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Effect of *Funneliformis mosseae* on growth, mineral nutrition, biochemical indexes and chlorophyll content of *Ziziphus spina-christi* seedlings at different salinities

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Vast area of the land around the world is saline. Knowledge of plant behavior and their interaction with mychorrizal fungi in saline areas may help seedling establishment in such environments. This study aimed to determine the effects of the inoculation of the fungus *Funneliformis mosseae* (FM) on *Ziziphus spina-christi* (Rhamnaceae) plants grown under salt stress. Mycorrhizal and non-mycorrhizal seedlings were exposed to different levels of NaCl in the soil (0, 50, 100, and 150 mM). The following parameters were measured in both inoculated and non-inoculated plants: root colonization rate, seedling height, root diameter, root and shoot dry weights, chlorophyll *a* and *b*, total nitrogen (N), phosphorus (P), potassium (K) and sodium (Na^+) content, proline accumulation in roots and leaves, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities. The results showed that soil salinity hampered the root colonization by the fungus, and decreased basal diameter, seedling height, root and shoot dry weights, as well as some nutrients and chlorophyll *a* concentration, while increased leaves and roots Na^+, SOD and POD activity, proline accumulation, as well as CAT activity in the roots. Contrastingly, no significant effect of soil salinity were detected on K and CAT of leaves, root N, and chlorophyll *b*. Inoculated plants had higher basal diameter, leaves and roots P, root and shoot dry weights, chlorophyll *a* and lower SOD content, proline accumulation in leaves and Na^+, as compared with non-inoculated plants. Seedling height, root N, CAT and POD content, and chlorophyll *b* were not affected by inoculation with FM. These results demonstrated that FM inoculation is a promising method for improving the growth of *Z. spina-christi* seedlings under salt stress.

Keywords: Salinity, Peroxidase, Chlorophyll, Arbuscular Mycorrhiza, *Ziziphus spina-christi*

Introduction

Soil salinity is a chronic problem increasing worldwide, especially in arid and semi-arid areas (Al-Karaki 2006). At least 6% of the global landmass is affected by salinity (FAO 2007). Three types of physiological stress affect plant growth in saline soils: (i) toxic effects of specific ions, such as sodium and chloride, on plant cells (Juniper & Abbott 1993); (ii) physiological drought in soil with low osmotic potential, due to the plant efforts to maintain a lower internal osmotic potential, thus preventing water egression from roots into the soil; and (iii) imbalances of the nutrient content caused by the decreased nutrient uptake and/or transport to the leaves (Adiku et al. 2001).

To mitigate the effects of soil salt on plant growth, many strategies have been developed, including the use of seedlings with roots colonized by arbuscular mycorrhizal fungi (AMF – Wu et al. 2010, Yang et al. 2014). AMF have symbiotic relationship with the roots of over 80% of the terrestrial plant species, including halophytes, hydrophytes and xerophytes (Hejiden et al. 1998). Indeed, It has been demonstrated that AMF colonization increases the tolerance of some plants to salt (Tian et al. 2004). AMF are mutually symbiotic and provide a direct physical link between the soil and plant roots (Gaur & Adholeya 2004).

AMF promotes salinity tolerance by increasing nutrient uptake (Evelin et al. 2012, Beltrano et al. 2013), improving rhizospheric and soil conditions (Asghari et al. 2005), increasing photosynthesis and water use efficiency (Hajiboland et al. 2010), the accumulation of compatible solutes (Evelin et al. 2012) and enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) as a defense system to protect the plant cell from oxidative stress (Wu et al. 2010, Lu et al. 2014).

Funneliformis mosseae (FM) is an arbuscular mycorrhiza used to alleviate salt stress in *Arachis hypogaea* (Al-Khaliel 2010), *Capsicum annuum* (Abdel Latef 2013), *Poncirus trifoliate* (Wu et al. 2010) and *Olea europaea* (Porras-Soriano et al. 2009) seedlings. Symbiotic relationships between *Funneliformis mosseae* and several species of the genus *Ziziphus* (Rhamnaceae) have been

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Tab. 1 - Physico-chemical characteristics of soil used in this experiment. (EC): electrical conductivity; (OC): organic carbon; (Ca): Calcium; (N): Nitrogen; (P): Phosphor; (K): Potassium; (Mg): Magnesium; (Na^+): Sodium.

