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**Effect of mycorrhiza-like endophyte (*Sebacina vermifera*) on growth, yield and nutrition of rice (*Oryza sativa L.*) under salt stress**

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This research was designed to evaluate the effect of mycorrhiza-like endophyte (*Sebacina vermifera*) on growth, yield and nutrition of rice (*Oryza sativa L.*) under salt stress. The experimental design was a completely randomized design in factorial with five replicates. Treatments included four solutions of NaCl with different salinity (non-saline (control), 3, 6 and 9 dS/m<sup>2</sup>) and fungi symbiosis (without fungi inoculation (-MLF) and with fungi inoculation (+MLF)). Results showed that panicle number, grain number, panicle weight and grain yield, which showed significant difference among treatments, were promoted by involving MLF. However, MLF colonization did not significantly change grain number, 1000 grain weight and harvest index of rice plants. Tolerance index (T<sub>i</sub>) of rice plants markedly decreased with an increase of salt stress; however, application of MLF caused a significant increase in T<sub>i</sub> under slightly to moderate salinity levels. MLF inoculation promoted P, N and Zn uptake under slightly and moderate salinity (S<sub>1</sub> and S<sub>2</sub>) as well as control plants. Microscopic inspection of roots inoculated with *S. vermifera* detected heavy colonization and abundant production of chlamydospores in root cells. Generally, the results of this experiment indicated a positive and ameliorate effect of MLF fungi on rice performance under salinity stress.

**Key words:** Tolerance index, colonization, yield and yield component, *Sebacina vermifera*, phosphorus,

**Abbreviations:** AM\_arbuscular mycorrhizal, EC\_Electrical Conductivity, LSD\_Least Significance Difference, MLF\_mycorrhizal-like fungi, Ti\_Tolerance index.

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## **Introduction**

Arbuscular mycorrhizal (AM) symbiosis is the most important mutualistic association between arbuscular mycorrhizal fungi (AMF) from soils and the roots of terrestrial plants (Gadkar *et al.*, 2001). It is well documented that AM symbiosis can improve plant water relations or enhance stress resistance of host plants, thus protecting host plants against detrimental effects caused by environmental stress (Augé, 2001). It is now accepted that the contribution of AM symbiosis to plant resistance is the result of accumulative physical, nutritional, physiological and cellular effects (Aliasgharzad *et al.*, 2006). Also, salinity is one of the most important environmental factors that limiting crop production of marginal agricultural soils in many parts of the world (Netondo *et al.*, 2004; Ievinsh, 2006; Ahmad *et al.*, 2009; Cha-um *et al.*, 2009; Nemati *et al.*, 2011). Salinity effects on plants include ion toxicity, osmotic stress, mineral deficiencies, physiological and biochemical perturbations and combinations of these stresses (Chartzoulakis and Klapaki, 2000; Netondo *et al.*, 2004; Ali *et al.*, 2004; Mansour *et al.*, 2005; Cha-um *et al.*, 2009). It has been well documented that salinity affects several growth parameters, e.g. decreased leaf cell expansion and leaf growth, reduced leaf area, dry matter accumulation, diminished rates of net CO<sub>2</sub> assimilation and relative growth (Ahmad *et al.*, 2009; Freipica and Ievinsh, 2010). Salinity also affects rice grain yield and yield components such as spikelet number and tiller number (Ali *et al.*, 2004). Furthermore, the plant responses to salinity, however, are complex and depend on duration of salinity, type of salt, development stage of plant at exposure (Zeng *et al.*, 2003), time of the day and many other factors, as well (Ahmad *et al.*, 2009). The term of mycorrhiza refers to the association between fungi and roots of higher plants (Prasad *et al.*, 2005), Arbuscular mycorrhizal (AM) fungi, forming the order Glomales of the Zygomycota, occur on the roots of 80% of vascular flowering plant species (Varma *et al.*, 1999). AM enhance plant growth by increasing nutrients and water uptake, prevent heavy metal toxicity, pathogenic infection and improve soil structure but they are obligate biotrophs and cannot be cultured without the plant (Varma *et al.*, 1999; Dolatabadi *et al.*, 2011). *Piriformospora indica* and *Sebacina vermicifera* (Arbuscular mycorrhizal-like fungi) belonging to Sebacinales (Basidiomycetes) referred to the novel plant-growth promoting symbiotic fungi (Singh and Varma, 2005) and were able to grow axenically on a variety of simple or complex media (Singh and Varma, 2005). These two cultivable strains of Sebacinales are commonly used as root inoculants on various plant hosts and improve plant growth and/or biotic and abiotic stress tolerance such as salinity, drought, heat and diseases (Varma *et al.*, 2001; Selosse *et al.*, 2009; Waller *et al.*, 2005; Bultruschat *et al.*, 2008; Kohler *et al.*, 2009; Kumar *et al.*, 2009;

