

Increasing NaCl - Salt Tolerance of a Halophytic Plant *Phragmites australis* by Mycorrhizal Symbiosis

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Abstract: Through biological inoculation technology, pot greenhouse experiments were conducted in attempt to increase salinity tolerance of a halophytic plants; *Phragmites australis*, by using arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* isolated from saline soil. Mycorrhizal and non-mycorrhizal plants were exposed to 0.0, 50, 100, 150, 200, 250 and 300 mM of NaCl. Plant growth was significantly stimulated at lower salinity (0 and 50 mM NaCl), but sharply decreased at higher salinity concentration (250 and 300 mM NaCl) but AM plants still much higher than that of non-AM one. A positive correlation was observed between plant growth and its level of succulence. Sodium ions (Na^+) were greatly accumulated in *Phragmites* plants by rising salinity levels however, AM plants accumulate more Na^+ ions in roots than that in shoots. NaCl salt-stress stimulated the levels of organic solutes such as soluble sugars and glycinebetaine. Arbuscular mycorrhizal plants significantly had higher levels of these solutes than that of non-AM. The results also revealed that the increase in salinity generated a decrease in water potential and osmotic potential experimented plants, with this decrease being lower in AM salinity - stressed plants. Mycorrhization also had positive effects on turgor potential and osmotic adjustment of salinity - stressed plants. It is evident that amelioration of salt stress concentrations by mycorrhizal association can be related to improved osmotic adjustment but independent of salt uptake of plants. Thus the results indicated that the mycorrhizal symbiosis had a beneficial effect on the water status, accumulation of osmotica and growth of *Phragmites australis* plants under salinity - stress conditions.

Key words: Salt tolerance • halophytic • phragmites • mycorrhiza

INTRODUCTION

Salt stress has become an over-increasing threat to food production, irrigation being a major problem of agriculture fields due to gradual salinization. The most common solution to this problem is to increase the salt tolerance of conventional crop plants, but the gain in yield is generally low [1]. Another response to the salinity problem is the development of salt tolerant crops through breeding and genetic engineering. A recent approach is to use domesticated halophytes to combat the salinity problem [2]. Among the large pool of suitable plants, it is expected to find candidates for domestication. Halophytes represent an important potential as they can be used for fodder, fuel, oil, wood and pulp and fiber production. They Also can be used for land reclamation, dune stabilization and landscaping. Some of 2500 species of halophytes (graminae, shrubs and trees) occur in saline

coastal environment and inland deserts. Increasing attention has been paid to research and development of halophytes utilizing undiluted seawater on a large scale for irrigation [3, 4].

The ability of plants to survive under high salt conditions is important for the ecological distribution of plant species and agriculture in semi arid, arid and salinized region. Halophytes are known for their ability to adapt to living in salty solution environment and these plants adapt to salinity by altering their energy metabolism [5-7]. The plant has to react physiologically at least to four major constraints for plant growth on saline substrates [6, 8-10]. Control mechanisms include (a) growth rate and plant morphology, (b) resistance to water stress (reduction of the water potential), (c) regulation of CO_2 and H_2O - exchange by stomata and (d) avoidance of ion toxicity and nutrient imbalance.

Arbuscular mycorrhizal (AM) fungi are obligately symbiotic soil fungi that colonize the roots of the majority of plants. AM fungi widely exist in salt - affected soils [11]. Many studies have demonstrated that, inoculation with AM fungi the plant receives a variety of benefits that may result in increased growth, improved water relations, enhanced nutrient uptake over non-inoculated control plants and modification of root morphology [12-14].

Phragmites australis is a widespread species occurring in both freshwater and saline habitats and whose typical habitats are the fresh and brackish water areas of swamps, riversides and lakesides. However, the interaction between AM fungus and this species has not been studied in depth. Therefore, the symbiosis of *Glomus fasciculatum* with *P. australis* plants could report a higher water use efficiency and osmotic adjustment with which the plant more cope during salinity stress. Thus this study is considered a good attempt to increase the salinity tolerance of one of the most widespread halophytic plant *P. australis* by using AM fungal symbiosis.