Parameter	Value
pH	7.32
EC (mmho cm^{-1})	0.52
OC (%)	1.5
Ca (%)	5.4
N (g kg^{-1})	0.12
P (ppm)	19.6
K (ppm)	601
Mg (ppm)	0.6
Na^+ (g kg^{-1})	1.1
Texture	Loamy-Clay

formerly reported (Bisen et al. 1995, Prasad et al. 2011).

The aim of this study was to determine the effects of the mychorrizal fungus *Funneliformis mosseae* (FM) on the growth of *Ziziphus spina-christi* seedlings exposed to different soil salinity levels. Our starting hypothesis was that the FM inoculum could alleviate the effect of salinity stress in *Z. spina-christi* seedlings.

Material and methods

Plant material and AM inoculum

Experimental plants (*Ziziphus spina-christi*) were initially produced from seeds. The mycorrhizal inoculum of *Funneliformis mosseae* (FM, formerly known as *Glomus mosseae*) were originally purchased from the Tarbiat Modares University, Tehran, (Iran), and then propagated by trap culture technique in the rhizosphere of maize (*Zea mays*) roots for 5 months.

Growth conditions and methodology

The experiment was carried out in a forest nursery located in Mehran (western Iran) between February and August 2013 (6 months). The mean temperature was 19.5 °C and precipitation was 245.5 mm. Seeds of *Z. spina-christi* were scarified to overcome hard seed coat dormancy by removing a small portion of the coat at the cotyledon end with nail clippers. The seeds were germinated in a mixture of clay, silt and perlite (2:1:1 v/v - Tab. 1). About 10% (w/w) inoculum of FM was placed in the pots at sowing time. The FM inoculum consisted of soil, spores (50 spores g^{-1} inoculum), hyphae and root fragments. To ensure uniform soil conditions, sterilized inoculum was also added to the control pots (non-mycorrhiza).

Seedling were grown for 5 weeks before being treated with one of four levels of NaCl (0, 50, 100 and 200 mM). The salt was added to the soil with the irrigation water. The soil was salinized step-wise to avoid subjecting the plants to osmotic shock. The NaCl concentration was gradually increased by 25 mM on alternative day to reach the required salinity. The pots were daily weighed to measure water loss, which was replaced with deionized water to avoid percolation and maintain the soil water potential at field capacity.

Determination of growth parameters and colonization

The plants were harvested 6 months after planting and the height and basal diameter of the seedlings were measured. The shoots (leaves and stems) and roots were then oven-dried at 70 °C for 72 h and their dry weight (DW) was calculated at 0.01 g precision (Meloni et al. 2004).

Determination of the percentage of roots colonization was carried out according to the method suggested by Phillips & Hayman (1970). Ten thin fragments of roots each with length of about 1 cm were collected from several seedlings for each treatment. The percentage of colonization (AM%) was determined by the following formula (eqn. 1):

$$AM\ \% = \frac{RL_i}{RL_o} \cdot 100$$

where RL_i and RL_o were the infected and the overall root length, respectively.

Leaf and root nutrient analysis

Physiologically mature leaves and roots were randomly collected from selected seedlings in each treatment. Leaves were pooled, ground finely and sieved through a 40 µm mesh screen. Total nitrogen (N) was measured using the semi-micro Kjeldahl method (Nelson & Sommers 1982). Potassium (K), phosphor (P) and sodium (Na) contents were determined by atomic absorption spectrophotometry (UV/VIS 9000).

Enzyme assays

Fresh matured leaves were detached from seedlings for enzyme measurement (Jin et al. 2012). Some 0.5 g of frozen leaves were ground in liquid nitrogen until a fine powder was obtained. The same method was applied for fine root samples. The powder was extracted using an ice-cold 50 nM phosphate buffer at pH 7.0. The extracts were centrifuged (Rotina 380; Hettich) at 4° C for 20 min at 13000 rpm, and the supernatant was collected for antioxidant enzyme analyses (Mirzaei & Yousefzadeh 2013). The superoxide dismutase (SOD) activity was determined using potassium phosphate (pH = 7.5), Na_2co_3 (pH = 10.2) according to the method described by Giannopolitis & Ries (1977). The peroxidase (POD) activity was determined using the Guaiacol oxidation method (Kar & Mishra 1976). The catalase (CAT) was measured using potassium phosphate (pH = 7.0) and H_2O_2 (Cakmak & Horst 1991). Proline accumulation was determined using ninhydrin and sulfosalicylic acid (Bates et al. 1973).