Ghahfarokhi and Goltapeh, 2010; Sun *et al.*, 2010). Since rice (*Oryza sativa L.*) is moderately sensitive to salinity (Flovers and Yeo, 1981) and cultivated as a major crop in northern Iran (Feizabadi, 2011), where flooding system is dominant, widely encountered to salinity. Accordingly, in this research we examined ameliorating effect of mycorrhiza-like fungi in rice plant under different salinity stress.

## Materials and methods

This study was conducted at greenhouse and environmental stress laboratory of Sari Agricultural Sciences and Natural Resources University. The experimental design was a completely randomized design in factorial with five replicates. Experimental treatments included four solutions of NaCl with different salinity (control, 3, 6 and 9 dS/m<sup>2</sup>) and fungi symbiosis (without fungi inoculation (-MLF) and with fungi inoculation (+MLF)).

**Fungi Culture:** *Sebacina vermifera* were cultured in Petri dish containing Kaefer culture media (Kaefer, 1977) for 2 weeks, then transferred to liquid culture media and incubated in 20-25°C and 50rpm in a dark condition for 15 days. 5 pieces with 5mm diameter of fungus were used for inoculation in each pot.

**Plant and soil preparation:** The recommend pot sizes (30 cm height and 25 cm diameter) were chosen to provide up good conditions for rice plants. The pots filled with soil of experimental farm which puddled under flooded conditions (The results of some physical and chemical characteristics of soil are given in Table 1), then three 25-days old seedlings of rice cultivar of Tarom Hashemi were transplanted in each pot.

**Table 1.** Some chemical and physical properties of the soil

CEC (meq/100 gr)	EC (dS.m <sup>-1</sup> )	pH	K (ppm)	P (ppm)	N	sand (%)	silt	clay	Texture
27.34	2.39	7.14	254.3	18.88	0.252	18	35	47	clay

**Salt treatment:** Rice plants were exposed to salinity treatments in three stages including tillering, flowering and grain filling (approximately with 20 days interval from each other). The pots irrigated with the same amount of tap water and salinity treatments according to rice plant needs. Irrigation solutions were prepared and electrical conductivity (EC) was measured by EC meter (Caberscan con 410, Singapore).

Tolerance index (Ti): Ti of MLF and non-MLF rice plants to different NaCl levels was determined according to Shetty *et al.* (1995):

$$Ti = \frac{\text{dry weight of plant at salinity level} \times 100}{\text{dry weight of plant at 0.0 level of salinity}}$$

Morphological characters were collected at 10 days after use of stress treatments in each stage, number of tillers, numbers of active leaves per plant and leaf area were measured. Yield and yield components were collected after full maturity, two plants of each pot harvested and panicle number, grain number per panicle and plant, panicle weight, paddy yield, 1000 grain weight, harvest index and biological yield were determined. Root characters were observed after harvesting, two plants up-rooted completely and gently from the pots and washed with tap water carefully and some root related parameters such as root volume, length, root fresh weight and dry matter, shoot/root ratio and plant biomass were recorded. The root biomass was measured after placing them for 48 hours at aerated oven (BM750 Iran) at 70 °C. Root staining was done to study the symbiosis, 1cm pieces of rice root were cleared with KOH (10%) for 8 min then were stained in %5 ink-vinegar (Vierheilig *et al.*, 1998).