MATERIALS AND METHODS

The soil used in the present study was an autoclaved sandy soil. Soil fertilization 15 days prior to planting included 273 mg of ammonium sulfate, 145 mg of simple super phosphate and 25 mg potassium chloride. Ca^{2+} 14.5 mmol kg^{-1} ; Mg^{2+} 34.7 mmol kg^{-1} ; K^+ 2.9 mmol kg^{-1} ; Na^+ 6.0 mmol kg^{-1} ; P 63.8 mg kg^{-1} ; N 0.84 g kg^{-1} ; clay 78g kg^{-1} ; silt 320 g kg^{-1} fine sand 33 g kg^{-1} ; coarse sand 570 g kg^{-1} ; bulk density 1.3 g cm^{-3} and particular density 2.5 g cm^{-3} .

A soil sample was collected from the rhizosphere area of grown *Phragmites australis*. The extraction of the mycorrhizal spores was carried out by the method reported by Gerdemann and Nicolson [15]. Soil water suspension (1: 5) was subjected to a series of sieves (50-500 μm). The remaining debris was transferred into Petri dishes to be finally examined microscopically and identified the author. Examination indicated that the extracted mycorrhizal spores were of the species *Glomus fasciculatum*. Spore population of the inoculum is being around 470 spore/ml. The seeds were sterilized with sodium hypochlorite (20%) for 2 min, rinsed repeatedly in sterilized distilled water. Inoculation was performed on seedling stage by pipetting 50 cm^3 of the inoculum on the soil surface at the base of each plant seedling. Two seedlings per container were transplanted.

The experiment was designed as a 7 x 2 factorial, which was compared of 7 NaCl concentrations (0, 50, 100,

150, 200, 250 and 300 mM) and 2 levels of mycorrhizal inoculation [inoculated (+M) and non-inoculated (-M)]. Increase doses of NaCl were applied to the soil 15 days before planting. After salinization process, all treatments had a soil pH of 7.3. The plants were harvested 70 days after planting. Succulence [g (H_2O) g^{-1} (dm)] and biomasses of shoot and root of the plants were recorded.

Leaf water potential was determined using the spanner type thermocouple psychrometers as described by Scholander *et al.* [16]. A part of the lamina of the same leaf was frozen for 2 weeks, thawed and sap extracted by crushing the material with metal rod. The sap was used directly for osmotic potential determination in an osmometer (Wescor 5500). Leaf turgor potential was calculated by subtracting osmotic potential from leaf water potential and the osmotic adjustment was calculated as the difference in osmotic potential between salinized and control plants [17].

Chlorophyll concentration was determined by measuring the absorbance (654 nm) of an ethanol extract solution as described by Winterman and De Mots [18]. Na concentration was measured by flame photometer according to Allen *et al.* [19].

For glycinebetaine measurements Lever *et al.* [20], 0.5 g of plant material was boiled in 10 ml of distilled water for 2 h at 100°C using a dry heat bath. Samples were diluted with a 50 mM potassium dihydrogen phosphate buffer adjusted to pH 4.6. This was the carrier buffer that was also used in the HPLC system. The sample was cooled and filtered using a 0.45 μm membrane filter (Gelman, Ann Arbor, MI, USA) and then used directly to measure glycinebetaine with Hewlett Packard HP 1050 modular 3D HPLC (Boise, ID, USA). The soluble sugars were extracted with 80% ethanol and were estimated by the method of Najuib [21].

The roots were cleared and stained by using the methods by Philips and Hayman [22] and the percentage of mycorrhizal colonization was estimated by the methods of Trouvelot *et al.* [23].

Statistical analysis of the results was subjected to the procedure of Steel and Torrie [24] and the treatments means were compared using the least significant difference (L.S.D.).