Leaf chlorophyll content

Semi-mature leaflets (n = 32) were collected from seedlings to measure their chlorophyll content (*a*, *b* and total), which was extracted using 80% acetone (Harborne 1998). The supernatant was quantified with a spectrophotometer at 645 and 663 nm and compared to a blank 80% acetone standard. Chlorophyll content was expressed as mg g^{-1} fresh weight (Al-Khaliel 2010).

Experimental design and statistical analysis

The experiment was performed using a random design with 4 replications on 4 seedlings per treatment. All parameters were analyzed using the analysis of variance (ANOVA). Treatment means were compared using the *post-hoc* Duncan' test at the significance level of 0.05.

Results

Root colonization

Overall, salt stress had a significant effect on colonization of *Z. spina-christi* seedling roots by *Funneliformis mosseae*. By increasing the soil salinity, FM colonization was decreased by 48% to 33%. However, no significant difference was observed between salt concentration in the soil of 100 and 150 mM. The highest level of FM colonization was in the control, while the lowest was in 100 and 150 mM treatments (Fig. 1).

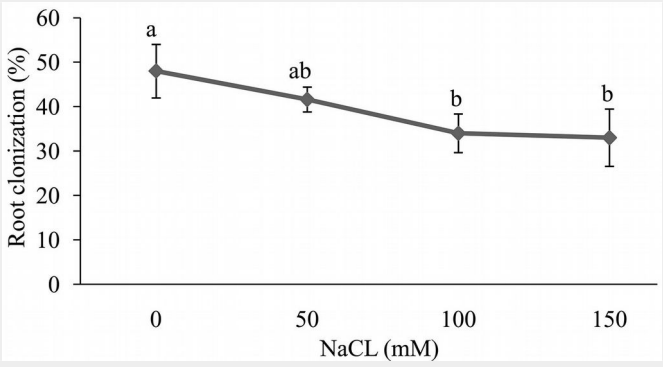

Fig. 1 - Effect of salt stress on root colonization of *Z. spina-christi* seedlings. Different letters indicate significant differences between treatments ($p < 0.05$).

Growth indexes

Tab. 2 shows the effects of FM on *Z. spina-christi* tolerance to salt stress as inferred from the change in growth indexes. Under salt stress seedlings' basal diame-

ters were significantly decreased, with the lowest values shown by seedlings grown at 150 mM soil salinity. At all levels of soil salinity, the basal diameter of FM-inoculated plants was significantly higher than that of non-inoculated seedlings. Likewise, salinity significantly decreased seedlings' height. Contrastingly, no significant difference was detected between the height of inoculated and non-inoculated seedlings at low levels of salinity (0 and 50 mM). The root dry weight (RDW), a proxy of total dry matter, was also decreased under salt stress, while FM-inoculation increased RDW at all levels of salt stress (Tab. 2).

Shoot dry weight (SDW) under salinity was highly decreased, as the highest and lowest levels were in control and 150 mM of salinity, respectively. Moreover, it was observed that SDW of FM-inoculated seedlings was increased as compared with non-mycorrhizal plans. Height values showed that salt-stressed mycorrhizal *Z. spina-christi* had significantly greater SDW than salt-stressed non-mycorrhizal plants. Furthermore, there was an increase in SDW of mycorrhizal plants at all levels of salinity (0, 50, 100 and 150 mM); however, at high levels of salt stress (50 and 100 mM) a severe decrease in SDW in both mycorrhizal and non-mycorrhizal plants was evident. This may be related to the adverse effects of salinity on photosynthesis (Fig. 2).

Tab. 2 - Effects of salt stress on diameter, height and RDW of mycorrhizal and non-mycorrhizal seedlings of *Z. spina-christi*. Means ± standard errors are reported. The signs (+, -) indicate the presence/absence of *Funneliformis mosseae*. Means in the same column followed by the same letter are not significantly different ($p > 0.05$) after the Duncan's *post-hoc* test. (ns): not significant.

