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Full Length Research Paper

Improving salinity tolerance of *Acacia saligna* (Labill.) plant by arbuscular mycorrhizal fungi and *Rhizobium* inoculation

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This study was carried out to investigate the alleviation of salt stress (0, 6.25, 12.50 and 25 dS/m) on growth and development of *Acacia saligna*, grown in sandy loam sterile soil by using arbuscular mycorrhizal fungi (AMF) and *Sinorhizobium terangae* (R), individually or in combination (AMF+R). Growth and nodulation parameters, leaf osmotic adjustment and chemical analysis were used as parameters. Salt stress increases the percentage of sodium (Na) and calcium (Ca) contents as well as proline; meanwhile, it reduces the leaf osmotic potential, growth parameters, nodulation parameters, Nitrogen, phosphorus, potassium (N. P. K.) contents, total carbohydrates percentages and chlorophyll contents. Co-inoculated (AMF+R) stressed plants were able to maintain a higher osmotic potential of cells leading to the significantly rapid growth, enhanced nodulation parameters, N, P, K, Ca, total carbohydrates percentages and chlorophyll contents as well as proline in leaves, and significantly reduced the Na percentage. In conclusion, Co-inoculated (AMF+R) enabled the plants to maintain osmotic adjustments and enhanced the plants tolerance against salinity.

Key words: *Acacia saligna*, salinity, Arbascular mycorrhizal fungi and *Rhizobium*.

INTRODUCTION

Many countries in arid and semi-arid Africa, such as Egypt are suffering from decline in fresh water resources available for agriculture. Thus there is a need to look for alternative methods to balance sustenance with demand. Irrigation with low quality water (up to salinity of 4.5 dS/m) is one of many reasons that cause secondary salinization in Egypt (El-Hendawy, 2004). Therefore, planting salt-tolerant species, particularly N_2-fixing species, is the most useful approach in rehabilitating salt-affected degraded lands (Rasmussen et al., 2009).

Acacia saligna is a multipurpose, fast growing tree species (MPTS), which belongs to family Fabaceae. It is a dense and multi-stemmed, thornless, spreading shrub or single stemmed small tree (Maslin, 1974). It occurs naturally in Southwest and Western Australia and has been introduced to the Mediterranean coast in Egypt for many different purposes, including re-vegetation, tanning, fodder, protein-rich seeds and fruits, firewood, agroforestry, windbreak, control of soil erosion, enhancement of bio-productivity and overcoming salt stress problems, which is reported to be salt tolerant (5 to 10dS/m), because these plants enrich soil nitrogen in symbiotic association with *Rhizobium* and form associations with Arbuscular mycorrhizal fungi (AMF) (Hobbs et al., 2006; Swelim et al., 2010).

The application of bio-inoculants (Arbuscular mycorrhizal fungi (AMF) and *Rhizobium*) for improving of salt-tolerant plants is one of great importance because it minimizes the production costs and environmental hazards (Javaid, 2010).

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Therefore, the objective of this study is to determine the effect of Arbuscular mycorrhizal fungi (AMF) and *Rhizobium* (R) inoculation; individually or in combination (AMF+R) on salt stress alleviation in *A. saligna* plants, in order to improve growth, nodulation, osmotic adjustment and chemical composition.

MATERIALS AND METHODS

This study was conducted at the Experimental Laboratories of the Natural Resources Department, Institute of African Research and Studies and the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during the two seasons of 2009/2010 and 2010/2011.

Seeds of *A. saligna* (Labill.) were obtained from Sadat Research Station, Desert Development Center, American University in Cairo, Menofia Governorate, Egypt. The laboratory work begins on the 1st of June of both seasons. The collected seeds were immersed in boiling water for 1 min to accelerate germination Fox (1995). Pre-treated seeds were sown in plastic pots, 25 cm diameter, filled with sandy loam sterile soil prepared specifically for this purpose by standard procedure.

Microbial inoculation and salt stress treatments

The following two bio-inoculants were obtained from Soils, Water and Environmental Resources, Institute of Agricultural Research Center, Giza, Egypt.

