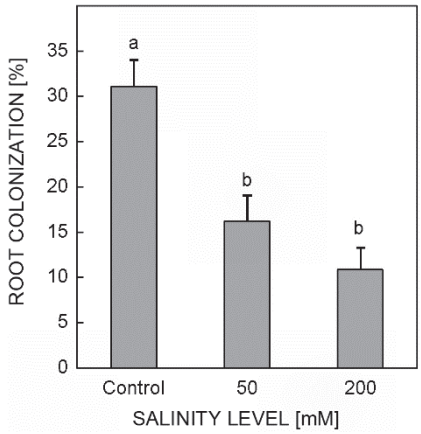

Fig. 2. Colonization rate of halophytic grass, *Aeluropus littoralis*, with *Claroideoglomus etunicatum* under three levels of NaCl salinity. Values are means ± SD, $n = 4$. Bars with *the same letter* are not significantly different ($p<0.05$).

concentration was higher in the +AMF plants than in the –AMF ones (Table 1).

The P_N increased significantly by both applied salinity levels. In addition, the +AMF plants showed higher P_N compared with the –AMF ones under Ct as well as both salinity treatments (Fig. 3*A*). In contrast, E was not significantly affected by salt or AMF colonization. Nevertheless, E was significantly higher in the +AMF plants treated with HS compared with the –AMF plants without salt treatment (Fig 3*B*). Salinity treatments had no effect on g_s, while mycorrhization of plants resulted in higher g_s in the salt-treated but not in the Ct plants (Fig. 3*C*). The ratio of C_i/C_a (the ratio of internal to the ambient CO_2 concentration), in general, decreased by both salinity treatments and AMF colonization. However, the significant effect of salt treatment was observed only at HS and after AMF colonization in the Ct plants without salt treatment (Fig. 3*D*).

The soluble sugar concentration in the leaves was higher in the salt-treated plants. AMF colonization, similarly, increased the leaf soluble sugars concentration (Table 2). In the roots, in contrast, salt treatments in the –AMF plants and AMF colonization of Ct or salt-treated plants did not affect the soluble sugar concentration. A significant effect of salt was observed in the +AMF plants under LS compared with the plants without salt (Table 2).

Starch concentration in the leaves remained unchanged in the –AMF plants but increased in the +AMF plants under the HS treatment. AMF inoculation did not affect the leaf starch concentration. In the roots, either salt treatment or AMF inoculation did not influence the starch concentration (Table 2).

The concentration of free α-amino acids increased in the leaves under HS up to 35% and 15% in the –AMF and +AMF plants, respectively. Colonization of plants resulted in accumulation of free α-amino acids in the leaves in the Ct and salt-treated plants. In the roots, in contrast, salt treatments or AMF colonization did not affect the free α-amino acid concentration (Table 2).

The proline concentration in the leaves was expectedly higher in the salt-treated plants. The increase was 68 and 84% for the +AMF and –AMF plants, respectively, under HS (Table 2). In contrast, the AMF colonization decreased the accumulation of proline in the leaves. This effect, however, was significant only for the Ct plants without salt (Table 2). In the roots, a similar effect of salt on the proline concentration was observed only for the –AMF plants. In the +AMF plants, in contrast, salt treatments rather decreased the root proline concentration. As related to the effect of AMF, plants without salt had the higher proline concentration upon mycorrhization, while in the salt-treated plants, AMF colonization either did not affect (at 50 mM, LS) or even significantly decreased (at 200 mM, HS) proline accumulation in the roots (Table 2).

Expectedly, Na^+ uptake was higher in the salt-treated plants, but the significant effect was observed only at HS (Fig. 4*A*). The +AMF plants showed significantly higher Na^+ uptake, this effect was not observed in the absence of the salt treatment. Uptake of K^+ was not affected significantly by salinity treatments and it was similar to Na^+; K^+ uptake increased by AMF colonization in the salt-treated plants. However, this effect was significant only in the plants treated with HS (Fig. 4*B*). Ca^{2+} uptake was affected neither by salt nor by AMF colonization (Fig. 4*C*).

The shoot:root ratio of Na^+ concentration was lower at HS compared with Ct and LS in both the –AMF and +AMF plants. This ratio was not affected by AMF

Table 1. Leaf concentrations of chlorophyll (Chl) *a*, *b*, and carotenoids (Car) in halophytic grass, *Aeluropus littoralis*, grown under three levels of NaCl salinity in the absence (–AMF) or presence (+AMF) of arbuscular mycorrhizal fungi. Data are mean ± SD ($n = 4$). Difference among data of each column followed by *the same letter* was not significant ($p<0.05$).

Treatment		Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Car [mg g^{-1}(FM)]
–AMF	Control	1.87 ± 0.23^a	0.50 ± 0.07^b	2.77 ± 0.22^b
	50 mM	2.35 ± 0.19^a	0.49 ± 0.11^b	3.87 ± 0.10^{ab}
	200 mM	2.56 ± 0.22^a	0.47 ± 0.05^b	4.12 ± 0.44^a
+AMF	Control	2.31 ± 0.22^a	0.50 ± 0.13^b	3.96 ± 0.22^a
	50 mM	2.53 ± 0.38^a	0.50 ± 0.06^b	4.23 ± 0.64^a
	200 mM	2.56 ± 0.20^a	0.74 ± 0.05^a	4.25 ± 0.98^a