Treatment (nM NaCl)	AM inoculation	Basal diameter (mm)	Height (cm)	RDW (g)
0	+ FM	3.93 ± 0.25 ^{a}	28.8 ± 4.5 ^{a}	4.12 ± 0.38 ^{a}
	- FM	1.33 ± 0.25 ^{d}	28.8 ± 4.8 ^{a}	1.52 ± 0.38 ^{cd}
50	+ FM	3.17 ± 0.22 ^{ab}	14.4 ± 1.9 ^{b}	2.76 ± 0.27 ^{b}
	- FM	0.63 ± 0.17 ^{de}	15.4 ± 1.9 ^{b}	0.62 ± 0.24 ^{ef}
100	+ FM	3.05 ± 0.40 ^{b}	12.0 ± 1.4 ^{b}	2.10 ± 0.20 ^{bc}
	- FM	0.61 ± 0.31 ^{de}	7.8 ± 2.9 ^{b}	0.30 ± 0.15 ^{f}
150	+ FM	2.20 ± 0.22 ^{c}	8.8 ± 0.7 ^{b}	1.10 ± 0.22 ^{de}
	- FM	0.20 ± 0.17 ^{e}	7.4 ± 2.0 ^{b}	0.04 ± 0.02 ^{f}
Main effects	NaCl	p<0.001	p<0.001	p<0.001
	AM	p<0.001	ns	p<0.001
Interaction effects	NaCl × AM	ns	ns	ns

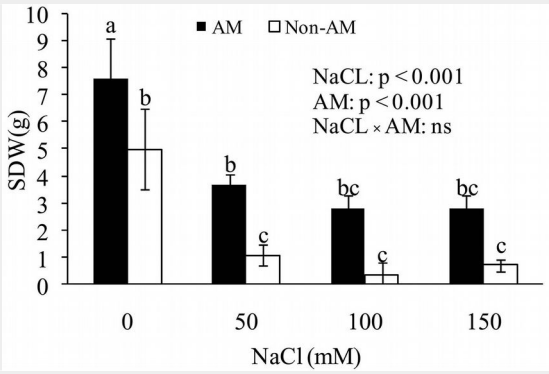

Fig. 2 - Effect of salt stress on shoot dry weight (SDW) of mycorrhizal and non-mycorrhizal seedlings of *Z. spina-christi*. Different letters indicate significant differences between treatments ($p < 0.05$). (ns): not significant.

Tab. 3 - Effect of salt stress on N, P and K absorption of mycorrhizal and non-mycorrhizal seedlings of *Z. spina-christi*. Means ± standard errors are reported. The signs (+, -) indicate the presence/absence of *Funneliformis mosseae*. Means in the same column followed by the same letter are not significantly different ($p > 0.05$) after the Duncan's *post-hoc* test. (ns): not significant.

Treatment (mM NaCl)	AM inoculation	N (gr kg^{-1})		P (ppm)		K (ppm)	
		Leaf	Root	Leaf	Root	Leaf	Root
0	+ FM	3.92 ± 0.29 ^{a}	2.82 ± 0.53 ^{a}	3.85 ± 0.47 ^{a}	4.11 ± 0.24 ^{a}	3.09 ± 1.02 ^{a}	2.98 ± 0.83 ^{a}
	- FM	2.72 ± 0.27 ^{b}	2.02 ± 0.36 ^{a}	3.05 ± 0.47 ^{ab}	3.31 ± 0.24 ^{ab}	2.31 ± 1.01 ^{a}	2.18 ± 0.83 ^{ab}
50	+ FM	2.94 ± 0.28 ^{b}	1.48 ± 0.10 ^{a}	3.52 ± 0.30 ^{a}	3.05 ± 0.12 ^{abc}	2.76 ± 0.68 ^{a}	2.59 ± 0.40 ^{a}
	- FM	2.74 ± 0.28 ^{b}	1.28 ± 0.10 ^{a}	2.72 ± 0.30 ^{b}	2.25 ± 0.12 ^{bcd}	1.96 ± 0.68 ^{a}	1.80 ± 0.40 ^{ab}
100	+ FM	2.42 ± 0.17 ^{b}	1.50 ± 0.23 ^{a}	2.12 ± 0.38 ^{b}	1.93 ± 0.27 ^{cd}	2.50 ± 1.42 ^{a}	1.60 ± 0.33 ^{ab}
	- FM	2.32 ± 0.20 ^{bc}	1.62 ± 0.24 ^{a}	1.32 ± 0.38 ^{bc}	1.13 ± 0.27 ^{d}	1.74 ± 0.41 ^{a}	0.80 ± 0.32 ^{b}
150	+ FM	2.20 ± 0.27 ^{bc}	1.78 ± 0.39 ^{a}	0.98 ± 0.17 ^{c}	1.85 ± 0.71 ^{d}	1.19 ± 0.28 ^{a}	1.44 ± 0.09 ^{ab}
	- FM	1.60 ± 0.23 ^{c}	1.58 ± 0.39 ^{a}	0.28 ± 0.12 ^{c}	1.26 ± 0.60 ^{d}	0.45 ± 0.25 ^{a}	0.64 ± 0.09 ^{b}
Main effects	NaCl	p < 0.001	ns	p < 0.001	p < 0.001	ns	p < 0.001
	AM	p < 0.001	ns	p < 0.001	p < 0.001	ns	p < 0.05
Interaction effects	NaCl × AM	ns	ns	ns	ns	ns	ns