One month after sowing the seeds in both seasons, the seedlings were inoculated with mixed spores of arbuscular mycorrhizal fungi (AMF) from genera (*Glomus*, *Gigaspora* and *Acaulospora*) (500 spores/g) at a rate of 10 g/hole, where spores dressed in a hole around the rhizosphere attached to secondary roots (Massoud, 2005). Once the mycorrhizal symbiosis was established, two different *Sinorhizobium terangae* strains (R) (10^9 CFU/ml) were applied at the rate of 10 ml/ pot. The salinity stress was applied one month after inoculation, in both seasons, to allow the time required for the symbiosis to occur. The plants were irrigated every three days using tap water (control, 0.42 dS/m) or saline water at concentrations of 6.25, 12.50 or 25 dS/m. The different saline water concentrations were prepared using a mixture of sodium chloride (NaCl) and calcium chloride [$CaCl_2$ (1:1, w/w)].

The experiment was conducted using completely randomized block design (CRBD) with two factors including 16 treatments and three replicates. The first factor had four inoculation treatments together with the control; the second factor had four irrigation water salinity treatments, with each block consisting of 80 plants (five plants/treatment). The seedlings were harvested 90 days after germination.

Growth parameters

Plant height (cm), root length (cm), number of branches/plant, and total dry weights (g)/plant were recorded. Leaf area (cm^2) was measured with area meter.

Nodulation parameters and mycorrhizal infection

The freshly harvested roots were immediately washed with potassium hydroxide (KOH) and stained with acid fuschin (0.01% in lactoglycerol), then the mycorrhizal infection was determined by the grid-line intersect slide method of Giovannetti and Mosse (1980), number of nodules and nitrogenase activity were also recorded (Somasegaran and Hoben, 1985).

Chemical analysis

Determination of leaf water relations

The osmotic potential (ψ_s) of the cell sap was measured using a vapor pressure osmometer (model 5,500; Wescor, Logan, UT, USA) one week after starting salt treatment. Osmotic adjustment (OA) was calculated as the difference in ψ_s between the treated (salinized) and control plants.

Total chlorophyll content was extracted using the method described by Nornai (1982). Total carbohydrates (%) in the dried leaves were also determined as described by Dubois et al. (1956). Dried leaves samples were digested and the extract was analyzed to determine nitrogen (N%) using the modified micro-Kjeldahl method, phosphorus (%) by Jackson (1967), K and Na% using a flame spectrophotometer (Jameel and Kahayri, 2002), while Ca was determined by atomic absorption (Allen et al., 1984). The proline content in fresh leaves was also determined according to Bates et al. (1973).

The data were subjected to statistical analysis of variance and the means were compared using the "least significant difference (LSD)" test at the 5% level, as described by Little and Hills (1978).

RESULTS AND DISCUSSION

It is well known that osmotic adjustment involves the net accumulation of solutes in a cell in response to salinity, and consequently, the osmotic potential decreases, which in turn attracts water into the cells enabling the turgor to be maintained (Taiz and Zeiger 2006). These results suggest that co-inoculation (Arbuscular mycorrhizal fungi + *Sinorhizobium* sp.) treatment was able to maintain higher osmotic potential of cells due to increase of their osmotic concentration, leading to the maintenance of plant growth and enhancement of the plant ability to tolerate salt stress. Co-inoculated (AMF+R) *A. saligna* plants under salt treatment gives higher osmotic adjustment values (O.A.) followed by R then AMF compared with the control treatment at the same salt concentration (Table 1).

The data indicate that co-inoculation (AMF+R) treatment improve salt tolerance by protecting the cellular protein contents against the damage caused by salt injury, while R or AMF treatment alone could not give the same protection.

Growth parameters

Salinity stress significantly reduces the growth parameters of young *A. saligna* compared with the control treatment due to direct effects of ion toxicity or indirect effects of saline ions that cause soil/plant osmotic imbalance (Table 2) (Abdel Latef, 2010).

Co-inoculation treatment (AMF+R) significantly improved the growth parameters in the salt-stressed plants

Table 1. Osmotic potential (ψ_s) and osmotic adjustment (O.A.) in *Acacia saligna* plants treated with bio-inoculants under salinity stress.