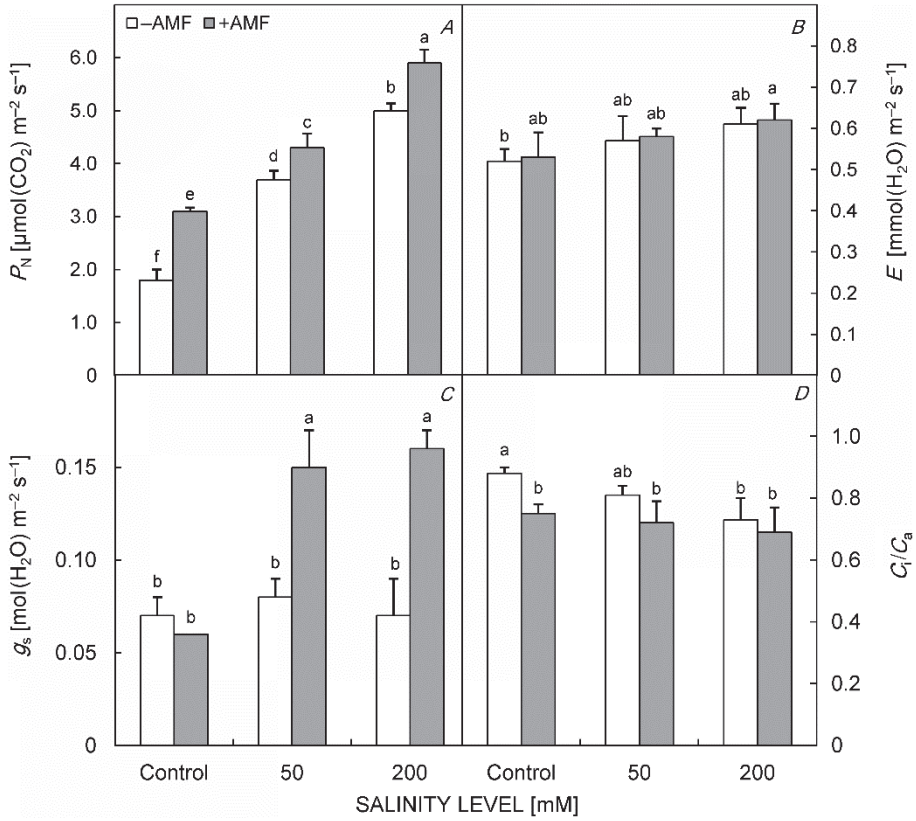

Fig. 3. Gas-exchange parameters including: net CO_2 assimilation rate (P_N) (*A*), transpiration rate (*E*) (*B*), stomatal conductance to water vapor (g_s) (*C*), and internal/ambient CO_2 concentration (C_i/C_a) ratio (*D*) in halophytic grass, *Aeluropus littoralis*, grown under three levels of NaCl salinity in the absence (–AMF) or presence (+AMF) of arbuscular mycorrhizal fungi. Values are means ± SD, $n = 4$. Bars with *the same letter* are not significantly different ($p<0.05$).

Table 2. Concentrations of soluble sugars, starch, free α-amino acids, and proline in the shoots and roots of halophytic grass, *Aeluropus littoralis*, grown under three levels of NaCl salinity in the absence (–AMF) or presence (+AMF) of arbuscular mycorrhizal fungi. Data are mean ± SD ($n = 4$). Difference among data of each column within each plant organ followed by *the same letter* was not significant ($p<0.05$).

Treatment		Soluble sugars [mg g^{-1}(FM)]	Starch [mg g^{-1}(FM)]	Free α-amino acids [μmol g^{-1}(FM)]	Proline [μmol g^{-1}(FM)]
Shoot					
–AMF	Control	3.60 ± 0.32^{e}	4.98 ± 0.90^{b}	188 ± 5^{d}	5.33 ± 0.70^{cd}
	50 mM	5.41 ± 0.44^{d}	5.39 ± 0.80^{ab}	173 ± 9^{d}	9.19 ± 0.70^{ab}
	200 mM	6.63 ± 0.35^{c}	5.65 ± 0.90^{ab}	254 ± 13^{c}	10.71 ± 1.40^{a}
+AMF	Control	6.13 ± 0.44^{cd}	4.86 ± 0.90^{b}	285 ± 9^{b}	4.83 ± 0.80^{d}
	50 mM	8.85 ± 0.34^{b}	5.16 ± 0.50^{ab}	279 ± 3^{b}	7.23 ± 1.70^{bc}
	200 mM	10.62 ± 0.72^{a}	7.01 ± 0.90^{a}	327 ± 14^{a}	8.90 ± 0.50^{ab}
Root					
–AMF	Control	2.52 ± 0.14^{b}	0.64 ± 0.24^{a}	157 ± 16^{a}	3.81 ± 0.30^{c}
	50 mM	3.55 ± 0.76^{ab}	0.88 ± 0.24^{a}	189 ± 10^{a}	4.80 ± 0.60^{c}
	200 mM	3.57 ± 0.82^{ab}	0.93 ± 0.17^{a}	179 ± 19^{a}	12.34 ± 1.20^{a}
+AMF	Control	2.45 ± 0.38^{b}	0.56 ± 0.12^{a}	193 ± 29^{a}	7.14 ± 0.60^{b}
	50 mM	4.54 ± 0.66^{a}	0.66 ± 0.16^{a}	184 ± 18^{a}	5.23 ± 0.90^{c}
	200 mM	3.13 ± 0.90^{ab}	0.64 ± 0.08^{a}	174 ± 14^{a}	4.84 ± 0.30^{c}

colonization (Fig. 4*D*). The ratio of K^+/Na^+ in the shoots increased under LS but not HS. The AMF colonization, in general, decreased this ratio being significant in the Ct plants and those treated with LS (Fig. 4*E*). In the roots, the ratio of K^+/Na^+ decreased by HS and in contrast to the shoots, the AMF colonization increased this ratio in the roots. However, this effect was significant only in the Ct plants (Fig. 4*F*).

Leaf osmotic potential was expectedly lower in the salt-treated plants. In the –AMF plants, this effect was significant only at HS, while in the +AMF plants both LS and HS treatments lowered the leaf osmotic potential. AMF colonization decreased leaf osmotic potential only in the LS plants. Salinity and AMF colonization did not