These stained samples were used in microscopically examine. Nutrient determination was investigated as nitrogen which determined using the Kjeltec Auto 1030 Analyzer (Tecator AB, Höganäs, Sweden). Phosphorus was photometrical determined using the molybdate-vanadate method according to Jackson (1973). Fe<sup>++</sup> and Zn<sup>+</sup> were determined using the Perkins-Elmer Atomic Absorption Spectrophotometer.

Data were statistically analyzed by using SAS (SAS Institute 2002) and GenStat12 software and the means were compared by using Fisher's Protected Least Significant Difference (LSD) test at 5 percent probability level.

## Results

Plant leaf area, active leaf number and tiller number per plant are presented in Table 2. Salinity at low level (S1) did not affect or slightly decreased plant leaf area and tiller number at 30 DAT, but at 50 and 70 DAT significantly decreased these parameters as compared to the control. However, tiller number per plant less respond to salinity compared to both plant leaf area and active leaf number (Table 2). By contrast, MLF colonization significantly improved these parameters in the salt-stressed plants but they remained lower than those described for control plants. Panicle number, grain number, panicle weight and grain yield, which showed significant difference among treatments,

were promoted by involving MLF. However, MLF colonization did not significantly change grain number, 1000 grain weight and harvest index of rice plants. There was no interaction between salt stress  $\times$  MLF for yield and yield components (Table 3). Changes in plant root parameters as affected by NaCl salinity and MLF are shown in Table 4. Root volume, fresh weight, dry weight and root biomass significantly improved by MLF inoculation. Application of MLF, however, did not change both shoot-to root ratio and root length of rice plants (Table 4). N, Fe and Zn concentrations were significantly lower in the salt treated plants than that in the control plants. However, concentration of P did not differ significantly in salinity levels (Table 5). In the present experiment, MLF inoculation promoted P, N and Zn uptake under slightly and moderate salinity ( $S_1$  and  $S_2$ ) as well as control plants. MLF inoculation did not improve Fe concentration under salinity stress. However, further increase in salt stress had no additional effect on Fe concentration. There was negative correlation (data not shown) between zinc and phosphorus in the leaves (Table 5). Tolerance index ( $T_i$ ) of rice plants markedly decreased with an increase of salt stress, however, application of MLF caused a significant increase in  $T_i$  under  $S_1$  and  $S_2$  levels of salinity.  $T_i$  did not differ significantly under either non-saline or sever ( $S_3$ ) salinity stress (Fig. 1). Microscopic inspection of roots inoculated with *S. vermicifera* detected heavy colonization and abundant production of chlamydospores in root cells (Fig. 2).

**Table 2.** The effect of salinity and MLF on plant leaf area, active leaf number and tiller number per plant at different dates after transplanting (DAT)

Treatments		30 days			70 days				
MLF inoculation	Salt stress	Plant leaf area	Active leaf number	Tiller number	Plant leaf area	Active leaf number	Plant leaf area	Active leaf number	Tiller number
-MLF	control	283.70	18.40	6.00	189.00	13.38	140.40	10.90	6.20
	$S_1$	281.10	18.20	5.38	121.20	10.60	32.30	4.70	5.40
	$S_2$	187.40	14.40	5.40	128.10	10.80	46.00	6.00	5.20
	$S_3$	150.70	13.10	5.60	79.60	9.10	16.10	1.76	5.00
	control	330.50	22.00	6.20	236.80	16.62	167.80	13.30	7.20
	$S_1$	280.90	19.70	6.30	153.60	11.62	42.50	5.50	6.50
+MLF	$S_2$	254.60	17.00	6.62	141.00	12.80	65.40	8.00	6.70
	$S_3$	188.90	14.70	6.12	86.30	9.20	12.50	1.90	4.38
	LSD (0.05)	36.29	2.78	0.7	33.81	1.66	14.94	1.86	0.91
Significance									
Salt stress (SS)		**	**	NS	**	**	**	**	**
MLF		**	**	**	**	**	**	**	**
SS $\times$ MLF		NS	NS	NS	NS	*	NS	*	*