Treatment	Salt concentration dS/m	OP (ψ_s)	O.A
Control	0	--5.95 ± 0.3	
	6.25	-6.92 ± 0.2	0.97
	12.50	-6.94 ± 0.5	0.99
	25	-7.93 ± 0.7	1.98
AMF	0	-3.64 ± 0.6	
	6.25	-4.96 ± 0.3	1.32
	12.50	-5.21 ± 0.1	1.57
	25	-6.44 ± 0.4	2.8
AMF+ R	0	-7.43 ± 0.3	
	6.25	-8.41± 0.5	0.98
	12.50	-9.50 ± 0.3	2.07
	25	-10.87 ± 0.1	3.44
R	0	-3.96 ± 0.4	
	6.25	-4.46 ± 0.3	0.5
	12.50	-5.69 ± 0.2	1.73
	25	-6.94 ± 0.5	2.98

Values are means of five replicates ± standard error (SE*), R, *Sinorhizobium* sp. AMF, Arbuscular mycorrhizal fungi; AMF+R, AM fungi + *Sinorhizobium* sp.

compared to un-inoculation plants. This effect may be attributed to the production of secondary metabolites (as antibiotic and plant hormones), which improve the physiological processes such as water absorption capacity of plants by increasing root hydraulic conductivity and increasing the uptake of essential macro- and micro-nutrients, which in turn improves the plants growth (de Varennes and Goss, 2007; Kaschuk et al*.,* 2010).

Nodulation parameters and mycorrhizal infection

Salinity similarly affected the nodulation parameters and mycorrhizal infection on *A. saligna* (Figure 1). Salinity decreased the hyphae growth and/or viability of AMF (Canrell and Linderman, 2001), and also decreased respiration, survival probability, inhibited enzyme function and multiplication of the rhizobia cells in the substrates, which affect the process of root colonization and nitrogenase activity (Mahmood et al*.,* 2008) .

In this present study, co-inoculated (AMF+R) test plants show less toxic effects of salts on nodulation parameters and mycorrhizal infection compared with control plants. This may be attributed to improve plants growth "root". In addition, root exudation is modified both qualitatively and quantitatively by arbuscular mycorrhizal symbiosis and this led to increase in nodulation parameters and mycorrhizal infection (Garg and Manchanda, 2009).

Chemical composition

As shown in Table 3, increasing of salinity concentration causes a reduction in total chlorophyll content due to the antagonistic effect of NaCl on N absorption, which is considered as an essential component of the structure of chlorophyll molecule (Grattan and Grieve, 1994).

Co-inoculation treatment significantly increased chlorophyll content. This suggests that co-inoculation can improve N nutrition and this may help to reduce the toxic effects of Na ions by reducing its uptake, and this may indirectly help in maintaining the chlorophyll content of the plant (Kaya et al*.,* 2009).

Increasing salt concentration in the irrigation water increased the total carbohydrates (%) (Table 3). This may be explained by the important role of carbohydrates as an abiotic stress protectant; stabilizing dehydrated enzymes and membranes and protecting biological structures from desiccation damage (Soliman, 2008).

Co-inoculated plants had the highest total carbohydrates (%). The favorable effect of co-inoculation may be attributed to hydrolysis of starch to sugars in the co-inoculated plants. In addition, favorably adjusting the osmotic balance and increasing the contents of chlorophylls increases the rate of photosynthesis and carbohydrate synthesis (Swaefy et al., 2007).

Proline concentration was significantly higher in the salt treated plants than that in the non-treated plants (Table 3). This appears to be the best indicator of some

Table 2. Influence of bio-inoculants and irrigation water salinity on growth parameters *of Acacia saligna* during the two seasons of 2009/2010 and 2010/2011.