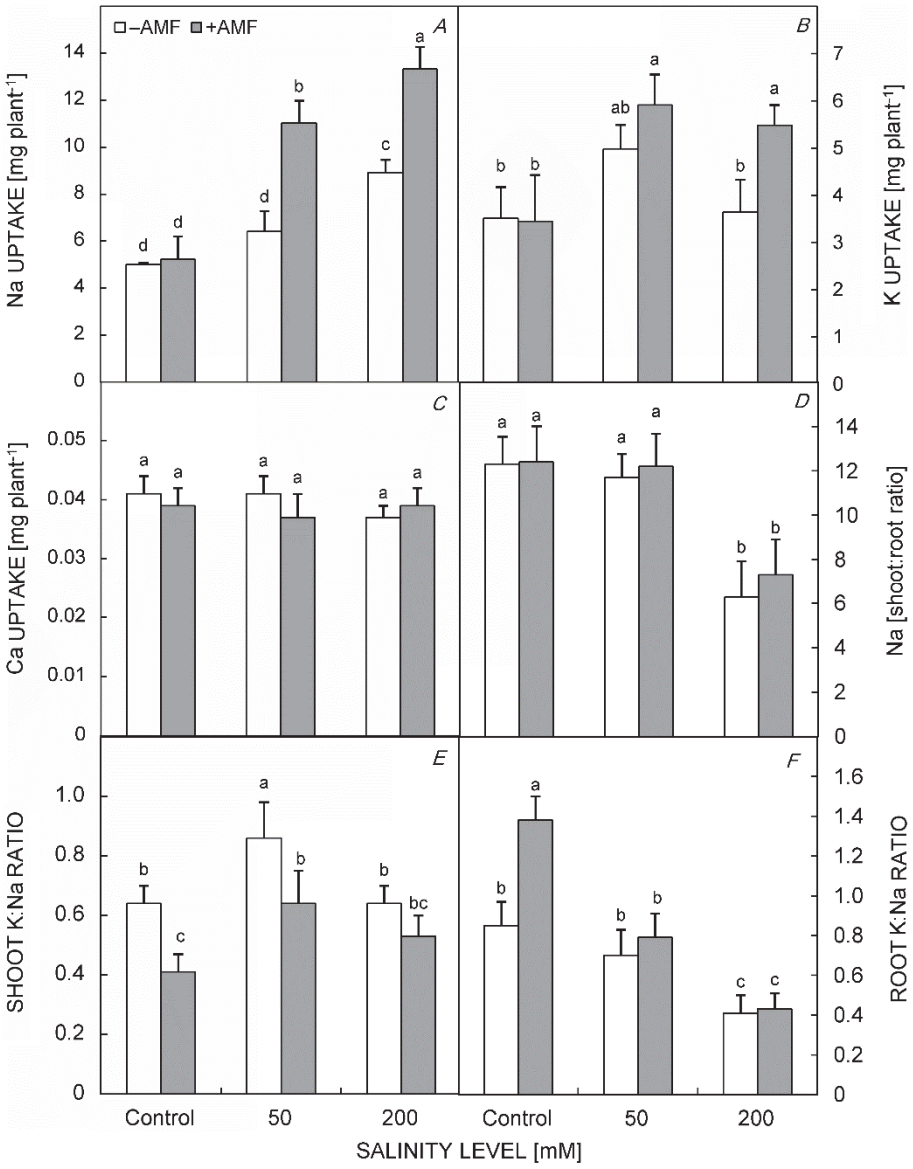

Fig. 4. Uptake of Na^+ (*A*), K^+ (*B*), and Ca^{2+} (*C*), the ratio of shoot:root Na^+ concentration (*D*), and the ratio of K^+/Na^+ concentration in the shoots (*E*) and roots (*F*) of halophytic grass, *Aeluropus littoralis*, grown under three levels of NaCl salinity in the absence (–AMF) or presence (+AMF) of arbuscular mycorrhizal fungi. Values are means ± SD, $n = 4$. Bars with *the same letter* are not significantly different ($p<0.05$).

influence significantly the osmotic potential in the roots, however, slight reductions imposed by salt was similar with that in the shoots (Table 3).

Leaf RWC remained stable under both salt and AMF-colonization treatment. In contrast, WUE increased significantly at both levels of salinity in the –AMF plants and under HS in the +AMF ones. AMF inoculation resulted in higher WUE significant in the Ct and HS plants (Table 3).

The activity of NR increased by salinity treatments in the leaves of both –AMF and +AMF plants. The AMF-colonized plants had higher leaf NR activity in the absence and presence of salt. In the roots, in contrast, salt treatments lowered NR activity and AMF colonization decreased it further (Table 4).

Concentrations of nitrate in the leaves and roots decreased with the increasing salinity level in both –AMF and +AMF plants. Comparison of nitrate concentration in the –AMF and +AMF plants showed only a slight reduction of leaf nitrate concentration upon mycorrhization. In the roots, this effect was significant at LS (Table 4).

The nitrite concentration was higher in the leaves of the salinized plants in the absence of AMF colonization. In the +AMF plants, however, salinity did not affect the leaf nitrite concentration. As related to the effect of AMF, the leaf nitrite concentration increased by inoculation only under nonsaline conditions. The same effect of salt treatments and AMF inoculation was observed on nitrite concentration of the roots, but to a greater extent than that in the shoots. In contrast to the leaves, effect of AMF colonization on increasing nitrite concentration was significant under all applied salt treatments (Table 4).

Leaf and root protein concentrations were not affected by AMF colonization and increases in the salt-treated plants were not statistically significant (Table 4).

Table 3. Osmotic potential in the shoots and roots, leaf relative water content (RWC), and water-use efficiency (WUE) in halophytic grass, *Aeluropus littoralis*, grown under three levels of NaCl salinity in the absence (–AMF) or presence (+AMF) of arbuscular mycorrhizal fungi. Data are mean ± SD (n = 4). Difference among data of each column followed by *the same letter* was not significant (p<0.05).

Treatment		Osmotic potential [MPa] Shoots	Roots	RWC [%]	WUE
–AMF	Control	–0.24 ± 0.02^{b}	–0.15 ± 0.01^{a}	82.4 ± 2.0^{a}	3.46 ± 0.39^{d}
	50 mM	–0.27 ± 0.01^{b}	–0.16 ± 0.05^{a}	83.7 ± 2.5^{a}	6.49 ± 0.29^{c}
	200 mM	–0.34 ± 0.02^{a}	–0.20 ± 0.05^{a}	84.0 ± 2.1^{a}	8.20 ± 0.08^{b}
+AMF	Control	–0.28 ± 0.02^{b}	–0.14 ± 0.02^{a}	80.7 ± 2.1^{a}	5.85 ± 0.09^{c}
	50 mM	–0.37 ± 0.05^{a}	–0.22 ± 0.04^{a}	81.0 ± 3.3^{a}	7.41 ± 0.08^{bc}
	200 mM	–0.36 ± 0.02^{a}	–0.17 ± 0.05^{a}	82.7 ± 6.8^{a}	9.52 ± 0.92^{a}

Table 4. Activity of nitrate reductase (NR) and phospho*enol*pyruvate carboxylase (PEPC) and concentrations of nitrate, nitrite, and soluble proteins in the shoots and roots of halophytic grass, *Aeluropus littoralis*, grown under three levels of NaCl salinity in the absence (–AMF) or presence (+AMF) of arbuscular mycorrhizal fungi. Data are mean ± SD (n = 4). Difference among data of each column within each plant organ followed by *the same letter* was not significant (p<0.05).

