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Arbuscular mycorrhizal (*Funneliformis mosseae*) improves alfalfa (*Medicago sativa* L.) re-growth ability in saline soil through enhanced nitrogen remobilization and improved nutritional balance

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Abstract

In current study, the influence of arbuscular mycorrhizal fungi (AMF) on salinity tolerance in terms of root's reserves remobilization to shoot and its relationship with re-growth ability and ionic status of alfalfa (*Medicago sativa* L.) plants were investigated. In a pot experiment, a factorial experiment in base of randomized complete blocks design in three replications was carried out. Alfalfa plants (Iranian cultivar-Baghdadi) inoculated with AMF (*Funneliformis mosseae*) or retained as un-inoculated, were grown in soil and irrigated with three salt concentrations including 1.4 (control), 7 and 12 dS/m. Three harvests were carried out at 10% of flowering stage. AMF inoculation increased the size of root sugars and soluble N pools at harvest time. The shoot biomass production following harvest had a close correlation with nitrogen (N) remobilization from root (r=0.92, P≤0.01). However salinity stress significantly reduced amount and percentage of N remobilization to re-growing shoot but AMF plants exhibited greater amount and percentage of root N pools dedicated to remobilization. AMF inoculation also affected ionic relations of plants as AM+ plants contained greater K+ within both root and shoot organs while Ca^{2+} and Na^{+} were affected by AMF only within shoot tissue. AMF plants exhibited higher K^{+}/Na^{+} within shoot and Ca^{+2}/Na^{+} within root organs. There was a high positive correlation coefficient between K^{+}/Na^{+}, Ca^{+2}/Na^{+} ratios and N remobilization from root (respectively, r=0.92, 0.88; P≤0.01). To sum up, ionic status within both root and shoot organs, got more balanced by AMF inoculation so that AMF reduced limitations within both source (root) and sink (re-growing shoot) organs concerning N remobilization to re-growing shoot.

Keywords: alfalfa, defoliation, ionic status, mycorrhiza, nitrogen remobilization, root reserves, salinity, shoot re-growth, soluble sugars

Introduction

Soil salinity is one of the environmental stresses that considerably restricts crops yield (Flowers, 2004). Almost 1,000,000,000 ha of lands (7% of all land area) are affected by soil salinity around the world (Pessarakli, 1994) and the possibility of irrigation by saline water in arid and semi-arid regions requires a better understanding of plant salt tolerance. Osmotic stress and ion toxicity are two main harmful aspect of salinity stress (Munns and Tester, 2008). Legumes and specially alfalfa (*Medicago sativa* L.) are proper crops for the enrichment of bio-productivity and for the restoring of marginal lands. These plants not only produce beneficial fodder, but also enrich soil nitrogen through a symbiotic association with *Rhizobium* spp. bacteria (Garg and Singla, 2004). Alfalfa has been known as a moderately tolerant crop to salinity (Noble et al., 1984).

Rapid re-growth ability after harvest makes alfalfa interested among other herbaceous species. In the last years it is well established that N reserves play a vital role in regrowth of perennial herbages following defoliation (Skinner et al., 1999). Alfalfa regrowth following defoliation also strongly depends on N remobilization from roots to re-growing shoots (Meuriot et al., 2005).

Microorganisms vary in their ability to overcome the unfavorable influences of increased salt. Specific bacteria or symbiotic eukaryotes could have the potential to ameliorate salt tolerance in plants. Among all fungi, arbuscular mycorrhizal fungi (AMF) have a considerable importance in this subject, since it has been reported to have numerous effects on plant growth and development (Van der Heijden and Sanders, 2002). Legumes species are able to form dual symbiosis with both AM fungi and *Rhizobium* spp. (Barea et al., 2002) and this dual symbiosis has great ecological and agricultural advantages regarding sustainable agriculture (Jeffries and Barea, 2001). Many studies have demonstrated that inoculation with arbuscular mycorrhizal fungi (AMF) improves growth of plants under salt stress (Giri and Mukerji, 2004; Al-Karaki, 2006; Cho et al., 2006).