RESULTS

The results of Table 1 clearly showed that the frequency of mycorrhizal infection (F percentage) was influenced by salinity. The mycorrhizal infection was reduced from 63% at 50 mM NaCl to 31% at 300 mM NaCl. It was observed that the highest infection was recorded

Table1: Effect of NaCl - salt stress on mycorrhizal root segments (%F); intensity of mycorrhizal colonization (%M) and arbuscule frequency in root systems (%A) in *Phragmites australis* inoculated with *Glomus fasciculatum*

NaCl levels	0	50	100	150	200	250	300
F%	55	63	57	53	41	35	31
M%	41	48	37	36	29	19	17
A%	19	21	19	14	12.6	10	8

NaCl mM	F%	M%	A%
5	55.0	41.0	19.0
50	63.0	48.0	21.0
100	57.0	37.0	19.0
150	53.0	36.0	14.0
200	41.0	29.0	12.6
250	35.0	19.0	10.0
300	31.0	17.0	8.0

Table 2: Effect of NaCl-salt stress on biomass of root and shoot of AM (+ M) and non-AM plants

NaCl mM	Root biomass (g plant ⁻¹)		Shoot biomass (g plant ⁻¹)		Chlorophyll content (µg ⁻¹ dry weight)	
	- M	+ M	- M	+ M	- M	+ M
0	128.1	147.9	355.2	372.0	1.21	3.91
50	141.0	164.6	361.7	397.0	1.23	4.53
100	91.0	122.4	204.7	316.7	1.06	3.42
150	43.5	88.5	125.6	227.1	0.95	2.66
200	16.2	44.3	53.2	156.8	0.81	2.31
250	11.5	40.7	32.6	141.6	0.79	2.10
300	10.8	40.1	30.1	123.9	0.72	1.66
L.S.D. (P≤0.05)	7.84	13.2	15.67	18.2	0.02	0.24

at low and moderate levels of salinity (50, 100, 150 mM NaCl). Evidence from the data in Table 1 indicates that the intensity of mycorrhizal colonization in the root tissue (M%) and the rate of orbicular formation in root (A%) follow similar trends of mycorrhizal infection (F%) with the maximum its values were observed at 50 mM NaCl salinity level.

The results given in Table 2 showed the effect of mycorrhizal inoculation (AM fungi) on biomass of root and shoot, chlorophyll content of leaves and total soluble sugar concentration of *Phragmites australis* plants under various levels of salinity. With increasing salinity level the biomass of root and shoot of plants were significantly decreased specially in plants non-inoculated with AM fungi. Both root and shoot, biomass was optimal at 50 mM NaCl in the presence and absence of AM fungi. Mycorrhizal association increases salinity tolerance of phragmites plants at all salinity levels used in this study relative to those of non-mycorrhizal ones.

The results also revealed that the effect of inoculation of AM fungi on chlorophyll content of

phragmites plants under salinity conditions follow similar trends of these inoculant's on plant biomass under the same salinity conditions (Table 2). The highest value of chlorophyll was recorded at 50 mM NaCl in both mycorrhized and non-mycorrhized plants.

The results of Table 3 indicated that sodium ions (Na⁺) were greatly accumulated in root and shoot of experimented plants by rising salinity levels during the course of experiment either in the presence and absence of AM fungi. In the absence of AM fungi, phragmites plants accumulate more Na⁺ ions in shoot system than that in root system at all salinity levels. While, in the presence of AM Fungi these plants accumulate more Na⁺ ions in roots than that in shoots. A close examination of the results showed that there is no significant differences between Na⁺ ions concentrations at low and moderate levels of salinity in shoot system of AM plant.

The effect of AM inoculation to phragmites plants grown under salinity stress on soluble sugars content were represents in Fig. 1. Soluble sugar content of phragmites plants were significantly increase by rising

Table 3: Effect of NaCl-salt stress on sodium (Na⁺) of root and shoot of AM(+M) and non-AM plants

NaCl (mM)	Na content of AM plant (mg g ⁻¹ dry weight)		Na content of non-AM plant (mg g ⁻¹ dry weight)	
	Root	Shoot	Root	Shoot
0	1.46	3.12	1.32	2.76
50	4.72	3.98	3.86	5.18
100	7.53	4.22	4.65	5.97
150	9.81	4.67	4.91	7.62
200	11.35	5.82	5.73	8.41
250	14.10	6.74	6.12	11.33
300	15.61	7.83	6.92	14.42
L.S.D. (P≤0.05)	2.74	3.23	0.67	3.79

