SHORT COMMUNICATION

Glomus intraradices improved salt tolerance *in Prosopis alba* seedlings by improving water use efficiency and shoot water content.

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ABSTRACT

The present work was aimed at testing the hypothesis that mycorrhizal *Prosopis alba,* an economically important tree species worldwide, presents increased salt-tolerance compared with non-mycorrhizal ones and at gaining insight into the possible mechanisms underlying that improvement. For this purpose, a randomized complete block experiment with two factors: mycorrhizal treatments with or without the arbuscular fungus *Glomus intraradices* and two salinity levels, 0 and 200 mM NaCl was performed. Plant growth in *P. alba* plants colonized by *G. intraradices* was less affected by salinity than that in non-arbuscular mycorrhizal (AM) plants, indicating that mycorrhizal colonization turned *P. alba* more tolerant to salinity. Photosynthesis was reduced by salinity in non-AM plants but not in AM ones. Salinity caused a significant decrease in mean stomatal conductance and transpiration rate, in mycorrhizal plants, but not in uninoculated ones. In this work, we detected two main mechanisms intervening in the salt tolerance enhancement of *P. alba* by the inoculation with *G. intraradices*: a- maintaining the net photosynthesis level and b- control of the transpiration rate. Taken together, the results suggest that inoculation with *G. intraradices* improves *P. alba* survival rates during the implantation period and seems to be a promising strategy to improve *P. alba* cultivation in saline lands.

Key words: photosynthesis, salinity, stomatal conductance, transpiration ratio, proline.

INTRODUCTION

Soil salinity induces decreases in plant water holding capacity, imbalance of nutrient uptake and ion toxicity towards photosynthesis, resulting in stunted growth and productivity of plants (Munns, 1993; 2002). In the Argentinean Chaco region, a semi-arid to arid zone spanning the provinces of Salta, Formosa, Chaco and Northern Santiago del Estero (Prado, 1993), logging and livestock grazing led to abundance of primary minerals and soluble salts, resulting in extended areas of saline soils (FAO and UNESCO, 1971), with EC values greater than 50 dSm^{-1} (Ragonese, 1951). Several *Prosopis* tree species, natives to the Chaco and other South America regions, were naturalized in Africa, Australia, and Asia (Pasiecznik et al., 2001). The facts that many of these species are highly tolerant to soil salinity and have a valuable lumber for furniture, forage and medicinal purposes (Rhodes

and Felker, 1988; Figueiredo, 1990; Felker, 1999; Velarde et. al., 2003; Felker and Guevara, 2003; Cagnolo et al., 2006; Lewis et al., 2009), make them economically attractive to use in governmental programs aimed to recovery wide zones with moderately saline soils (Meloni et al., 2004; Felker et al., 2008). Among them, the argentine mesquite *P. alba* is the most important commercial Prosopis species in Argentina. In addition, this species is able to grow even at the seawater salinity concentration (Velarde et al., 2003). However, growth of *P. alba* showed an approximate 50% drop in survival at 10 to 25 dSm^{-1} salinity during the early stages of seedling development (Velarde et al., 2003). Therefore, it becomes relevant to find methods increasing salt-tolerance in order to improve the rate of *P. alba* survival during the implantation period. Several authors have shown that arbuscular mycorrhizal (AM) fungi diminish detrimental effects of salinity on plants (Feng et al., 2002). Among the mechanisms intervening on plant growth enhancement by AM fungi are the improvement of water use efficiency (WUE) (Ruiz-Lozano et al., 1996), K^+/Na^+ ratios (Sannazzaro et al., 2006) and photosynthesis (Mukerji and Chamol, 2003; Al-Karaki, 2006). On the other hand, it has been shown that *P. alba* roots may be colonized by the AM fungus *Glomus intraradices* (Martin et al., 1994). The present work was aimed at testing the hypothesis that mycorrhizal *P. alba* seedlings present increased salt-tolerance compared with non-AM ones during the first stages of their development and at gaining insight into the possible underlying mechanisms of that improvement.