Improved AM plants growth under stress condition is attributed to numerous mechanisms; more efficient osmotic adjustment (Kubikova et al., 2001), enhancement of plant gas exchange, water use efficiency (Ruiz-Lozano and Azcón, 1995) and increased mineral nutrition acquire (Wu et al., 2010). The symbiotic association between AMF and roots makes a significant contribution to plant growth and nutrition. Enhanced mineral nutrition uptake by mycorrhizal plants has been attributed to hyphae growing beyond the rhizosphere soil which may increase the absorptive surface of the root (Al-Karaki, 2000).

As pointed out, alfalfa re-growth following harvest depends on remobilization of root nitrogen reserves to shoot. So, limitation in alfalfa yield under saline condition may result from limited nitrogen availability and remobilization. In current study, the possibility of specific influence of AMF on formation of root N and sugars reserves and remobilization process under saline conditions was investigated. Since the

harmful effects of NaCl mainly result from excessive toxic ions, changes in ion relations by AMF also were studied.

Materials and methods

The experiment was carried out from 21th of April to 27th of August, 2016 in Meymeh (33.4476° N, 51.1716° E), Iran. In a greenhouse experiment, Iranian semi tolerant cultivar (Baghdadi) was exposed to NaCl salinity (three levels including 1.4, 7 and 12 dS/m) and arbuscular mycorrhizal fungus inoculation (inoculated plants and non-inoculated plants) treatments using a factorial experiment in base of completely randomized blocks design in three replications. Plants were grown in plastic pots, containing 3.5 kg of soil comprising a mixture of clay, farmyard manure and sand in the ratio of 2:2:1, respectively. The basic soil properties were as follows: total N 0.89 mg/g, total K 159 mg/kg, total P 16.9 mg/kg, available P ($NaHCO_3$-extractable) 5.28 mg/kg, electrical conductivity 2 dS/m and pH 6.9. Supplementary light in the greenhouse was provided for 16 hours per day. The day/night temperatures were 26/18 °C. Three levels of salinity based on electrical conductivity including 1.4 (tap water), 7 and 12 dS/m were exerted and included in irrigation water through the use of tap water with or without sodium chloride. The salinity treatments were started 16 days after sowing when the plants were at the early vegetative stage. Saline irrigation (in 7 and 12 dS/m was started with conductivity of 2 dS/m and increased gradually up to 12 dS/m during 10 days to avoid of incurring salt shock to plants.

Three harvests were carried out at the 10% flowering stage. The averages of three replications within each harvest were used for statistical analysis (statistical analysis was carried out on obtained data from harvests 1, 2 and 3). The plants were defoliated at each harvest and 10 days later. The first harvest was carried out 62 days after sowing and two subsequent harvests were carried out almost 1 month later.

In order to determine the nitrogen remobilization the method of Kim et al. (1993) was performed. Briefly, the plants within 2 pots in each plot were defoliated respectively at harvest time (10% flowering) and 10 days later (considered as second defoliation). The samples then were washed with distilled water and weighed after being dried in oven at 70 °C for 48 h. Amount of root N remobilization from root to shoot was calculated as: Root soluble N content at first defoliation (10% flowering stage or harvest time) – Root soluble N content 10 days after first defoliation (second defoliation). This process was repeated for harvests 2 and 3 too. Percentage of N remobilization was also calculated as: Amount of remobilized N*100 divided by root soluble N content at first defoliation (10% flowering or harvest time). Determination of total (soluble plus insoluble) and soluble nitrogen concentration was carried out by using the Kjeldahl analysis procedure. The separation of buffer soluble and insoluble nitrogen (within root) was carried out as described by Barber et al. (1996). Nitrogen content within each organ was also determined as N concentration (per gram dry weight) × dry weight of organ. Contribution of remobilized N to nitrogen accumulation within shoot also determined as: amount of soluble N lost from roots between two sequential of defoliation / the accumulated N within shoots over the same time. The same method (as described for nitrogen) was used to determine the amount and percentage of sugars remobilization to re-growing shoots. Root's sugar content (per

plant) was determined by the anthrone sulphuric acid method as described by Badour (1959). Briefly, the dried roots were extracted by HCl. One cm^3 of the obtained extract was mixed with 9 cm^3 of anthrone sulphuric acid reagent and heated for 7 min at 100 °C. The absorbance was recorded at 620 nm by spectrophotometer (model: Cintra 6 GBC) and reported as root's sugar concentration. Root's sugar content was determined as root's sugar concentration × root's dry weight.