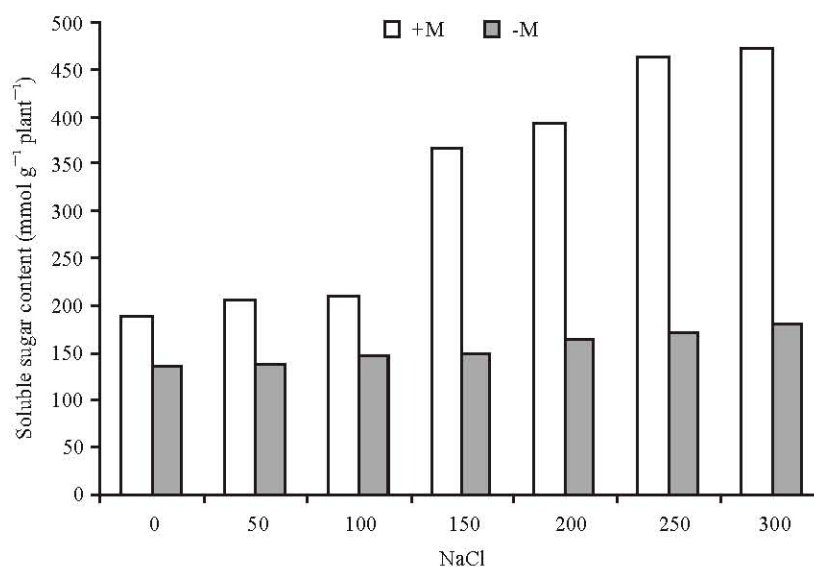


Figure 1: Effect of different salinity levels on soluble sugar content (mmol g⁻¹ plant⁻¹) of AM (+M) and non-AM plants (-M)

NaCl concentrations during this experiment. Under NaCl-stress conditions, AM plants had higher levels of soluble sugars than that of non-AM plant especially at higher levels of NaCl concentration (250-300 mM NaCl).

Figure 2 clearly showed that glycinebetaine content in *Phragmites australis* increased significantly with increasing NaCl concentration from about 1.14 and 1.87 mmol g⁻¹ at control (0.0 mM NaCl) to 9.1 and 18.73 mmol g⁻¹ at the 300 mM NaCl treatment in AM and non-AM plants, respectively. At the same NaCl level, plants inoculated with AM fungi had significantly higher glycinebetaine content than non-inoculated plants. It was found that at higher salinity levels glycinebetaine content of AM plant was about two fold than that of non-AM plants.

Results of Table 4 showed that both leaf water potential and osmotic potential values of phragmites leaves were high in non-stressed plants either in absence or in the presence of AM fungi. Leaf water potential and osmotic potential were exhibited progressive decrease with increasing salinity level. Leaf water potential was decreased from -0.67 MPa at 50 mM NaCl to -2.41 MPa at 300 mM NaCl in AM plant. While, osmotic potential was decreased from -1.01 MPa to -561 MPa in AM plants at 50 and 300 mM NaCl, respectively. Comparisons of leaf water potential and osmotic potential values at corresponding salinity levels showed that AM plants significantly maintained lower than that of non-AM plants especially at higher salinity levels. On the other hand, turgor potential and osmotic adjustment showed an increases in

Table 4: Effect of different salinity levels on Leaf water potential (Ψ) osmotic potential (π) Turgor potential Ψ_p Osmotic adjustment (OA) and succulence of AM (+M) and non-AM (-M) *P. australis* under salinity levels

NaCl (mM)	Ψ (Mpa)		π (Mpa)		Turgor potential (Ψ_p)		Osmotic adjustment (OA)		Succulence (g H ₂ O g ⁻¹ leaf dry weight)	
	(+M)	(-M)	(+M)	(-M)	(+M)	(-M)	+M	-M	+M	-M
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0	-0.56	-0.44	-0.85	-0.72	0.29	0.28			5.39	4.81
50	-0.67	-0.55	-1.01	0.84	0.34	0.29	0.16	0.12	6.89	5.66
100	-0.98	-0.65	-1.35	-0.96	0.37	0.31	0.5	0.24	6.55	4.84
150	-1.16	-0.98	-1.91	-1.31	0.75	0.33	1.06	0.59	5.21	3.61
200	-1.43	-1.41	-2.84	-2.05	1.41	0.64	1.99	1.33	2.72	0.94
250	-1.8	-1.68	-3.91	-2.29	2.11	0.61	3.06	1.57	2.10	0.62
300	-2.41	-1.83	-5.61	-2.40	3.20	0.57	4.76	1.68	1.95	0.55
L.S.D. (P≤05)	0.29	0.034	0.006	0.16					0.041	0.23