Tab. 4 - Effect of salt stress on Na^+ absorption and chlorophyll (*a* and *b*) on mycorrhizal and non-mycorrhizal seedlings of *Z. spina-christi*. Means ± standard errors are reported. The signs (+, -) indicate the presence/absence of *Funneliformis mosseae*. Means in the same column followed by the same letter are not significantly different ($p > 0.05$) after the Duncan's *post-hoc* test. (ns): not significant.

Treatment (mM NaCl)	AM inoculation	Na^+ (g kg^{-1})		Chlorophyll (Mg g^{-1} fresh weight)	
		Leaf	Root	*a*	*b*
0	+ FM	22.90 ± 4.19 ^{c}	16.56 ± 3.08 ^{c}	3.02 ± 0.39 ^{a}	0.43 ± 0.10 ^{a}
	- FM	22.90 ± 4.19 ^{c}	22.80 ± 3.08 ^{c}	1.62 ± 0.19 ^{bc}	0.15 ± 0.08 ^{a}
50	+ FM	37.26 ± 2.58 ^{bc}	34.40 ± 2.40 ^{b}	1.68 ± 0.18 ^{bc}	0.27 ± 0.06 ^{a}
	- FM	43.50 ± 2.58 ^{ab}	39.74 ± 2.40 ^{ab}	1.14 ± 0.09 ^{c}	0.19 ± 0.07 ^{a}
100	+ FM	36.10 ± 3.58 ^{bc}	32.80 ± 3.33 ^{b}	1.38 ± 0.18 ^{c}	0.25 ± 0.14 ^{a}
	- FM	42.40 ± 3.58 ^{ab}	39.10 ± 3.33 ^{ab}	1.42 ± 0.24 ^{c}	0.17 ± 0.14 ^{a}
150	+ FM	45.60 ± 3.41 ^{ab}	40.50 ± 4.26 ^{ab}	2.38 ± 0.60 ^{ab}	0.11 ± 0.02 ^{a}
	- FM	51.90 ± 3.41 ^{a}	46.82 ± 4.26 ^{a}	0.98 ± 0.25 ^{c}	0.02 ± 0.02 ^{a}
Main effects	NaCL	p < 0.001	p < 0.001	p < 0.05	ns
	AM	p < 0.05	p < 0.05	p < 0.001	ns
Interaction effects	NaCl × AM	ns	ns	ns	ns

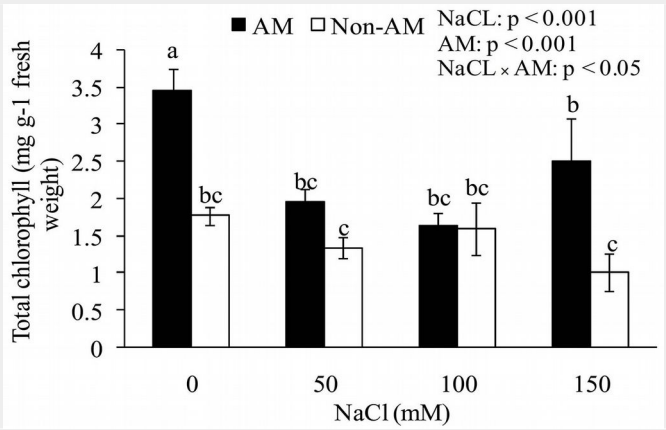

Fig. 3 - Effects of salt stress on total chlorophyll of mycorrhizal and non-mycorrhizal seedlings of *Z. spina-christi*. Different letters indicate significant differences between treatments ($p < 0.05$).