Arbuscular mycorrhizal fungal inoculum was provided by the Institute of Soil and Water Research, Karaj, Iran. The used AM fungus species was *Funneliformis mosseae* and was used at 5 gram pot-1 consisting of soil, spores (10 spores per 1 g), mycelia and infected root fragments. Five milliliters of inoculum filtrate containing the microbial population was obtained by suspending 100 g mycorrhizal inoculum from *F. mosseae* in 600 ml sterile water. The suspension was filtered after shaking and decanting.

In order to measure the K^+, Na^+ and Ca^{+2} within plant organs, the dried plant materials were digested in 5 mL of a mixture of H_2SO_4 and $HClO_4$ (9:1) and diluted to the desired volume then K^+, Na^+ and Ca^{+2} concentrations were estimated by using flame photometry. Concentration of each ions was determined by using standard curves obtained from stock solutions for Na, K and Ca.

Analysis of variance (ANOVA) for all data was carried out by using the GLM procedure in SAS. The mean comparisons were made following Duncan's multiple range test ($P \leq 0.05$). The correlation coefficients were calculated by the SPSS statistical package (version 19).

Results

Biomass production and nitrogen status

Table 1 summarizes the results of analysis of variance for shoot and root biomass production. AMF inoculation had a significant effect on biomass production. AM+ plants exhibited higher root and shoot biomass (Figure 2). Shoot and root biomass decreased as salinity level increased (Figure 1). Root soluble N content was significantly decreased by salinity stress (Table 2). Furthermore, AM+ plants contained greater amount of root soluble N content at harvest time (Table 3). Amount of N remobilization from root to shoot following harvest was decreased by increasing salinity (Table 4). AMF inoculation noticeably offset reduction in N remobilization caused by salinity. AM+ plants exhibited greater N remobilization from root under all levels of salinity (Table 4). However portion of N remobilization from root N pools to re-growing shoot was reduced by salinity within both AM+ and AM- plants, this portion was considerably higher in AM+ plants (Table 3). Contribution of remobilized root N reserves in N accumulation within shoot following harvest was defined through comparing accumulated N within shoots between two sequential of defoliation within each harvest (harvest time and 10 days after) with disappeared N from roots at the same time. This parameter increased under higher levels of salinity and was higher in AM- plants (Table 4).

Root sugars content and sugars remobilization (Disappeared from root)

Root sugars content and remobilization increased when salinity level reached to 7 dS/m while under more sever salinity level (12 dS/m), both root sugars content and remobilization declined (Table 2). AMF inoculation induced significant increase in root's sugars content and remobilization as AM+ plants exhibited greater root's sugars content and remobilization from root (Table 3).

Ionic status

Generally, Na^+ concentration of root and shoot tissues increased when salinity level increased from 1.4 to 12 dS/m whereas K^+ and Ca^{2+} concentrations reduced at the same time. AMF affected ionic status of different plant organs in different ways. Root Na^+ concentration wasn't influenced by AMF (Table 3) while AM+ plants exhibited significantly lower Na^+ concentration within shoot (Table 4). AM+ plants contained significantly higher concentration of K^+ and K^+/Na^+ ratio within both root and shoot organs (Table 6). In contrast with Na^+, the differences between AM+ and AM- plants regarding Ca^{2+} concentration was more considerable within root tissue when compared with shoot. AM+ plants exhibited higher root Ca^{2+} concentration at all salinity levels whereas only under severe salinity stress, AM+ plants contained higher shoot Ca^{2+} concentration (Table 7). K^+/Na^+ and Ca^{2+}/Na^+ ratios within both root and shoot tissues significantly reduced at more sever levels of salinity (Table 8) yet AMF inoculation positively affected these ratios as K^+/Na^+, Ca^{2+}/Na^+ ratios were greater in AM+ plants (Table 6).