Treatment		NR activity [pmol(NO_2^-) mg^{-1}(protein) s^{-1}]	Nitrate [µmol g^{-1}(FM)]	Nitrite [nmol g^{-1}(FM)]	Protein [mg g^{-1}(FM)]	PEPC activity [nmol(NADH) mg^{-1}(protein) s^{-1}]
Shoot						
–AMF	Control	10.0 ± 0.7^{e}	5.28 ± 0.92^{a}	7.58 ± 0.96^{b}	10.6 ± 3.64^{a}	7.02 ± 0.12^{a}
	50 mM	14.7 ± 1.1^{d}	4.07 ± 0.56^{ab}	10.82 ± 1.02^{a}	13.5 ± 0.21^{a}	7.13 ± 0.20^{a}
	200 mM	21.6 ± 0.8^{b}	3.31 ± 0.85^{b}	12.54 ± 1.43^{a}	10.7 ± 1.72^{a}	3.92 ± 0.18^{b}
+AMF	Control	18.3 ± 1.4^{c}	4.48 ± 0.79^{a}	10.62 ± 1.44^{a}	9.6 ± 2.63^{a}	3.85 ± 0.17^{b}
	50 mM	25.1 ± 1.5^{a}	3.60 ± 0.64^{ab}	10.49 ± 1.53^{a}	11.2 ± 1.92^{a}	3.97 ± 0.15^{b}
	200 mM	26.6 ± 1.4^{a}	2.25 ± 0.68^{b}	10.06 ± 1.60^{a}	13.4 ± 3.71^{a}	2.27 ± 0.21^{c}
Root						
–AMF	Control	133.0 ± 11.0^{a}	8.03 ± 1.81^{a}	0.15 ± 0.01^{d}	1.31 ± 0.09^{a}	-
	50 mM	69.0 ± 2.0^{b}	7.83 ± 0.07^{a}	0.31 ± 0.16^{cd}	1.46 ± 0.12^{a}	-
	200 mM	26.0 ± 2.0^{c}	3.93 ± 0.39^{b}	1.06 ± 0.20^{b}	1.96 ± 0.07^{a}	-
+AMF	Control	72.0 ± 3.0^{b}	7.06 ± 0.54^{a}	0.73 ± 0.41^{bc}	1.37 ± 0.65^{a}	-
	50 mM	31.0 ± 3.0^{c}	5.69 ± 0.95^{b}	0.97 ± 0.08^{b}	1.58 ± 0.58^{a}	-
	200 mM	26.0 ± 1.7^{c}	4.82 ± 0.90^{b}	2.01 ± 0.16^{a}	1.66 ± 0.44^{a}	-

Activity of PEPC decreased under HS in both –AMF and +AMF plants. The AMF-colonized plants had significantly lower PEPC activity compared with –AMF counterparts in the absence and presence of salt (Table 4).

Discussion

Plant growth: Plant biomass increased by salt treatment and the highest dry matter production of both shoots and roots was obtained at HS treatment. Optimal growth in dicotyledonous halophytes can be achieved at salinity concentrations as high as 200 mM (Caldwell 2012). Monocotyledonous halophytes often show optimum growth in the absence of salt or, if growth is stimulated, under a low (50 mM or lesser) concentration of NaCl (Flowers and Colmer 2008, Shabala and Mackay 2011, Rozema and Schat 2013). In contrast, our data indicated clearly that growth of *A. littoralis* was stimulated in the presence of 200 mM salt. *A. littoralis* grown hydroponically under 100 mM NaCl decreased significantly growth and at 200 mM NaCl, shoot growth was inhibited by about 50% (Barhoumi *et al.* 2007). Such discrepancy may be related to different growth conditions or different plant material collected from different habitats, *i.e.*, an ecotypic difference. Study of another species of this genus, *A. lagopoides*, showed a slight stimulation of growth at 200 mM (Gulzar *et al.* 2003). Optimal growth of halophytes under higher salt concentrations could not be regarded as an indication of a nutritional role for Na^+. Although Na^+ is an essential micronutrient for some C_4 halophytes (Kronzucker *et al.* 2013), nutritional requirement of Na^+ is satisfied by very small quantities (0.1 mM) (Caldwell 2012).

Root AMF colonization rate was diminished by salt treatment. It was most likely due to a direct effect of salt

on AMF, *i.e.*, inhibition of hyphal growth and/or its spreading after initial infection (Porcel *et al.* 2012, Hajiboland 2013). Though lower colonization rate of the salt-treated plants, the +AMF plants produced higher biomass of shoots and roots compared with the –AMF ones.

These data suggested that a higher benefit of AMF association for the host plants is not necessarily accompanied by a higher colonization rate. Indeed a few active fungal structures in only a small number of roots may help plants to cope with all kinds of stress including salinity (Füzy *et al.* 2008).

Interestingly, growth stimulation mediated by the AMF colonization in *A. littoralis* was observed only in the salt-treated plants. Since AMF association requires extra energy and C sources, it may rather inhibit growth of host plants under conditions of a limited photosynthetic rate because of competition between AMF and the host for photoassimilates (Smith and Read 2008). Higher P_N in the salt-treated *A. littoralis* plants may better provide AMF with photoassimilates compared with the Ct plants.

Plants AMF responsiveness was greater under LS (54 and 48%) compared with HS (27 and 10% considering shoot and root DM, respectively). This led in turn to a lower shoot (but not root) DM of the +AMF plants under HS compared with LS. Variation in AMF responsiveness can be partitioned into dependence and independence components; dependence variation relates to plant performance under stress but independence variation describes differences in the interaction between a plant and AMF (Sawers *et al*. 2008). Considering parameters determined in this work, we could assume that reduction of the leaf osmotic potential, the K^+/Na^+ ratio in the shoot and roots, root NR activity (as dependence parameters), and colonization rate (as a non-dependence parameter) under HS compared with LS concentration, were probably the mechanisms for lower AMF responsiveness of *A. littoralis* at 200 mM salt concentration.