Salt concentration (SC)** dS/m	1st					2nd				
	Inoculum (I)*				Mean (SC)	Inoculum (I)*				Mean (SC)
	Control	R	AMF	AMF+R		Control	R	AMF	AMF+R	
Plant height (cm)										
Control	25.33	28.33	31.67	34.00	29.83	23.50	26.33	30.00	32.67	28.13
6.25	24.33	27.50	31.00	33.67	29.13	22.17	25.33	29.17	32.00	27.17
12.50	23.17	26.50	29.83	33.00	28.13	19.83	23.83	27.83	31.17	25.67
25	19.50	24.33	27.83	32.00	25.92	16.00	22.00	26.33	30.00	23.58
Mean (I)	23.08	26.67	30.08	33.17	---	20.38	24.38	28.33	31.46	---
LSD (0.05)	I = 2.27		SC = 1.63		IX SC = 3.27	I = 3.49		SC = 2.15		IX SC = 4.29
Root length (cm)										
Control	14.17	16.00	17.17	18.50	16.46	12.83	15.17	16.00	16.33	15.08
6.25	12.33	14.67	16.00	17.83	15.21	11.17	14.00	15.00	15.50	13.92
12.50	10.33	13.00	14.67	16.67	13.67	8.50	12.00	13.33	14.17	12.00
25	7.83	11.00	13.00	15.33	11.79	5.33	9.50	11.33	12.50	9.67
Mean (I)	11.17	13.67	15.21	17.08	---	9.46	12.67	13.92	14.63	---
LSD (0.05)	I = 1.25		SC = 1.53		IX SC = 3.05	I = 1.53		SC = 1.29		IX SC = 2.59
Number of branches/plant										
Control	10.33	16.00	17.00	19.33	15.67	9.67	13.67	15.67	18.67	14.42
6.25	9.00	15.00	16.33	19.00	14.83	8.00	12.67	15.00	18.33	13.50
12.50	7.33	13.67	15.33	18.67	13.75	6.00	11.33	14.00	17.67	12.25
25	5.33	12.00	14.00	18.00	12.33	3.667	9.67	12.67	16.67	10.67
Mean (I)	8.00	14.17	15.67	18.75	---	6.83	11.83	14.33	17.83	---
LSD (0.05)	I = 1.70		SC = 2.05		IX SC = 4.10	I = 1.45		SC = 1.64		IX SC = 3.28
Total dry weight g/plant										
Control	16.47	23.87	24.57	33.07	24.49	14.53	20.63	22.97	25.77	20.98
6.25	13.80	22.27	23.40	32.03	22.88	12.10	19.43	21.87	24.93	19.58
12.50	10.23	20.13	21.43	30.33	20.53	9.40	17.70	20.37	23.57	17.76
25	5.80	17.17	19.20	28.17	17.58	5.87	15.13	17.93	21.33	15.07
Mean (I)	11.57	20.86	22.15	30.90	---	10.48	18.23	20.78	23.90	---
LSD (0.05)	I =2.64		SC = 3.00		IX SC = 6.00	I = 2. 46		SC = 1.75		IX SC = 3.50

* R, *Sinorhizobium* sp. AMF, Arbuscular mycorrhizal fungi; AMF+R, AM fungi + *Sinorhizobium* sp. LSD, Least significant difference

mechanism of stress resistance (Jampeetong and Brix, 2009).

The proline concentration in the leaves of co-inoculated plants was increased significantly with the salinity stress, compared to controlled plants. The high level of proline enables the plants to maintain osmotic balance when growing under salinity (Feng et al., 2002), and acts as a major reservoir of energy and nitrogen for utilization by plants subjected to salinity stress (Rabie and Almadini, 2005).

The results in Table 4 also show that an accumulation of Na and Ca% in leaves of *A. saligna* seedlings were accompanied by a significant decrease in N, P, and K% which raises the salt concentration. This indicates that during salt stress, the plants tend to take up more Na resulting in decreased K uptake. Na ions compete with K for binding sites essential for various cellular functions. The Ca concentration which acts as a second messenger is also increased to transduce signals, while phosphate ions precipitate with Ca, Mg and Zn ions in salt stressed soils and become unavailable to plants. Also, salinity interferes with nitrogen (N) acquisition and utilization by influencing different stages of N metabolism, such as, nitrate (NO_3) uptake and reduction, and protein synthesis (Canrell and Linderman, 2001; Ramoliya et al., 2006; Abdel Latefa and Chaoxing, 2011).