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Table 1a. Summary of analysis of variance for the traits investigated in response of alfalfa plants to AMF inoculation under saline condition

	RDW[a]	SDW[b]	RSN[c]	RNC10[d]	NR[e]	PNR[f]	SNC10[g]	SNFR[h]	SN Concentration[i]	RSC[j]	DSR[k]	PDS[l]
Salinity	**	**	**	*	**	**	**	**	**	**	**	**
AMF status	**	**	**	**	**	**	**	*	**	**	**	ns
AMF×Salinity	ns	ns	ns	**	**	ns	**	*	**	ns	ns	ns

[a]Root dry weight, [b]Shoot dry weight, [c]Root soluble nitrogen at harvest time, [d]Root soluble N content 10 days after harvest, [e]Nitrogen remobilization, [f]Percent of N remobilization, [g]Shoot N content 10 days after harvest, [h]Shoot N received from remobilization, [i]Shoot N concentration at harvest time, [j]Root sugars content at harvest time. [k]Disappeared sugars from root 10 days after harvest, [l]Percent of disappeared sugars from root during 10 days after harvest. [**]Significant at (P≤0.01),[*]Significant at (P≤0.05), [ns]Non-significant.

Table 1b. Summary of analysis of variance for the traits investigated in response of alfalfa plants to AMF inoculation under saline condition

	Root					Shoot				
	K	Na	K/Na	Ca	Ca/Na	K	N	K/Na	Ca	Ca/Na
Salinity	**	**	**	**	**	**	**	**	**	**
AMF status	**	ns	*	**	*	**	**	**	**	*
AMF×Salinity	ns	ns	ns	**	ns	*	*	ns	*	ns

[a]Root dry weight, [b]Shoot dry weight, [c]Root soluble nitrogen at harvest time, [d]Root soluble N content 10 days after harvest, [e]Nitrogen remobilization, [f]Percent of N remobilization, [g]Shoot N content 10 days after harvest, [h]Shoot N received from remobilization, [i]Shoot N concentration at harvest time, [j]Root sugars content at harvest time. [k]Disappeared sugars from root 10 days after harvest, [l]Percent of disappeared sugars from root during 10 days after harvest. [**]Significant at (P≤0.01),[*]Significant at (P≤0.05), [ns]Non-significant.

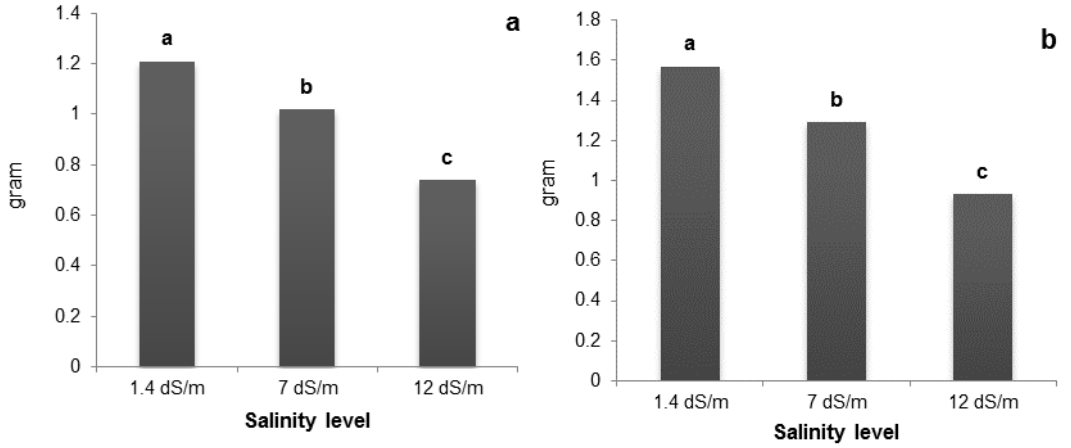

Figure 1. Effect of different salinity levels on root (a) and shoot (b) dry weight at harvest time (10% flowering). The values are means of three sequential harvests (three replications within each harvest). The same letters indicate non-significant difference between compared means at P≤0.05