G. intraradices was propagated in 1 L pot cultures with soil-perlite (1: 3 V/V) and *Sorghum halepense* (L.) Pers. (=*Andropogon halepensis* Brot.) as host for 4 months. *P. alba* seeds were washed under running tap water and surface-sterilized by washing them for 1 min in 10% (v/v) NaOCl and 30 s in 5% (v/v) ethanol. Sterilized *P. alba* seeds were germinated in plastic trays containing sterilized sand. One week-old plants were transferred to 3 l pots filled with a mixture of perlite- vermiculite (1:1 V/V) and inoculated with 5 g of the AM-fungal inoculum consisting of root fragments with no less than 70% of their root length infected. Control plants received an equal amount of autoclaved inoculum. Plants of similar height were selected in order to avoid intra-specific growth differences bias. Plants were weekly supplied with nutrient solution containing 1.5 mM $CaCl_2$, 0.25 mM $MgSO_4$, 0.02 mM KH_2PO_4 and micronutrients equivalent to ¼ of Hoagland solution. After two months of pot culture, AM-inoculated plants showed 60% of mycorrhizal length colonization. At that moment, half of the non-AM and AM plants were subjected to saline treatment during 4 weeks. For saline treatment, the nutrient solution was supplemented with 200 mM NaCl, which conferred 40 dS m^{-1} soil electrical conductivity at harvest time. The experiment was performed under controlled environmental conditions ($27 \pm 1/22 \pm 2$ °C day/night, 14 h photoperiod, 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD from fluorescent lamps, 50-55 % relative humidity).

Plants were harvested and shoot and root dry weights recorded for each individual plant. Assessment of root colonization was performed according to McConigle et al. (1990). Leaf area was determined by using an area meter analyser (LiCor 3000). Foliar gas exchange, water relations, and growth parameters were measured at the end of the experiment. All physiological parameters were determined at midday. Mean stomatal conductance, transpiration and net photosynthesis were measured on intact, fully expanded mature leaves with infrared gas analyzers built into a leaf cuvette in an open-flow gas exchange system (LiCor 6400, USA). The LI-6400 light source was used to control photosynthetic photon flux densities (PPFD) at 1500 $\mu mol\ m^{-2}\ s^{-1}$. The airstream entering the cuvette was maintained at 350 $\mu mol\ CO_2 \cdot mol^{-1}$ with a computer-controlled CO_2 mixing system supplied with the LI-6400. Leaf and air temperatures were measured with thermocouples linked to the LI-6400 computer. Leaf was maintained at desired temperature with a computer controlled Peltier module mounted on the cuvette. The leaf-to-air vapour pressure deficit in the chamber was kept at approximately 1.6 KPa. The WUE was calculated as the mass of fixed CO_2 ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) over the mass of transpired H_20 ($mol\ H_2O\ m^{-2}\ s^{-1}$). Na^+ and K^+ were extracted from oven-dried (70 °C) shoots and roots with 100 mM HCl and their levels estimated by standard flame photometry (Chen et al., 2001). Proline content was estimated spectrophotometrically by the ninhydrin reaction under conditions described elsewhere (Maiale et al., 2004).

The experiment consisted of a randomized complete block with two factors: 1) mycorrhizal treatments, with (M+) or without (M-) AM fungus and 2) 0 (S-) and 200 (S+) mM NaCl. Only one plant was grown in each pot and there were 20 pots (replicates) per treatment. The experiment was performed twice, with similar results. Only results from the most representative experiment are shown. Data were analyzed by ANOVA of two

factors: salinity (0 and 200 mM) and symbiotic status (with or without *G. intraradices*).