Gas exchange and PEPC activity: The P_N was significantly higher in the salt-treated plants. This effect was not associated with higher g_s suggesting nonstomatal mechanisms for elevated P_N of *A. littoralis* under saline conditions (*see* below). The lower C_i/C_a ratio under HS concentration, which indicated partial CO_2 depletion in the interspaces of the leaves, may confirm this suggestion. Data on the photosynthetic responses of halophytic species to saline conditions are scattered and even in a recent review (Lovelock and Ball 2002) a definite pattern on the differences between halophytes and glycophytes has not been presented. Significant reduction of P_N under saline conditions has been reported for C_3 halophytes, *Aster tripolium* (Geissler *et al.* 2009), *Arthrocnemum perenne*, and *A. fruticosum* (Nieva *et al.* 1999), and *Phragmites australis* (Ge *et al.* 2014). In contrast, under 200 mM NaCl salinity, P_N of C_3 halophytic grass, *Puccinellia distans*, was 30% higher than control plants without salt (Dashtebani *et al.* 2014). In spite of a significant dominance in arid and saline regions, C_4 photosynthesis as affected by salinity was studied less than C_3 photosynthesis. In *Andropogon greenwayi* and *Sporobolus ioclados* (Hamilton *et al.* 2001), *Spartina maritima* and *S. densiflora* (Nieva *et al.* 1999), *S. patens* (Maricle *et al.* 2007), and *S. alterniflora* (Maricle *et al.* 2007, Ge *et al.* 2014) as C_4 grass species, reduction of photosynthesis under saline conditions has been reported. In *Atriplex nummularia*, a C_4 dicotyledonous halophyte, P_N decreased under even mild salinity though higher biomass of plants was observed at this salt concentration (Hussin *et al.* 2013). In contrast, in *Haloxylon aphyllum* (Rakhmankulova *et al.* 2014), a C_4 dicotyledonous halophyte, and *Sporobolus spicatus* (Hamilton *et al.* 2001), a C_4 halophytic grass, P_N were higher at salt concentrations of 200 mM and 400 mM, respectively. These data indicated that the response of photosynthesis in halophytes to salinity is highly dependent on plant species, but not on their photosynthetic pathway. *In situ* field measurements of P_N in two halophytes from Chenopodiaceae (*Eurotia lanata*, a C_3 species, and *Atriplex confertifolia*, a C_4 species) revealed that throughout the season both species exhibited similar rates of photosynthesis even under different light intensities (Caldwell 2012). It has been stated that since both C_3 and C_4 photosynthetic pathways have been successful strategies for desert halophytes throughout the world, a clear-cut advantage of one system over the other has not been widely accepted (Caldwell 2012).

Mycorrhizal colonization increased P_N in both control and salt-treated plants. In contrast to the effect of salt, higher P_N of the +AMF compared with –AMF plants was mainly associated with higher g_s. It is noteworthy that the elevation of P_N in the salt-treated +AMF *A. littoralis* plants was the result of a combined effect of stomatal and nonstomatal mechanisms. Mycorrhizal plants have often higher CO_2 assimilation capacity because of elevated g_s (Hajiboland 2013). Mycorrhization of plants results in greater sink strength of roots and this has been suggested as a reason for the often observed mycorrhizal promotion of g_s (Augé 2000). Our data showed that AMF-mediated elevation of P_N in *A. littoralis* relied mainly on the CO_2 diffusion through stomata. It implies likely that CO_2 availability for enzymatic reactions was still limiting for photosynthesis of this C_4 species. In *Atriplex lentiformis*, relative leakage of CO_2 from the bundle sheath increased with increasing salinity (Meinzer and Zhu 1999). These results suggest that salinity stress reduces the efficiency of CO_2 concentrating mechanism of the C_4 pathway.

As related to the nonstomatal mechanisms, our data could not explain elevated P_N since PEPC activity was rather reduced (*see* below). Nevertheless, slightly or significantly higher leaf protein, Chl *a*, Chl *b*, and Car concentrations observed here under different treatment combinations could at least partly explain the enhancement of P_N independent from the leaf CO_2 diffusion parameters. A positive correlation among the leaf N and protein content and leaf Chl content and its photosynthetic

efficiency has been well documented (Hawkesford *et al.* 2012). Car as the main biochemical component of nonphotochemical quenching plays an important role in protecting photosystems against damage caused by excessive excitation energy, particularly under salt stress (Hajiboland 2014). It has been also shown that upregulation of Chl *b* biosynthesis regulates the expression of several thylakoid membrane proteins that increase both the antenna size and the electron transport rates and enhance CO_2 assimilation, starch content, and dry matter accumulation (Biswal *et al.* 2012) and increases thermostability and function of PSII (Lin *et al.* 2005).

Unexpectedly, in contrast to the P_N, the activity of PEPC was diminished by higher salt concentration as well as by AMF colonization. It has been reported that the ratio of C_3-cycle activity to that of C_4-cycle in the C_4 halophyte, *Atriplex lentiformis*, decreased from 0.96 in control plants to 0.37 in plants grown at 600 mM NaCl (Meinzer and Zhu 1999). In our experiment, the lower PEPC activity indicated that the C_4 cycle was also affected adversely under severe saline conditions in *A. littoralis*. Despite the lower PEPC activity, higher P_N in the +AMF compared with –AMF plants could be partly attributed to the elevated g_s confirming again CO_2 limitation of photosynthesis in *A. littoralis* under salinity conditions. In the –AMF plants, however, the higher P_N in the absence of elevated g_s was surprising and needs further detailed examinations. Regardless of the mechanisms involved, the CO_2 fixed under salinity and AMF colonization ultimately supported metabolic and energy reactions to be translated as growth and development. The elevated P_N was likely the main reason for growth stimulation under these conditions in *A. littoralis*.

In contrast to the P_N, E was not affected by salt obviously because of stable g_s under these conditions. However, AMF-mediated elevation of g_s was not associated with higher E because of a lower hydraulic conductivity in the leaves. Increasing evidence suggests that AMF colonization changes root and leaf hydraulic properties *via* differential modifications in the expression of various aquaporin subgroups (Ruiz-Lozano *et al.* 2012).

N assimilation: Higher accumulation of N in shoots of mycorrhizal plants than that of nonmycorrhizal controls has been reported (Giri and Mukerji 2004). Improved N nutrition in AMF plants may help to reduce the toxic effects of Na^+ by reducing its uptake and this may indirectly help in maintaining the Chl content of the plant (Giri and Mukerji 2004). However, the exact mechanism used by AMF to improve N nutrition under saline conditions is not clearly understood (Evelin *et al.* 2009). In this work, concentration of proteins was not much affected by both treatments, while the activity of NR increased by both treatments in the shoots. The NR activity is regulated at transcriptional and posttranslational levels and the availability of nitrate, carbohydrates, and C skeletons and the redox state of the cells are important factors regulating NR activity in the shoots and roots (Foyer *et al.* 2003). We assume that higher P_N, which increases availability of reducing equivalents and carbohydrates, was responsible for the elevated NR activity in the shoots of *A. littoralis* under salinity and mycorrhizal conditions. A significant correlation between the NR activity and P_N ($r^2 = 0.78$, $p<0.05$) and soluble carbohydrate concentrations ($r^2 = 0.92$, $p<0.001$) in the shoots confirmed the link between the C-assimilation rate and NR activity.