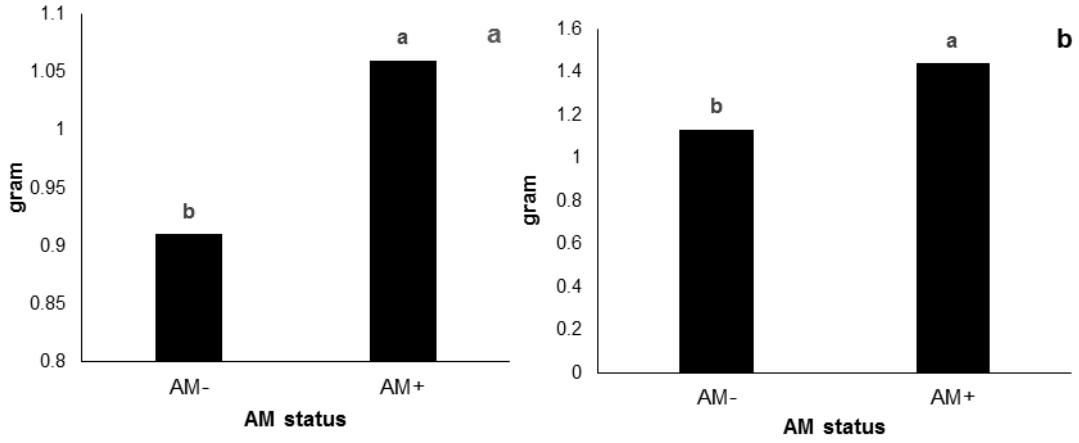

Figure 2. Effect of AMF inoculation on root (a) and shoot (b) dry weight at harvest time (10% flowering). The values are means of three sequential harvests (three replications within each harvest). The same letters indicate non-significant difference between compared means at P≤0.05

Table 2. Effects of salinity levels on nitrogen and sugar accumulation and partitioning in Alfalfa plants at harvest time (10% flowering) and 10 days after. Mean values followed by the same letters do not differ significantly according to Duncan's multiple range test (P≤0.05)

Salinity level (dS/m)	RSN	Changes[a] (%)	PNR	Changes (%)	RSC	Changes (%)	DSR	Changes (%)	PDSR	Changes (%)
1.4	15.7a		76.4a		118a		58.7b		49.3a	
7	10.45b	-33	77.6a	1.6	130a	11	80.8 a	38	61.9b	25
12	6.71c	-57	59.3b	-22	66b	-44	55.1b	-6	71.4c	45

[a]Percent of changes compared with non-saline condition (1.4 dS/m).

Table 3. Effects of AMF inoculation (*Funneliformis mosseae*) on nitrogen and sugar accumulation and partitioning in Alfalfa plants at harvest time (10% flowering) and 10 days after. Mean values followed by the same letters do not differ significantly according to Duncan's multiple range test (P≤0.05)

AMF status	RSN	Changes (%)[a]	PNR	Changes (%)	RSC	Changes (%)	DSR	Changes (%)	PDSR	Changes (%)
AM-	9.5b		60.7b		91b		52.5b		60a	
AM+	12.4a	31	81.5a	34	126a	39	77.3a	47	62a	3

[a]Percentage of changes compared with AM-plants.

Table 4. Effects of salinity and AMF inoculation (*Funneliformis mosseae*) on nitrogen accumulation and partitioning in alfalfa plants at harvest time (10% flowering) and 10 days after. Mean values followed by the same letters do not differ significantly according to Duncan's multiple range test (P≤0.05)

AMF status	Salinity level (dS/m)	RSNC 10 (mg/plant)	Changes (%)	NR (mg/plant)	Changes (%)	SNC 10 (mg/plant)	Changes (%)	SNFR (%)	Changes (%)	SN concentration (mg*g/DW)	Changes (%)
	1.4	5.51a		9.14c		15.19b		61.3c		26.93b	
AM-	7	2.94b	-47	5.9d	-35	7.04c	-54	84.2b	37	19.4d	-28
	12	2.32b	-58	2.46e	-73	2.81d	-81	88.1ab	44	12.2e	-54
	1.4	1.69b		14.89a		28.46a		52.3d		31.08a	
AM+	7	1.52b	-10	10.54b	-29	16.59b	-41	63.7c	22	21.4c	-21
	12	2.88b	70	5.75d	-61	6.24c	-78	92a	76	17.42d	-44