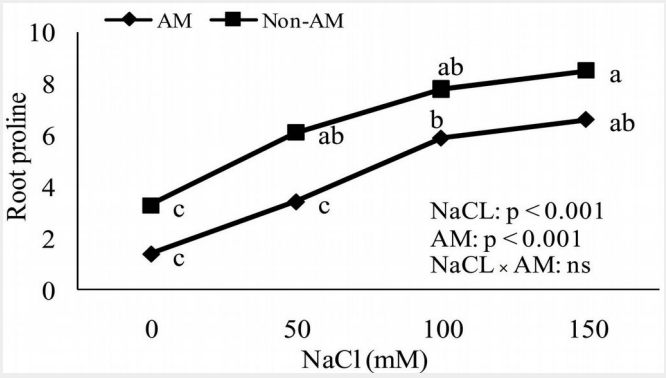

Fig. 4 - Effect of salt stress on proline accumulation in roots of mycorrhizal and non-mycorrhizal seedlings of *Z. Spina-christi*. Different letters indicate significant differences between treatments ($p < 0.05$). (ns): not significant.

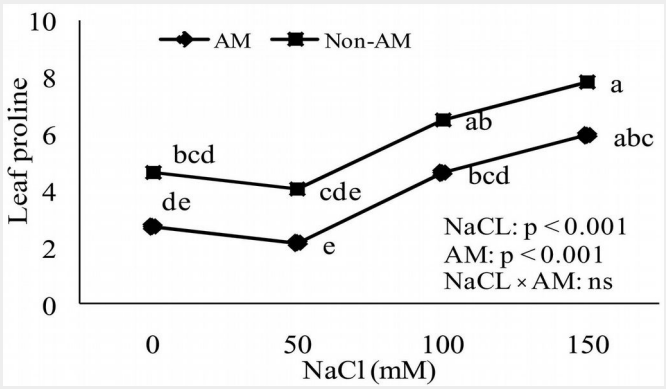

Fig. 5 - Effect of salt stress on proline accumulation in leaves of mycorrhizal and non-mycorrhizal seedlings of *Z. spina-christi*. Different letters indicate significant differences between treatments ($p < 0.05$). (ns): not significant.

N, P, K, and Na⁺ concentrations

Leaf N significantly decreased by increasing NaCl concentration in the soil for both mycorrhizal and non-mycorrhizal plants. The FM inoculation increased the N absorption in leaves of seedlings grown at the lowest salinity by 44.1%, while such increase was 37.5% for seedlings grown at the highest salinity treatment. Contrastingly, no significant differences were observed in N absorption in the roots in seedlings grown under any soil salinity and FM treatments (Tab. 3).

In general, a decreased in the P content of leaves and roots of seedlings grown under salinity stress was observed. However, the P content in leaves and roots increased respectively by 26.2% and 24.2% in FM-inoculated seedlings grown under the lowest salinity treatment, as compared to non-mychorrizal plants, while at the highest salinity treatment such increase was 28.0% in leaves and 20.8% in roots.

Concerning K content, salt stress and FM inoculation had no significant effects on seedling leaves. On the contrary, soil salinity decreased the K absorption in the roots. However, as compared with non-inoculated plants, mychorrizal seedlings showed an increase of K in the roots by 36.7% at the lowest and by 125.0% at the highest salinity treatments (Tab. 3).

Tab. 4 shows that the Na^+ content increased in the leaves and roots of both mycorrhizal and non-mycorrhizal plants as the NaCl concentration in the soil increased. At all levels of soil salinity, the Na^+ content in the leaves and roots of FM-inoculated seedlings was lower than that observed for non-inoculated seedlings.

Chlorophyll content

Total chlorophyll and chlorophyll *a* in mycorrhizal plants were significantly higher than in non-mycorrhizal plants at 0, 50, and 150 mM NaCl (Tab. 4, Fig. 3). In addition, soil salinity levels and the interaction between salinity and the fungus had significant effect on the total chlorophyll content. In other words, by increasing the salinity in the soil the total chlorophyll content of seedling leaves decreased. There were no significant differences between FM-inoculated and non-inoculated plants as for chlorophyll *b*. Similarly, the salt stress

Tab. 5 - Effect of salt stress on catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) of mycorrhizal and non-mycorrhizal seedlings of *Z. spina-christi*. Means ± standard errors are reported. (+, -): presence/absence of *Funneliformis mosseae*. Means in the same column followed by the same letter are not significantly different ($p > 0.05$) after the Duncan's *post-hoc* test. (ns): not significant.