Salt treatment caused no effect on AM fungal colonization, given that plants presented 70% of their root length colonized, regardless the saline treatment. However, despite the acknowledged salt tolerance of mature *P. alba* plants, the present study showed a noticeable detrimental effect of salinity on seedling growth, which was in the order of that observed in *P. juliflora* (Hussain and Alshammary, 2008) and *P. cinerea* (Ramoliya et al., 2006). At harvest, dry weights, number of leaves per plant and total leaf area were significantly reduced in salt-stressed plants, either they were or not colonized by *G. intraradices* (Table 1). Such diminution could be assigned to the extremely high salt content in the soil towards the end of the experiment (40 dS m^{-1} soil electrical conductivity). However, dry biomass and leaf area decreased in a much lower extent in AM plants due to salt treatment, compared with the uninoculated control, and salinity reduced the stem length in non-AM plants, but not in that of AM ones. These findings are consistent with previous reports showing improved plant growth response in mycorrhizal plants under salinity (Feng et al., 2002; Sannazzaro et al., 2006, 2007). A salt-derived reduction in the shoot/root ratio was found in non-AM plants, in accordance with previous results in plants of this species subjected to 600 mM NaCl (Meloni et al., 2004). In contrast, no significant change in this ratio was observed in salt-treated AM *P. alba* plants. Our results in *P. alba* confirmed that shoot reduction due to salinity is higher than that observed in the roots, as pointed out by Munns and Termaat (1986) and showed that AM colonization reduces organs susceptibility to saline stress, particularly in the case of shoots. Besides, mycorrhizal colonization itself led to increased *P. alba* growth regardless the saline condition, in line with results obtained in *P. juliflora* inoculated with *G. fasciculatum* (Tarafdar and Praveen-Kumar, 1996) and *G. aggregatum* (Duponnois et al., 2001). High levels of salt-induced defoliation were found in AM and non-AM plants. However, AM plants presented a higher total leaf area than non-AM plants, which along with a lower sensitivity to salinity by net photosynthesis (Table 2), could account for the lower magnitude of salt-induced growth reduction, compared with non-AM ones. It has been suggested that strong reductions in stomatal conductance and transpiration rate represent adaptive mechanisms to cope with excess salt, rather than merely negative consequences of this stress (Flanagan and Jefferies, 1989). Our results showing that upon long-term saline stress, reduced stomatal aperture and transpiration rate (Table 2) corresponded with improved plant growth in mycorrhizal *P. alba* plants (in contrast with non-AM ones) support that view, and put forward that the presence of *G. intraradices* in roots might have activated some mechanism for transpiration control, probably mediated by the enhancement of the absicic acid (ABA, an inhibitor of stomatal opening; Mittlehanser and Van Steveninck, 1969; Jones and Mansfield, 1970; Duan et al., 1996; Goicoechea et al., 1997), given that AM fungi apparently can produce ABA (Esch et al., 1994). In the M+S+ treatment, the sustainment of photosynthetic activity, along with a significantly reduced mean stomatal conductance led to an improved WUE (Table 2), which was 2.0, 2.8 and 1.9 orders of magnitude higher than those of M+S-, M-S+ and M-S- treatments, respectively. These facts denote a lower requirement in the amount of water to produce a unit of plant dry matter in these plants, what eventually might contribute to explain the higher plant biomass found in this treatment, compared with the corresponding non-AM one.

Table 1: Growth parameters and water contents of mycorrhized (M+) and non-mycorrhized (M-) *Prosopis alba* plants, after exposure to 0 or 200 mM NaCl (S- or S+, respectively) during 4 weeks. Standard deviation is shown in italics. Averages with the same letter are not statistically different ($P<0.05$).