Table 5. Effects of salinity levels on ionic status of alfalfa roots at harvest time (10% flowering). Mean values followed by the same letters do not differ significantly according to Duncan's multiple range test (P≤0.05)

Salinity level (dS/m)	R Na^+ (mg*g/DW)	Changes (%)	R K (mg*g/DW)	Changes (%)	R K^+/Na^+	Changes (%)	R Ca^{2+}/Na^+	Changes (%)	S K^+/Na^+	Changes (%)	S Ca^{2+}/Na^+	Changes (%)
1.4	1.52a		9.23a		6.28a		7.68a		21.1a		10.3a	
7	2.44b	61	7.88b	-15	3.33b	-47	4.43b	-42	10.53b	-50	5.65b	-45
12	2.97c	95	5.61c	-39	1.95c	-69	3.16c	-59	4.94c	-76	2.76c	-73

Table 6. Effects of AMF inoculation (*Funneliformis mosseae*) on ionic status of alfalfa roots at harvest time (10% flowering). Mean values followed by the same letters do not differ significantly according to Duncan's multiple range test (P≤0.05)

AMF status	R Na (mg*g/DW)	Changes (%)	R K (mg*g/DW)	Changes (%)	R K/Na	Changes (%)	R Ca/Na	Changes (%)	S K/Na	Changes (%)	S Ca/Na^{+}	Changes (%)
AM-	2.41a		6.49b		3.21b		4.54b		9.86b		5.54b	
AM+	2.2a	-9	8.66a	33	4.49a	40	6.64a	46	14.5a	47	6.93a	26

Table 7. Effects of salinity and AMF inoculation (*Funneliformis mosseae*) on ionic status of alfalfa roots at harvest time (10% flowering). Mean values followed by the same letters do not differ significantly according to Duncan's multiple range test (P≤0.05)

AMF status	Salinity level (dS/m)	Ca (mg*g/DW)	Changes (%)	S K (mg*g/DW)	Changes (%)	S Ca (mg*g/DW)	Changes (%)	S Na (mg*g/DW)	Changes (%)
AM-	1.4	10.64bc		22.1b		11.39ab		1.16a	
	7	10.16c	-5	17.67c	-20	10.77b	-5	2.28c	96
	12	7.82d	-27	9.81d	-56	7.18d	-37	3.62d	212
AM+	1.4	11.99a		25.26a		11.62a		1.11a	
	7	10.95b	-8	22.5ab	-11	11.06ab	-5	1.72b	54
	12	10.52bc	-0.12	17.96c	-29	8.88c	-24	2.54c	129

Table 8. Correlation coefficients between traits from alfalfa plants grown under different salinity levels in response to inoculation with arbuscular mycorrhizal fungus (*Funneliformis mosseae*)

	RDW	RSN	NR	PNR	SNC10	SNFR	RS	DSR	PDS	R K/Na	R Ca/Na	S K/Na	R Ca/Na
SDW	0.95**	0.9**	0.92**	0.76**	0.92**	-0.78**	0.85**	0.55*	-0.66**	0.81**	0.77**	0.86**	0.83**
RDW		0.91**	0.87**	0.62**	0.87**	-0.72**	0.8**	0.44ns	-0.75**	0.82**	0.79**	0.89**	0.86**
RSN			0.93**	0.61**	0.93**	-0.81**	0.68**	0.27ns	-0.83**	0.9**	0.89**	0.96**	0.95**
NR				0.83**	0.98**	-0.84**	0.77**	0.45ns	-0.67**	0.87**	0.83**	0.92**	0.88**
PNR					0.83**	-0.66**	0.82**	0.74**	-0.3ns	0.56*	0.5*	0.63**	0.55*
SNC10						-0.84**	0.76**	0.45 ns	-0.68**	0.87**	0.83**	0.92**	0.88**
SNFR							-0.56**	-0.23 ns	0.67**	-0.8**	-0.77**	-0.83**	-0.81**
RSC								0.85**	-0.47ns	0.58*	0.53*	0.62**	0.59**
DSR									0.05ns	0.16 ns	0.1 ns	0.21ns	0.15ns
PDS										-0.78**	-0.8**	-0.8**	-0.86**
R K/Na											0.98**	0.88**	0.85**
R Ca/Na												0.85**	0.86**
S K/Na													0.98**