The activity of NR in the roots was constitutively higher than that in the shoots. However, in contrast to the shoots, it was diminished by salt and AMF treatments leading to a comparable shoot and root NR activity under high salt concentration in both +AMF and –AMF plants. The mechanism for organ-dependent response of NR to salt and AMF colonization is not known and needs further elucidation. The lack of any significant rise of carbohydrates availability in the roots in contrast to the shoots might at least partly explain differential responses of shoot and root NR activity to the applied treatments. Higher C and energy requirements for vacuolar sequestration of Na^+ under salinity (Shabala and Mackay 2011) and the presence of AMF as a strong sink for root C (Smith and Read 2008) imposed likely limitations for C sources in the roots. The proportion of nitrate reduction carried out in roots and shoots depends on various factors, including plant species and age, nitrate supply, and light intensity (Hawkesford *et al.* 2012). Ecological and energetic advantages and higher efficiency of shoot- *vs*. root-localized nitrate assimilation have been proposed by many authors (Hawkesford *et al.* 2012). In our experiment, the reduction of the root NR activity in *A. littoralis* was compensated by the correspondingly elevated the shoot NR activity. An increased contribution of shoots to the whole plant N assimilation under these conditions could be considered an adaptive response in this species leading to the maintenance of N assimilation capability of the whole plant. Reports on the effect of salt on NR activity are restricted mainly to glycophyte species and little is known about the N metabolism of halophytes, particularly of desert halophytes (Caldwell 2012). Activity of NR decreased in both shoots and roots in *Suaeda physophora* at 100–450 mM NaCl (Yuan *et al.* 2010). Effect of AMF inoculation has been reported in glycophyte species as increase in the leaf NR activity in wheat (Talaat and Shawky 2013).

Higher NR activity in the leaves upon salt and AMF inoculation treatments was associated with lower nitrate concentrations suggesting a partial depletion of nitrate in the leaves following elevated assimilation. In the roots, however, depletion of nitrate occurred despite the lower NR activity probably because of lower nitrate uptake under these conditions (Talaat and Shawky 2013).

In contrast to the nitrate, the nitrite concentration increased under salinity in the leaves of the –AMF plants and in the roots of both –AMF and +AMF plants. The higher nitrite concentration in the leaves (but not in the

roots) was associated with the higher NR activity; it might indicate disturbances in the coupling between nitrate assimilation and nitrite reduction and/or a partial shortage of reducing equivalents. The lack of nitrite accumulation in the leaves of the +AMF plants may imply an adequate supply of reducing equivalents in the +AMF plants as the consequence of higher Chl *b* concentration as discussed above. Nevertheless, plants growth parameters under the corresponding treatments indicated that nitrite concentration was not obviously at toxic levels either in the leaves or in the roots.

Water-relation parameters and osmolyte concentrations: Data of RWC and osmotic potentials suggested that effect of AMF on the improvement of growth in *A. littoralis* under saline conditions was not mediated by an improvement in the water relation parameters. However, WUE mainly increased due to the elevated P_N associated with often unchanged E in both salt-treated and +AMF plants.

Not much is known about the role of carbohydrates as osmolytes in halophytes, but there is evidence that they also contribute to osmoregulation, as they do in many glycophytes (Gil *et al.* 2011). Soluble sugar accumulation under both salt and AMF colonization in the leaves of *A. littoralis* was correlated well with higher P_N under these conditions. The increase in total carbohydrates is positively correlated with mycorrhization of host plants (Evelin *et al.* 2009) and is attributed to the hydrolysis of starch to sugars and the sink effect of the fungus demanding sugars from the shoot tissues (Augé 2000). Here the stable amounts of starch in the leaves of the +AMF plants suggested that soluble sugars accumulated under these conditions were derived from photosynthesis and not from starch degradation. In the roots, the changes brought about by salinity or AMF inoculation were much less pronounced than that in the leaves. It implies likely limitations in the allocation of photoassimilates to the roots and/or lesser functional role of soluble sugars in protecting roots in *A. littoralis*.

The proline concentration in *A. littoralis* was higher under salinity in both leaves and roots of the –AMF and in the leaves of +AMF plants. As related to the effect of AMF, the proline concentration of leaves and roots remained unchanged or even decreased in the salt-treated *A. littoralis* plants. In addition of its function as a reservoir of energy and N for utilization during exposure to salinity, proline plays key roles in osmotic adjustment, protection of enzymes and membranes, ROS scavenging (Ashraf and Harris 2004), and improvement of K^+ selectivity (Shabala and Mackay 2011). Data in the literature on the effect of mycorrhizal association on proline accumulation include both higher and lower proline concentrations in mycorrhizal plants (Evelin *et al.* 2009, Porcel *et al.* 2012, Ruiz-Lozano *et al.* 2012, Hajiboland 2013). Lower proline content of +AMF plants under saline conditions compared with –AMF plants may be regarded as a reflection of an increased salt resistance in plants upon mycorrhization, *i.e.*, less injury (Hajiboland 2013). Proline is a biochemical marker of salt stress (Ashraf and Harris 2004) and its accumulation does not necessarily confer higher salt tolerance (Porcel *et al.* 2012, Hajiboland 2013). Here the lower proline content of the +AMF plants under salt stress suggests that the +AMF plants were likely less strained by salinity than the –AMF plants.

The concentration of total free α-amino acids [in the range of 170–330 μmol g^{-1}(FM)] was considerably higher than the proline [in the range of 5–12 μmol g^{-1}(FM)] concentration in the shoots and roots. It may suggest the important role of all free α-amino acids instead of proline alone in osmoregulation of *A. littoralis*. Concentrations of proline and even free α-amino acids were probably not sufficient for providing osmotic adjustment of the cytosol in *A. littoralis* regarding Na^+ tissue concentrations of about 80–157 mg g^{-1}(FM) [~3,500–6,800 μmol g^{-1}(FM)] in the leaves and roots (data not shown), respectively, and considering an estimated ion accumulation of about 500 mM (~500 μmol g^{-1} cell sap) in the vacuoles of halophytes (Shabala and Mackay 2011). It has been suggested that the major role of compatible solutes in halophytes is scavenging of free radicals and regulation of K^+-permeable ion channels in the plasma membranes (Shabala and Mackay 2011).