Treatment (mM NaCl)	FM inoculation	CAT (U mg^{-1} protein)		POD (U mg^{-1} protein)		SOD (U mg^{-1} protein)	
		Leaf	Root	Leaf	Root	Leaf	Root
0	+ FM	5.31 ± 1.70 ^{a}	9.95 ± 2.73 ^{c}	6.19 ± 1.50 ^{b}	2.75 ± 1.73 ^{c}	0.98 ± 0.23 ^{d}	1.57 ± 0.54 ^{c}
	- FM	7.21 ± 1.70 ^{a}	11.85 ± 2.73 ^{c}	8.09 ± 1.50 ^{b}	2.75 ± 3.60 ^{c}	2.88 ± 0.23 ^{bcd}	3.47 ± 0.54 ^{bc}
50	+ FM	7.56 ± 2.90 ^{a}	14.60 ± 3.70 ^{bc}	6.61 ± 2.10 ^{b}	6.16 ± 1.82 ^{bc}	2.11 ± 0.41 ^{cd}	2.34 ± 0.73 ^{bc}
	- FM	11.46 ± 2.40 ^{a}	18.90 ± 3.46 ^{bc}	8.51 ± 2.10 ^{b}	6.16 ± 3.70 ^{bc}	4.41 ± 0.51^{abc}	4.24 ± 0.73 ^{ab}
100	+ FM	15.36 ± 7.20 ^{a}	11.02 ± 2.25 ^{c}	15.20 ± 4.90 ^{ab}	2.84 ± 0.46 ^{c}	3.28 ± 1.08 ^{bcd}	3.05 ± 0.66 ^{bc}
	- FM	19.06 ± 7.70 ^{a}	14.90 ± 2.37 ^{bc}	17.10 ± 4.90 ^{ab}	2.84 ± 0.65 ^{c}	5.18 ± 1.08 ^{ab}	4.95 ± 0.66 ^{ab}
150	+ FM	12.57 ± 2.60 ^{a}	27.10 ± 5.70 ^{b}	15.98 ± 3.90 ^{ab}	10.93 ± 5.80 ^{ab}	3.07 ± 0.76 ^{bcd}	4.63 ± 1.25 ^{ab}
	- FM	22.47 ± 8.90 ^{a}	40.90 ± 7.11 ^{a}	21.80 ± 5.20 ^{a}	10.93 ± 7.70 ^{a}	6.17 ± 0.85 ^{a}	6.78 ± 1.19 ^{a}
Main effects	NaCl	ns	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001
	AM	ns	ns	ns	ns	p < 0.001	p < 0.001
Interaction effects	NaCl × AM	ns	ns	ns	ns	ns	ns

showed no significant effect on chlorophyll *b* content (Tab. 4).

Enzyme activity assessment

The levels of POD, SOD and CAT antioxidant enzymes in inoculated plants exposed to salt stress were lower than for non-inoculated plants. Salinity of the soil was observed to increase the SOD, POD and CAT activity (Tab. 5). In comparison to non-mycorrhizal plants, the activity of SOD enzymes decreased in mycorrhizal plants. In addition, FM inoculation did not significantly affect the activity of CAT and POD enzymes in both roots and leaves of *Z. spina-christi* seedlings.

Proline accumulation

Accumulation of proline in non-mycorrhizal and mycorrhizal plants increased as soil salinity increased. The proline accumulation in leaves and roots of non-mycorrhizal plants increased significantly compared to mycorrhizal plants at all levels of soil salinity (Fig. 4, Fig. 5).

Discussion

The results of this study indicate that FM inoculation markedly improved the growth characteristics of *Z. spina-christi* seedlings under salt stress. Tian et al. (2004) demonstrated that inoculation with FM fungi could improve growth of cotton plants under a variety of salt stress conditions. In the present investigation, the tolerance of FM-inoculated plants to salt stress increased compared to non-mychorrizal seedlings, as demonstrated by the increase of fresh weight and other parameters, including the level of colonization, basal diameter and RDW. Indeed, the abundance of fungus hyphae around the host roots may help absorbing poorly mobile nutrients such as P in the depletion zone of roots. These nutrients are transported into the host plants, resulting in the improvement of seedling growth (Okurowska 2008).

According to Beltrano et al. (2013), we found that the FM colonization of *Z. spina-christi* roots was inversely correlated to NaCl concentration in the soil (Fig. 1). This decrease in the fungus colonization under salt stress may be due to a reduced germination of fungal spores (Van Aarle et al. 2002, Al-Khaliel 2010).