**Significant at (P≤0.01), *Significant at (P≤0.05), nsNon-significant.

Discussion

As shown in Table 4, 10 days after harvest, root soluble N pools shrunk regardless of the effect of salinity. In recent study, the average of allocated root N pools to remobilization was almost 70% and was variable depended on either salinity level or AMF inoculation. The previous investigations also suggested that alfalfa re-growth depends on utilization of previously acquired N present within the organs remaining after defoliation (Meuriot et al., 2005). Taproots act as the source organs and re-growing shoots as the main sink after alfalfa defoliation (Dhont et al., 2004). Nitrogen remobilization to shoot has been considered as the main reason for depletion of root N reserves during the first days after defoliation (Barber et al., 1996; Skinner et al., 1999). In this respect, prior investigations clarified that N compounds such as proteins and amino acids are taproot components that support shoot re-growth after defoliation (Dhont et al., 2003; Meuriot et al., 2004). Strong correlation (Table 8) between disappeared N from root and N accumulated within re-growing shoot during 10 days after harvest (introduced as shoot N content 10 days after harvest) suggests that root N reserves serve as a main source for shoot's demanded N. Nitrogen sources for re-growing alfalfa may divide to two distinct parts: a) freshly acquired N from biological fixation or uptake from soil, b) received N from remobilization (from root). In present study, AMF inoculation increased utilization of root N pools for remobilization (introduced as percent of N remobilization from root) and on the other hand promoted the contribution of freshly acquired N (total N accumulated in shoot during 10 days minus amount of remobilized N from root) in shoot regrowth (Table 4). Contribution of N remobilization in N accumulation within re-growing shoot (introduced as shoot N received from remobilization) was higher in AM-plants (Table 4). Higher limitation in N acquire from biological fixation and/or uptake in AM-plants leads to higher dependence of shoot re-growth to remobilized N. In current study, there was also a close correlation between amount of root N reserves (total soluble N within root) at harvest time and amount of N remobilization to shoot following harvest (Table 8). Thus, AMF by enhancing root dry weight (Figure 1) and particularly root soluble N concentration (Table 2), may increase N remobilization and shoot dry matter production. It seems that positive effects of AMF on biological nitrogen fixation (Barea et al., 2002) and/or N uptake from soil (Abdel-Fattah and Asrar, 2012) may provide higher level of available N for remobilization to re-growing shoots. In this regard, AMF inoculation increased amount of N remobilization by 62, 78 and 133% respectively under 1.4, 7 and 12 dS/m. However shoot biomass production was subordinate to remobilization of both nitrogen and sugar reserves from root but had a closer correlation with N remobilization (0.92** vs 0.55*, $P \leq 0.01$). In fact, it's suggested that rapid depletion of root sugar reserves following alfalfa defoliation mainly results from respiration and provides C skeleton for shoot re-growth (Avice et al., 1996). Dhont et al. (2002) also reported that there is a poor correlation between root non-structural carbohydrates and shoot re-growth after harvest. In current study, there was a positive correlation between root sugars content and shoot regrowth ability (Table 8). Increased sugars content within AM+ plants (Table 2) may provide higher energy and C skeleton for respiration which leads to higher dry matter production following harvest. Increased sugars content under saline condition has physiological advantages in terms of salinity tolerance. Several physiological studies suggested that nonstructural carbohydrates such as sucrose and hexoses are accumulated under osmotic stress condition (Streeter et al., 2001; Taji et al., 2002).

Sugars may interact with polar head groups of phospholipids in membranes and inhibit the membrane fusion under osmotic stress (Bartels and Sunkar, 2005).