Ion homeostasis and distribution between shoots and roots: Mycorrhizal colonization of *A. littoralis* increased uptake of K^+ in the salinized plants; the effect was more pronounced under higher salt concentration. Effect of AMF colonization on the enhancement of K^+ absorption under saline conditions is well documented and is accomplished by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps (Evelin *et al.* 2009). However, the same effect of AMF on K^+ uptake was observed for Na^+ uptake under saline conditions in *A. littoralis*. Mycorrhizal association enhances or reduces Na^+ uptake depending on plant and fungus species (Evelin *et al.* 2009, Porcel *et al.* 2012, Ruiz-Lozano *et al.* 2012, Hajiboland 2013). It has been reported that AMF induces a buffering effect on the uptake of Na^+ indicating possibility of a regulatory mechanism operating in the plant for K^+ and Na^+ selectivity depending on external Na^+ concentrations (Ruiz-Lozano *et al.* 2012). It is likely that cyclic nucleotide-gated ion channels (CNGCs) as nonselective cation channels that contribute to Na^+ reallocation within the plant tissues are involved in salt stress amelioration in AMF plants (Porcel *et al.* 2012).

Mycorrhizal colonization of salinized *A. littoralis* plants resulted in the lower K^+/Na^+ ratio in the leaves but higher in the roots. The reason for such differential effect of AMF on the K^+/Na^+ ratio in the leaves and roots is not known but suggests that improvement of leaf ion homeostasis was not the mechanism for AMF-mediated growth promotion in *A. littoralis*.

Conclusion: Grass species from natural populations provide outstanding materials for the investigation of

mechanisms, responsible for adaptation to high salt concentrations, particularly, those related to AMF associations. We observed here that both salt and AMF treatments improved performance of *A. littoralis* in a synergistic manner mainly *via* elevated net CO_2 and nitrate assimilation rates. These data provide evidence for the feasibility of using this species, particularly in association with AMF, as vegetation cover, for preventing erosion, stabilizing soil, and as a fodder crop in salt-affected soils.

References

Akhani H.: Biodiversity of halophytic and Sabkha ecosystems of Iran. – In: Khan M.A., Barth H., Kust G.C., Böer B. (ed.): Sabkha Ecosystems. Vol II: The Southern and Central Asian Countries. Pp. 71-88. Springer, New York 2006.

Aliasgharzadeh N., Saleh Rastin N., Towfighi H., Alizadeh A.: Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. – Mycorrhiza **11**: 119-122, 2001.

Ashraf M., Harris P.J.C.: Potential biochemical indicators of salinity tolerance in plants. – Plant Sci. **166**: 3-16, 2004.

Ashton R.A., Burnell J.N., Furbank R.T. *et al.*: Enzymes of C_4 photosynthesis. – In: Dey P.M., Harborne J.B. (ed.): Methods in Plant Biochemistry. Vol 3: Enzymes of Primary Metabolism. Pp. 39-72. Academic Press, London 1990.

Augé R.M.: Stomatal behavior of arbuscular mycorrhizal plants. – In: Kapulnik Y., Douds D.D. (ed.): Arbuscular Mycorrhizas: Physiology and Function. Pp. 201-237. Kluwer Academic, Dordrecht 2000.

Barhoumi Z., Djebali W., Smaoui A. *et al.*: Contribution of NaCl excretion to salt resistance of *Aeluropus littoralis* (Willd) Parl. – J. Plant Physiol. **164**: 842-850, 2007.

Bates L.S., Waldren R.P., Teare I.D.: Rapid determination of free proline for water-stress studies. – Plant Soil **39**: 205-207, 1973.

Biswal A.K., Pattanayak G.K., Pandey S.S. *et al.*: Light intensity-dependent modulation of chlorophyll *b* biosynthesis and photosynthesis by overexpression of chlorophyllide *a* oxygenase in tobacco. – Plant Physiol. **159**: 433-449, 2012.

Caldwell M.M.: Physiology of desert halophytes. – In: Mold R.J. (ed.): Ecology of Halophytes. Pp. 357-378. Elsevier, New York 2012.

Cataldo D.A., Maroon M., Schrader L.E. *et al.*: Rapid colorimetric determination of nitrate in plant tissues by nitration of salicylic acid. – Commun. Soil Sci. Plan. **6**: 71-80, 1975.

Chaves M.M., Flexas J., Pinheiro C.: Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. – Ann. Bot.-London **103**: 551-560, 2009.

Clayton W.D., Vorontsova M.S., Harman K.T., Williamson H.: Grass Base – The Online World Grass Flora. – http://www.kew.org/data/grasses-db.html [accessed 13 Aug 2014].

Dashtebani F., Hajiboland R., Aliasgharzad N.: Characterization of salt-tolerance mechanisms in mycorrhizal (*Claroideoglomus etunicatum*) halophytic grass, *Puccinellia distans*. – Acta Physiol. Plant. **36**: 1713-1726, 2014.

Eimanifar A., Mohebbi F.: Urmia Lake (Northwest Iran): a brief review. – Saline Syst. **3**: doi:10.1186/1746-1448-3-5, 2007.

Evelin H., Kapoor R., Giri B.: Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. – Ann Bot.-London **104**: 1263-1280, 2009.

Feldmam S.R., Bisaro V., Biani N.B., Prado D.E.: Soil salinity determines the relative abundance of C_3/C_4 species in Argentinean grasslands. – Global Ecol. Biogeogr. **17**: 708-714, 2008.

Flowers T.J., Colmer T.D.: Salinity tolerance in halophytes. – New Phytol. **179**: 945-963, 2008.

Foyer C.H., Parry M., Noctor G.: Markers and signals associated with nitrogen assimilation in higher plants. – J. Exp. Bot. **54**: 585-593, 2003.

Füzy A., Biró B., Tóth T. *et al.*: Drought, but not salinity, determines the apparent effectiveness of halophytes colonized by arbuscular mycorrhizal fungi. – J. Plant Physiol. **165**: 1181-1192, 2008.

Ge Z.-M., Zhang L.-Q., Yuan L., Zhang C.: Effects of salinity on temperature-dependent photosynthetic parameters of a native C_3 and a non-native C_4 marsh grass in the Yangtze Estuary, China. – Photosynthetica **52**: 484-492, 2014.