Parameters	M-S-	M-S+	M+S-	M+S+
Total dry weight (g)	1.93 b	0.44 c	3.68 a	2.46 b
	0.43	*0.03*	*0.02*	*0.24*
Root dry weight (g)	0.46 b	0.17 c	1.11 a	0.78 b
	0.1	*0.04*	*0.12*	*0.17*
Stem dry weight (g)	0.8 b	0.2 c	1.5 a	1.1 b
	0.21	*0.01*	*0.13*	*0.09*
Leaf dry weight (g)	0.67 b	0.062 c	1.082 a	0.57 b
	0.21	*0.02*	*0.02*	*0.12*
Number of leaves per plant	12.8 a	4.5 b	12 a	2 b
	0.8	*1.5*	*4.5*	*0*
Total leaf area (cm^2)	157 b	23 c	256 a	148 b
	55	*9.2*	*24.1*	*34.2*
Stem length (cm)	53 a	29 b	51 a	47 ab
	6.04	*1.73*	*4.67*	*3.13*
Shoot/root ratio	3.15 a	1.63 b	2.34 a	2.31 ab
	0.54	*0.38*	*0.39*	*0.28*
Shoot water content	40 b	53 b	50 b	65 a
	12	*8*	*8*	*10*
Root water content	63 a	60 a	68 a	66 ab
	10	*12*	*7*	*1*

Table 2: Water relations and gas exchange parameters of mycorrhized (M+) and non-mycorrhized (M-) *Prosopis alba* plants, after exposure to 0 or 200 mM NaCl (S- or S+, respectively) during 4 weeks. Standard deviation is shown in italics. Averages with the same letter are not statistically different ($P<0.05$)

Water relations and gas exchange parameters	M-S-		M-S+		M+S-		M+S+	
Net photosynthesis (μmol CO^2 m^{-2} s^{-1})	10.8 *0.9*	a	7.4 *2.6*	b	11.2 *0.7*	a	9.9 *0.7*	ab
Stomatal conductance (mmol H_2O m^{-2} s^{-1})	0.022 *0.011*	a	0.021 *0.004*	ab	0.024 *0.011*	a	0.007 *0.005*	b
Transpiration rate (mol H_2O m^{-2} s^{-1})	0.67 *0.35*	a	0.71 *0.15*	ab	0.77 *0.35*	a	0.22 *0.19*	b
Water use efficiency (μmol $mmol^{-1}$)	1.89 *0.31*	b	1.28 *0.28*	b	1.78 *0.44*	b	3.66 *0.71*	a

The accumulation of various organic metabolites for osmotic adjustment is part of the plant adaptation to the osmotic stress caused by high salt build up in the soil, being proline the most widely distributed osmolyte among plants (Delauney and Verma, 1993; Hasegawa et al., 2000). Our results showed that *P. alba* plants do not accumulate proline in shoots as response to salt stress (data not shown), in accordance with previous results by Meloni et al. (2004). However, the root proline level was increased due to saline treatment in mycorrhizal plants, what could have reduced the water potential of this organ, thus enhancing the water inflow to the plant. Such response, in addition to the reduction in mean stomatal conductance and transpiration rate observed in salinized plants could explain the observed increase in the shoot water content in the AM-plants.

Salinity augmented Na^+ contents, regardless mycorrhizal treatment, whereas it led to a higher K^+ accumulation in roots of non-AM plants, and mycorrhizal colonization increased both shoot and root K^+ contents (data not shown). However, the intense defoliation observed in *P. alba* upon salt-treatment suggests that basipetal re-translocation of Na^+ excess and a further toxic accumulation of this cation might occur in older leaves, as a mechanism to tolerate salinity (Lessani and Marschner, 1978). Unfortunately, this fact prevents any conclusion linking shoot ion balances and plant growth from our data, since it masks the actual amount of Na^+ and K^+ reaching the shoot.

In this work, we detected two main mechanisms intervening in the salt tolerance enhancement of *P. alba* by the inoculation with *G. intraradices*: a- the net photosynthesis maintenance and b- control of the transpiration rate. Taken together, the information emerged from this work encourages future field experiments to test the hypothesis that inoculation with *G. intraradices* improves *P. alba* survival rates during the implantation period. Moreover, these results could help at designing a technological strategy for the inoculation of arbuscular mycorrhizal fungi at the greenhouse stage, in order to improve *P. alba* implantation in saline fields.

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