However, N, P, K and Ca% were significantly higher in co-inoculated plants at all salinity levels compared to un-

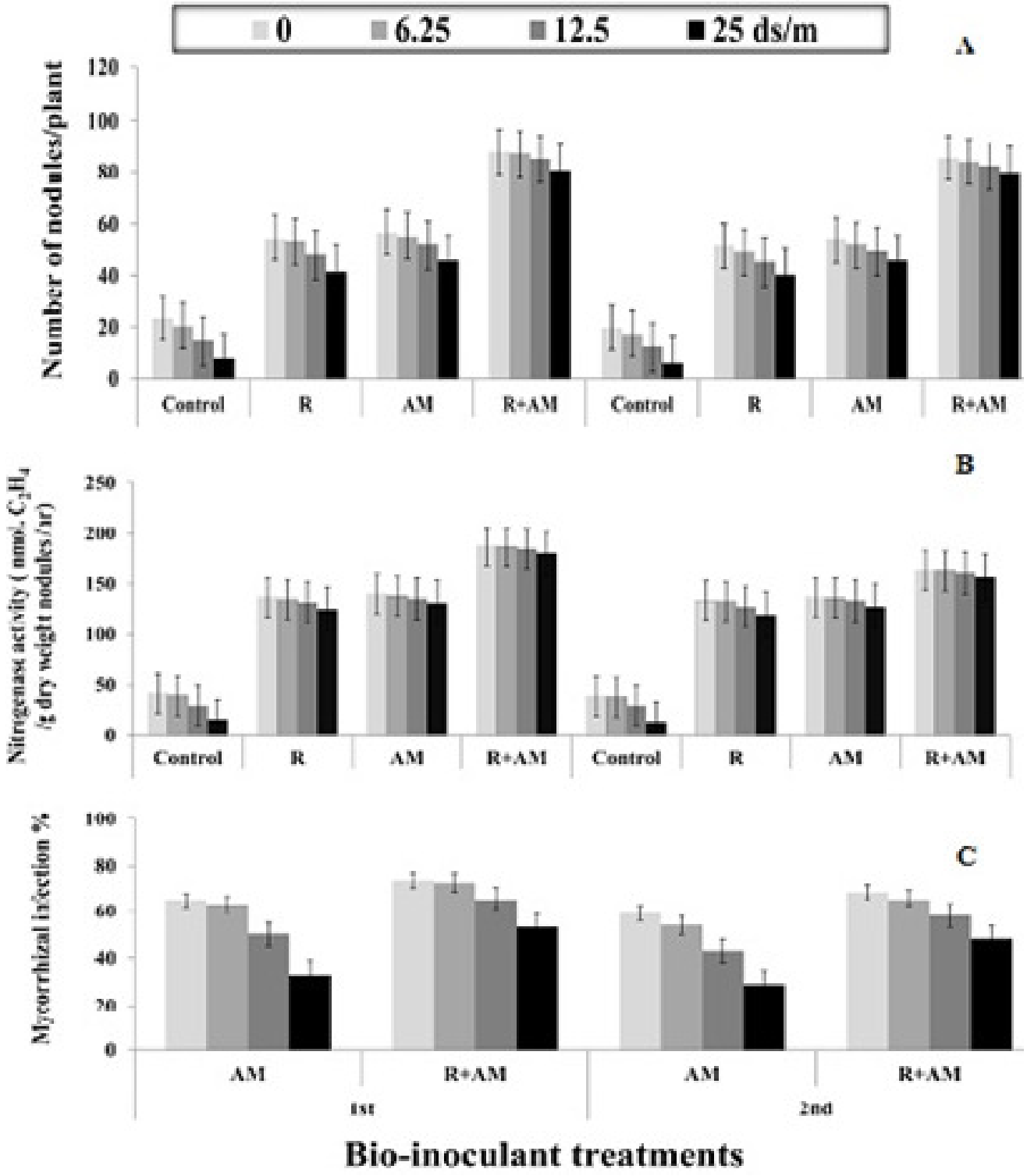

Figure 1. Influence of bio-inoculums and irrigation water salinity on nodulation parameter (A, B) and mycorrhizal infection (C) of Acacia saligna during the two seasons of 2009/2010 and 2010/2011.

inoculated plants, while Na% was lower. Increased nutrients uptake in co-inoculated plants may be due to a change in N metabolism brought about by changes in the enzymes associated with N metabolism, enhancing its uptake facilitated by the extensive hyphae of the fungus which allows them to explore more soil volume than the

Table 3. Influence of bio-inoculants and irrigation water salinity on leave area and chemical analysis of *Acacia saligna* during the two seasons of 2009/2010 and 2010/2011.