Geissler N., Hussin S., Koyro H-W.: Interactive effects of NaCl salinity and elevated atmospheric CO_2 concentration on growth, photosynthesis, water relations and chemical composition of the potential cash crop halophyte *Aster tripolium* L. – Environ. Exp. Bot. **65**: 220-231, 2009.

Gil R., Lull C., Boscaiu M. *et al.*: Soluble carbohydrates as osmolytes in several halophytes from a Mediterranean salt marsh. – Not. Bot. Horti. Agrobo. **39**: 9-17, 2011.

Giovanetti M., Mosse B.: An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. – New Phytol. **84**: 489-500, 1980.

Giri B., Mukerji K.G.: Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. – Mycorrhiza **14**: 307-312, 2004.

Gulzar S., Khan M.A., Ungar I.A.: Effects of salinity on growth, ionic content, and plant-water status of *Aeluropus lagopoides*. – Commun. Soil Sci. Plan. **34**: 1657-1668, 2003.

Hajiboland R.: Reactive oxygen species and photosynthesis. – In: Ahmad P. (ed.): Oxidative Damage to Plants, Antioxidant Networks and Signaling. Pp. 1-63. Elsevier, London 2014.

Hajiboland R.: Role of arbuscular mycorrhiza in amelioration of salinity. – In: Ahmad P., Azzoz M.M., Prasad M.N.V. (ed.): Salt Stress in Plants, Signaling, Omics and Adaptations. Pp. 301-354. Springer, New York 2013.

Hamilton E.W., McNaughton S.J., Coleman J.S.: Molecular, physiological and growth responses to sodium stress in C_4 grasses from a soil salinity gradient in the Serengeti ecosystem. – Am. J. Bot. **88**: 1258-1265, 2001.

Hawkesford M., Horst W., Kichey T. *et al.*: Functions of macronutrients. – In: Marschner P. (ed.): Marschner's Mineral Nutrition of Higher Plants, 3rd Edition. Pp. 135-189. Elsevier, Oxford 2012.

Hussin S., Geissler N., Koyro H.W.: Effect of NaCl salinity on *Atriplex nummularia* (L.) with special emphasis on carbon and nitrogen metabolism. – Acta Physiol. Plant. **35**: 1025-1038, 2013.

Jaworski E.G.: Nitrate reductase assay in intact plant tissues. – Biochem. Bioph. Res. Co. **43**: 1274-1279, 1971.

Kronzucker H.J., Coskun D., Schulze L.M. *et al.*: Sodium as nutrient and toxicant. – Plant Soil **369**: 1-23, 2013.

Lin Z., Peng C., Xu X. *et al*.: Thermostability of photosynthesis in two new chlorophyll *b*-less rice mutants. – Sci. China C. Life Sci. **48**: 139-147, 2005.

Lichtenthaler H.K., Wellburn A.R.: Determination of total carotenoids and chlorophylls *a* and *b* of leaf in different solvents. – Biol. Soc. Trans. **11**: 591-592, 1985.

Lovelock C.E., Ball M.C.: Influence of salinity on photosynthesis of halophytes. – In: Läuchli A., Lüttge U. (ed.): Salinity: Environment, Plants, Molecules. Pp. 315-339, Kluwer Academic Publishers, Dordrecht 2002.

Maricle B.R., Lee R.W., Hellquist C.E. *et al*.: Effects of salinity on chlorophyll fluorescence and CO_2 fixation in C_4 estuarine grasses. – Photosynthetica **45**: 433-440, 2007.

Meinzer F.C., Zhu J.: Efficiency of C_4 photosynthesis in *Atriplex lentiformis* under salinity stress. – Aust. J. Plant Physiol. **26**: 79-86, 1999.

Nieva F.J.J., Castellanos E.M., Figueroa M.E., Gil F.: Gas exchange and chlorophyll fluorescence of C_3 and C_4 saltmarsh species. – Photosynthetica **36**: 397-406, 1999.

Porcel R., Aroca R., Ruiz-Lozano J.M.: Salinity stress alleviation using arbuscular mycorrhizal fungi. – Agron. Sustain. Dev. **32**: 181-200, 2012.

Rakhmankulova Z.F., Voronin P.Yu., Shuyskaya E.V. *et al*.: Effect of NaCl and isoosmotic polyethylene glycol stress on gas exchange in shoots of the C_4 xerohalophyte *Haloxylon aphyllum* (Chenopodiaceae). – Photosynthetica **52**: 437-443, 2014.

Rozema J., Schat H.: Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. – Environ. Exp. Bot. **92**: 83-95, 2013.

Ruiz-Lozano J.M., Porcel R., Azcón C., Aroca R.: Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. – J. Exp. Bot. **63**: 4033-4044, 2012.

Sage R.F.: Environmental and evolutionary preconditions for the origin and diversification of the C_4 photosynthetic syndrome. – Plant Biol. **3**: 202-213, 2001.

Sawers R.J.H., Gutjahr C., Paszkowski U.: Cereal mycorrhiza: an ancient symbiosis in modern agriculture. – Trends Plant Sci. **13**: 93-97, 2008.

Shabala S.N., Mackay A.: Ion transport in halophytes. – Adv. Bot. Res.**57**: 151-199, 2011.

Smith S.E., Read D.J.: Mycorrhizal Symbiosis. Pp. 787. Academic Press, San Diego 2008.

Talaat N.B., Shawky B.T.: Modulation of nutrient acquisition and polyamine pool in salt-stressed wheat (*Triticum aestivum* L.) plants inoculated with arbuscular mycorrhizal fungi. – Acta Physiol. Plant. **35**: 2601-2610, 2013.

Wang F.Y., Liu R.J., Lin X.G., Zhou J.M.: Arbuscular mycorrhizal status of wild plants in saline-alkaline soils of the Yellow River Delta. – Mycorrhiza **14**: 133-137, 2004.

Yemm E.W., Cocking E.C.: The determination of amino acids with ninhydrin. – Analyst **80**: 209-213, 1955.

Yemm E.W., Willis A.J.: The estimation of carbohydrates extracts by anthrone. – Biochem. J. **57**: 508-514, 1954.

Yuan J.-F., Feng G., Ma H.-Y., Tian C.-Y.: Effect of nitrate on root development and nitrogen uptake of *Suaeda physophora* under NaCl salinity. – Pedosphere **20**: 536-544, 2010.