The beneficial effects of mycorrhiza on growth under saline conditions have been studied in various plant species and families (Al-Khaliel 2010, Evelin & Kapoor 2013). In the present study, when the plants were exposed to high concentrations of NaCl in the soil, seedling SDW substantially decreased regardless the presence or absence of mycorrhizal fungi (Fig. 2). The main reasons for the detrimental effects of salinity may be related to the negative osmotic pressure created by salt in the root zone (Jacoby 1994) or to growth inhibition caused by cell injury in transpiring leaves (Tuteja 2007).

In general, FM helped to partially alleviate NaCl stress; this was evident in the growth of inoculated plants compared to non-inoculated plants. The beneficial effect of FM symbiosis on plant growth has been largely attributed to the higher uptake of phosphorus (Moyersoen et al. 1998). In the present study, plants inoculated with FM showed higher P contents at all salinity levels, primarily in the roots (Tab. 3). This suggests that the effect of FM on P uptake constitutes a major mechanism for increasing plant tolerance to salinity.

In this study, the Na^+ concentrations in mycorrhizal seedlings were significantly smaller than in non-mycorrhizal plants. Low Na^+ concentration in leaves and roots of mycorrhizal plants may be due to positive effect of FM fungus on water absorption. Previous studies have also indicated that FM fungi increase plant growth by reducing Na^+ uptake (Tian et al. 2004, Al-Karaki 2006) and increasing the uptake of other nutrients such as P, K, and N (Al-Karaki 2006, Daei et al. 2009).

The results shown in Fig. 3 indicate that the total chlorophyll and chlorophyll *a* contents increased in the leaves of FM-inoculated plants as compared with non-mychorrizal plants. However, at 100 mM salinity the chlorophyll contents were at very close range and showed no statistically significant difference ($p > 0.05$). The higher chlorophyll content of FM-inoculated seedlings may reflect the higher photosynthetic rate necessary to support the carbon cost of association with the fungus (Wright et al. 1998). The increased photosynthesis in FM plants may be mediated by the increased P nutrition, as evidenced by increased plant growth. At higher NaCl concentrations in the soil, the total chlorophyll content decreased (Fig. 3). It has previously been reported that salinity decreased chlorophyll content (Singh et al. 2000); therefore, high levels of NaCl can decrease the chlorophyll content of leaves.

On the other hand, salt stress also enhanced the SOD and POD activity in roots and leaves and CAT activity just in roots of *Z. spina-christi* seedlings. It is well known that these enzymes represent an effective mechanism for preventing the negative effects of reactive oxygen species (ROS) under salinity stress (Mirzaei & Yousefzadeh 2013). In addition, if the stress lasts for a long time, these enzymes will negatively influence the plant (Abdel Latef & Miransari 2014). FM inoculation acts as a preventive mechanism by decreasing SOD in leaves and roots, thus favoring the avoidance of oxidative damage induced by salt stress (Hildebrandt et al. 2007). Finally, this leads to survive the plant under salt stress (Ouziad et al. 2005).

Proline accumulation is a symptom of stress in less salt-tolerant plants. Proline plays multiple roles in stress tolerance as a mediator of osmotic adjustment (Yoshiba et al. 1997). It also protects macromolecules during dehydration (Sanchez et al. 1998). In the present study, both salt-stressed mycorrhizal and non-mycorrhizal *Z. spina-christi* accumulated free proline (Fig. 4, Fig. 5). The increase in free proline in salt-stressed non-mycorrhizal plants was significantly higher than in inoculated plants at all levels of salinity. This suggests that FM inoculation may favor osmotic adjustments in seedlings by promoting the synthesis of solutes such as proline.

Conclusion

This study focused on the effects of the mychorrizal fungus *Funneliformis mossae* (FM) on the growth of *Ziziphus spina-christi* seedlings under different levels of soil salinity. The results showed that FM inoculation improved the tolerance of plants to salt stress, alleviated the detrimental effects of salinity on growth and improved the nutrition uptake, as evidenced by the higher K, P, N and lower Na^+ concentrations in leaf tissues. The use of FM-inoculated seedlings is a sustainable and environmentally safe treatment to improve tolerance to salinity in *Ziziphus spina-christi* seedings. Therefore, root inoculation and colonization by FM can be recommended as an effective strategy to alleviate the deleterious effects of salt stress.

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