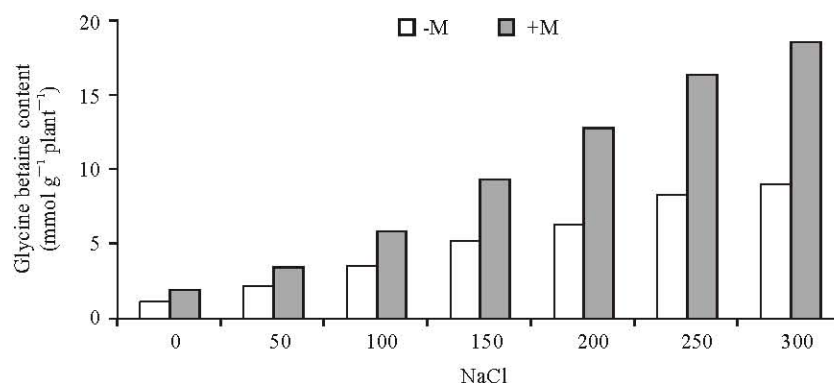


Figure 2: Effect of different salinity levels on Glycine betaine content (mmol g⁻¹ plant⁻¹) of AM (+M) and non-AM plants (-M)

AM plants with rising NaCl concentrations and still much higher than that of non-AM plants especially at higher salinity levels (Table 4). Although, it revealed that NaCl concentration in the soil solution significantly affected the tissue water content (succulence) of plants on a unit dry weight basis. Succulence slightly increased at low salinities but declined at higher level of NaCl concentrations. It was decreased from 6.89 to 1.95 in AM plant and from 5.66 to 0.55 in non-AM plant as a salinity levels raised from zero, 50 to 300 mM NaCl concentrations. AM plants were attained a higher levels of tissue succulence, especially at the higher salinity levels, relative to the non-AM plants.

DISCUSSION

Phragmites australis is a true halophyte because it remains viable and normally grows in salt marshes and coastal mudflat areas. Additionally, halophytic plants do

not only tolerate salinity but can be stimulated by NaCl. In the present study, inoculated and non-inoculated *P. australis* with AM fungus *Glomus fasciculatum* were examined under NaCl stress, plant biomass was significantly inhibited by salt stress. The plant biomass increased under a low NaCl concentration (50 mM) and then decreased with increasing NaCl concentrations. Similar results were previously reported in the halophytic legume *Alhagi pseudalhagi* where 50 mM NaCl increased the plant dry weight and 200 mM NaCl decreased the plant dry weight by 42.7% compared with control plants [25]. The present data agree with previous data reported on *Plantago maritima* in which salt treatment at low levels improves plant growth [26].

AMF efficiency can be measured in terms of host plant growth under different environmental conditions [13]. In this sense, the inoculation of *Glomus fasciculatum* used in this study maintained and stimulated the growth in inoculated *phrgmites* plants

in salinity stress conditions compared to control (non-inoculated) plants. This effect was more pronounced in aerial biomass than in root biomass (Table 1), which may be because arbuscular mycorrhizal colonization caused a proportionally greater allocation of carbohydrates to the shoot than to the root tissues [27]. In this study, the overall growth and measured physiological parameters of *phragmites* plants under salinity stress increased upon mycorrhizal association with *Glomus fasciculatum* as compared to non-AM plants. The present data agree with previous data reported by Muhsin and Zwiazek [28]; Bohrer *et al.* [11] and Sanchez-Blanco [12] suggesting that mycorrhizal symbiosis is a key component in helping plants to cope with adverse saline conditions.