Salt concentration (SC)** dS/m	1st					2nd				
	Inoculum (I)*				Mean (SC)	Inoculum (I)*				Mean (SC)
	Control	R	AMF	AMF+R		Control	R	AMF	AMF+R	
Leave area (cm^2/ plant)										
Control	20.93	24.83	27.79	36.66	27.55	18.45	21.88	24.42	33.49	24.56
6.25	19.08	23.06	26.61	35.86	26.15	17.15	20.23	23.06	33.11	23.39
12.50	13.01	19.51	23.65	33.11	22.32	11.23	17.27	20.22	30.75	19.87
25	5.91	15.96	20.82	30.75	18.36	4.73	12.42	17.15	27.79	15.52
Mean (I)	14.73	20.84	24.72	34.10	---	12.89	17.95	21.21	31.29	---
LSD (0.05)	I = 1.83	SC = 1.77		IX SC = 3.54		I = 2.64	SC = 2.57		IX SC = 5.14	
Total chlorophylls content (mg/g fresh matter)										
Control	2.11	2.69	2.80	3.11	2.68	1.76	2.30	2.59	2.80	2.36
6.25	2.07	2.65	2.77	3.08	2.64	1.72	2.26	2.56	2.78	2.33
12.50	1.94	2.55	2.69	3.02	2.55	1.65	2.18	2.50	2.73	2.27
25	1.68	2.31	2.53	2.87	2.35	1.46	2.03	2.41	2.65	2.14
Mean (I)	1.95	2.55	2.70	3.02	---	1.65	2.19	2.51	2.74	---
LSD (0.05)	I = 0.06	SC = 0.13		IX SC = 0.26		I = 0.25	SC = 0.14		IX SC = 0.28	
Total carbohydrates (% of dry matter)										
Control	21.00	29.00	37.00	43.67	32.67	25.33	32.33	39.33	47.00	36.00
6.25	23.00	30.67	38.00	44.33	34.00	26.67	33.00	40.00	47.33	36.75
12.50	26.33	33.00	39.67	45.33	36.08	29.33	34.67	41.00	48.00	38.25
25	31.33	37.00	42.33	47.00	39.42	33.00	37.00	42.67	49.33	40.50
Mean (I)	25.42	32.42	39.25	45.08	---	28.58	34.25	40.75	47.92	---
LSD (0.05)	I = 3.07	SC = 2.52		IX SC = 5.03		I = 2.51	SC = 3.17		IX SC = 6.34	
Proline content (µ moles/g fresh matter)										
Control	13.00	15.33	17.33	23.33	17.25	16.33	18.00	21.33	26.33	20.50
6.25	13.33	16.00	18.67	25.00	18.25	17.00	19.00	23.00	28.67	21.92
12.50	15.00	19.67	23.33	30.33	22.08	20.00	23.67	28.33	34.67	26.67
25	18.67	25.33	29.67	37.33	27.75	24.67	29.67	35.00	42.33	32.92
Mean (I)	15.00	19.08	22.25	29.00	---	19.50	22.58	26.92	33.00	---
LSD (0.05)	I =2.64	SC = 3.00		IX SC = 6.00		I= 2. 46	SC = 1.75		IX SC = 3.50	

* R, *Sinorhizobium* sp. AMF, Arbuscular mycorrhizal fungi; AMF+R, AM fungi + *Sinorhizobium* sp. LSD, Least significant difference.

non-inoculated plants, and can reverse the effect of salinity on K and Na nutrition; while preventing Na translocation to shoot tissues and its negative effects from interfering in growth metabolic pathways.Co-inoculation strongly affects Ca in the plants. High Ca has a beneficial effect on toxic effects of NaCl by facilitating higher K/Na, selectivity leading to salt adaptation.

Moreover, high Ca was also found to enhance colonization and sporulation of AMF (Giri et al., 2007; Zuccarini and Okurowska, 2008; Shokri and Maadi, 2009).

In conclusion, results from this study provide the evidence that AM-fungus aid *Rhizobium* in protecting *A. saligna* plants against the lethal effects of salt by enhancing salt-avoidance mechanisms, such as de-creasing Na%, and increasing proline accumulation, protecting its contents from the salt injury as compared to un-inoculated plants.