Greater growth of AM+ plants under salinity stress (Figure 2) induced lower Na^+ content (total Na^+) which may decrease tribulation in cell metabolism caused by excessive Na^+. Maintenance of a higher cytosolic K^+/Na^+ ratio has been considered as a key factor regarding salinity tolerance in plants (Chinnusamy et al., 2005).

Potassium is not only a nutritive ion but also has been known to play a critical role in alleviating the toxic effects of salinity stress in plants (Cakmak, 2005). Other investigations also have indicated that AM fungi improve plant growth under salinity because of more balanced nutrient uptake (Cantrell and Linderman, 2001; Al-Karaki 2006). With regard to salt tolerance induced by AMF, the reported effects may be based on enhanced nutrient acquisitions and growth, better water availability and conductance in host plant or alleviation of the toxicity resulted from ions such as Na^+ and Cl^- within cell (Bothe, 2012). In contrast with root tissue, AM+ plants exhibited lower Na^+ accumulation within shoot tissue compared with AM- plants under saline condition (Table 7). Accumulation of Na^+ by AMF within root may prevent of its translocation to shoot tissues and this may be another strategy to alleviate harmful effect of salinity by AMF (Cantrell and Linderman, 2001).

The close correlation between K^+/Na^+, Ca^{2+}/Na^+ ratios within root and the size of root soluble N pools (Table 8) suggests that better ionic balance in AM+ root may reduce source (root) limitation for N remobilization to shoot. In this respect, there was also a high correlation between amount and percentage of N remobilization to shoot and root K^+/Na^+, Ca^{2+}/Na^+ ratios at harvest time (Table 8). Wu et al. (2010) reported that AMF symbiosis significantly increases K^+/Na^+ ratio within plant organs yet has no effect on Ca^{2+}/Na^+, Ca^{2+}/Na^+ ratios. In recent study, however K^+/Na^+ ratio in both root and shoot organs when compared with Ca^{+2}/Na^+ ratio (Table 8), had a greater correlation with biomass production and N remobilization, but still there was a considerable correlation between Ca^{2+}/Na^+ ratio with regrowth ability (Table 8). In plant cells, calcium functions as a second messenger which induces intracellular responses to a wide range of extracellular stimuli (Snedden and Fromm, 2001).

It seems that AMF inoculation may change N partitioning by induction more balanced ion status in both root and shoot tissues. The observed difference regarding N remobilization between AM- and AM+ plants could result from mechanisms within source (roots) or sink (shoots) of nitrogen. As presented in Table 8, N remobilization exhibited higher correlations with the shoot K^+/Na^+, Ca^{2+}/Na^+ when compared with corresponding ratios within root. Then, probably, more balanced nutrition in sink organ (shoot) caused by AMF inoculation may reduce sink limitation for remobilization of N. Potassium is a key nutrient essential for mobilization and translocation of N pools from alfalfa's roots following defoliation and rapid shoot regrowth (Li et al., 1998). In this respect, Berg et al. (2009) also reported that balanced potassium nutrition increases formation and utilization of carbon and nitrogen reserves within taproots and improves alfalfa re-growth ability following defoliation. In current study, although there was a positive significant correlation between root sugar content and K^+/Na^+, Ca^{2+}/Na^+ ratios in both root and shoot organs (Table 8), but there was not any significant correlation between mentioned ionic ratios and disappeared sugars from root during 10 days after harvest (Table 8).

This topic suggests that sugars accumulation in root is involved in salinity tolerance induced by AMF but the amount of disappeared sugars from root during the first days after harvest probably isn't a proper index about salinity tolerance in alfalfa.

Conclusions

Overall, alfalfa regrowth as a perennial forage is highly dependent on N remobilization from root following harvset. This process is significantly limited by salinity stress. Using AMF as biological fertilizer under salinity stress remarkably improved the regrowth ability of alfalfa plants following harvest. It seems that the positive influence of AMF on ionic balance, accumulation of N and sugar within root helps plants to somewhat cope with salinity stress and offset the detrimental implications caused by salinity on N remobilization and shoot regrowth process.

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