It has been widely accepted that plants are stressed in three ways by salinity (1) low water potential of the root medium leads to water deficits in crop plants, (2) toxic effect of ions, mainly Na and Cl and (3) nutrient imbalance caused by depression in uptake and / or shoot transport [29]. In this study, as expected, Na⁺ concentration was significantly increased with increasing salinity levels in AM and non-AM plants. Surprisingly, while mycorrhizal plants accumulated more Na⁺ in their roots with increasing salinity, shoots of these plants had lower Na⁺ content and showed limited accumulation with increasing salinity (Table 3). These results are in agreement with previous work of Rabie [13] and suggested that AM fungi protect leaf metabolism from Na⁺ toxicity. In this connection, Moghaieb *et al.* [17] reported that to maintain an osmotic gradient for the uptake of water from surrounding medium, many halophytic plants accumulate inorganic ions to a concentration equal to or greater than that of the surrounding root solution. Furthermore, Parks *et al.* [30] showed that vacuolar accumulation of Na⁺ provides an osmotic driving force for the uptake of water in highly saline environments, which would explain the observed higher succulence (leaf water content) in AM plants in our study.

Plants develop a plethora of biochemical and molecular mechanisms to cope with salt stress. A quaternary ammonium compound betaine acts as an osmotic solute that accumulates in response to osmotic stress and the accumulation of this osmolyte represents an important adaptive response to salt stress [31]. The present data indicated that AM plants accumulate higher levels of betaine in their tissues under salt conditions than non-AM plants especially at higher levels of salinity where AM plants accumulate two fold of betaine than in non-AM plant. This result indicated the experimental evidence that implicates that AM fungus

G. fasciculatum functions in salt adaptation of *phragmites* plants. The accumulation of betaine in both non-AM and AM plants increased with increasing NaCl concentration in our study. These results are also in harmony with those previous works [10, 17, 32].

The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity despite a significant decrease in net CO₂ assimilation rate [33]. The present study indicated that the total soluble sugar of *phragmites* plants were increased with rising NaCl concentrations. Meanwhile, soluble sugars of AM plants increased by about three folds more than that of non-AM plants especially at higher levels of salinity. This may reflect the potential role of AM fungi for increasing soluble sugars accumulation of *Phragmites* plants under salinity stress. These results are consistent with Rabie [13] who reported that soluble sugar of AM mungbean plants was increased by increasing level of salinity. It is well known that symbiotic interactions in AM associations are based on the exchange of carbohydrates and mineral nutrients between the plant and the fungus. Therefore, plant roots becoming a strong sink for carbohydrates when colonized by AM fungi and mycorrhizal sink strength influences the whole plant carbon balance [13]. Based on these data and existing literatures it is conceivable to conclude that the requirement for carbohydrates by AM fungi could cause an increased allocation to and accumulation of soluble sugars in roots. The higher accumulation of soluble sugars in mycorrhizal plant tissue, especially in roots, could make mycorrhizal plants more resistant to osmotic stress induced by exposure to NaCl salt.

Osmotic adjustment is a mechanism used for maintaining turgor and reducing the deleterious effects of water stress on vegetative and reproductive tissue [17]. In the present study, all the parameters of leaf water relations decreased with increasing NaCl concentration. It is well known that osmotic adjustment involves the net accumulation of solutes in a cell in response to salinity. Consequently, the osmotic potential decreases, which in turn attracts water into the cell and enables turgor to be maintained. Osmotic adjustment increased with NaCl concentration and was greater in AM plants and lowest in non-AM plants. The observed difference in osmotic potential and osmotic adjustment seemed to be related to the accumulation of sodium ions, betaine and soluble sugar.

The results of this study suggest that the inclusion of Na⁺ and its subsequent sequestration in vacuoles and the synthesis of compatible solutes (betaine and sugar) to

maintain osmotic adjustment is the main strategy that has evolved in *P. australis* plants to maintain growth under high Na⁺ concentrations. AM *P. australis* have a greater ability to maintain osmotic adjustment due to the accumulation of higher solute concentrations in cells in response to salinity compared with non-AM plants. The difference in the ability to maintain osmotic potential under salt conditions between the AM and non-AM plants reflects the efficiency of AM fungus (*G. fasciculatum*) to overcome the deleterious effect of salinity stress. If so AM symbiosis could be used to increase the salinity tolerance of a halophytic plants under salinity conditions.

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