Table 4. Influence of bio-inoculants and irrigation water salinity on nutrients of Acacia saligna during the two seasons of 2009/2010 and 2010/2011.

Salt concentration (SC)** dS/m	1st					2nd				
	Inoculum (I)*					Inoculum (I)*				
	Control	R	AMF	AMF+R	(SC)	Control	R	AMF	AMF+R	(SC)
N (% of dry matter)										
Control	1.70	2.56	3.00	3.34	2.65	2.05	2.77	3.08	3.45	2.84
6.25	1.67	2.54	2.98	3.33	2.62	2.02	2.76	3.07	3.45	2.83
12.50	1.55	2.46	2.92	3.28	2.55	1.93	2.69	3.02	3.40	2.76
25	1.40	2.35	2.83	3.21	2.45	1.81	2.60	2.95	3.35	2.68
Mean (I)	1.58	2.48	2.93	3.29	---	1.95	2.70	3.03	3.41	---
LSD (0.05)	I = 0.05	SC = 0.04		IX SC = 0.08		I = 0.06	SC = 0.05		IX SC = 0.09	
P (% of dry matter)										
Control	0.18	0.27	0.32	0.36	0.28	0.25	0.31	0.37	0.40	0.33
6.25	0.16	0.26	0.31	0.35	0.27	0.24	0.30	0.36	0.39	0.32
12.50	0.12	0.24	0.30	0.34	0.25	0.21	0.27	0.35	0.38	0.30
25	0.08	0.20	0.27	0.32	0.22	0.17	0.25	0.33	0.36	0.28
Mean (I)	0.13	0.24	0.30	0.34	---	0.22	0.28	0.35	0.38	---
LSD (0.05)	I = 0.03	SC = 0.04		I X SC= 0.08		I= 0.03	SC = 0.03		I X SC = 0.05	
K (% of dry matter)										
Control	1.56	1.62	1.67	1.78	1.66	1.53	1.58	1.64	1.75	1.63
6.25	1.54	1.60	1.65	1.77	1.64	1.51	1.57	1.63	1.74	1.61
12.50	1.50	1.57	1.63	1.76	1.61	1.46	1.52	1.60	1.72	1.57
25	1.44	1.52	1.60	1.73	1.57	1.40	1.47	1.57	1.70	1.54
Mean (I)	1.51	1.58	1.64	1.76	---	1.48	1.53	1.61	1.73	---
LSD (0.05)	I = 0.03	SC = 0.04		I X SC = 0.07		I = 0.03	SC = 0.03		I X SC= 0.05	
Na (% dry matter)										
Control	0.34	0.29	0.26	0.23	0.28	0.38	0.32	0.28	0.26	0.31
6.25	0.38	0.33	0.29	0.24	0.31	0.43	0.36	0.31	0.27	0.34
12.50	0.65	0.58	0.52	0.36	0.53	0.68	0.60	0.51	0.40	0.55
25	1.04	0.89	0.77	0.53	0.81	1.17	0.91	0.70	0.55	0.83
Mean (I)	0.61	0.52	0.46	0.34	---	0.66	0.55	0.45	0.37	---
LSD (0.05)	I = 0.06	SC = 0.08		I X SC = 0.16		I = 0.11	SC = 0.07		I X SC = 0.13	
Ca (% dry matter)										
Control	0.31	0.37	0.52	0.82	0.51	0.37	0.43	0.68	0.94	0.61
6.25	0.38	0.41	0.55	0.84	0.54	0.44	0.48	0.71	0.95	0.64
12.50	0.50	0.55	0.71	1.01	0.70	0.54	0.60	0.84	1.15	0.78
25	0.63	0.78	0.96	1.38	0.94	0.68	0.76	1.09	1.48	1.00
Mean (I)	0.46	0.53	0.69	1.01	---	0.51	0.57	0.83	1.13	---
LSD (0.05)	I = 0.18	SC = 0.13		I X SC = 0.26		I = 0.18	SC = 0.14		I X SC = 0.28	

* R, *Sinorhizobium* sp. AMF, Arbuscular mycorrhizal fungi; AMF+R, AM fungi + *Sinorhizobium* sp. LSD, Least significant difference

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