\*significant at  $P < 0.05$ , \*\* significant at  $P < 0.01$ , NS Non significant

**Table 3.** The effect of salinity and MLF on yield and yield components of rice plant

Treatments		Panicle number	Grain number (per Panicle)	Grain number (per plant)	Panicle weight (g/plant)	Grain yield (g/plant)	1000 grain weight (gr)	Harvest index
MLF inoculation	Salt stress	Control	7.50	41.24	309.0	5.19	4.59	24.16
		S1	6.10	43.56	264.9	4.61	4.19	22.84
	-MLF	S2	5.25	43.74	229.4	3.69	3.29	22.32
		S3	4.70	50.28	232.3	3.16	2.76	20.80
		Control	8.70	40.94	354.6	5.50	4.91	23.84
	+MLF	S1	6.30	47.76	299.4	5.13	4.66	22.80
		S2	6.80	40.95	276.2	4.16	3.73	22.40
		S3	5.10	46.09	232.6	3.41	2.99	21.16
	LSD (0.05)		0.91	5.88	29.57	0.68	0.65	4.67
	Significance							
	Salt stress (SS)		**	**	**	**	**	**
	MLF		**	NS	**	*	NS	NS
SS × MLF		NS	NS	NS	NS	NS	NS	NS

\*significant at P< 0.05, \*\* significant at P< 0.01, NS Non significant

**Table 4.** The effect of salinity and MLF on root characters

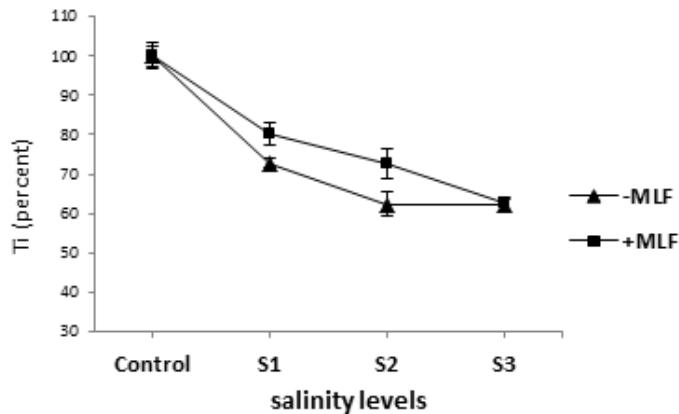
Treatments		Biological yield (gr)	Shoot /Root	Root length (cm)	Root volume (cm <sup>3</sup> )	Root fresh weight (gr)	Root dry weight (gr)	Biomass (gr)
MLF inoculation	Salt stress	Control	14.69	4.52	41.20	50.20	31.64	3.31
		S1	10.64	3.29	46.40	42.60	27.72	3.28
	-MLF	S2	9.13	3.61	42.20	42.50	20.39	2.6
		S3	9.09	3.58	49.88	35.80	19.77	2.58
		Control	15.19	4.44	40.00	54.43	34.51	3.47
	+MLF	S1	12.11	3.58	45.10	44.91	30.04	3.43
		S2	10.97	3.70	44.20	46.67	24.75	3.03
		S3	9.48	3.02	46.10	37.26	21.00	3.17
	LSD (0.05)		1.14	0.76	6.52	5.59	3.85	0.6
	Significance							
	Salt stress (SS)		**	**	*	**	**	**
	MLF		**	NS	NS	*	*	**
SS × MLF		NS	NS	NS	NS	NS	NS	NS

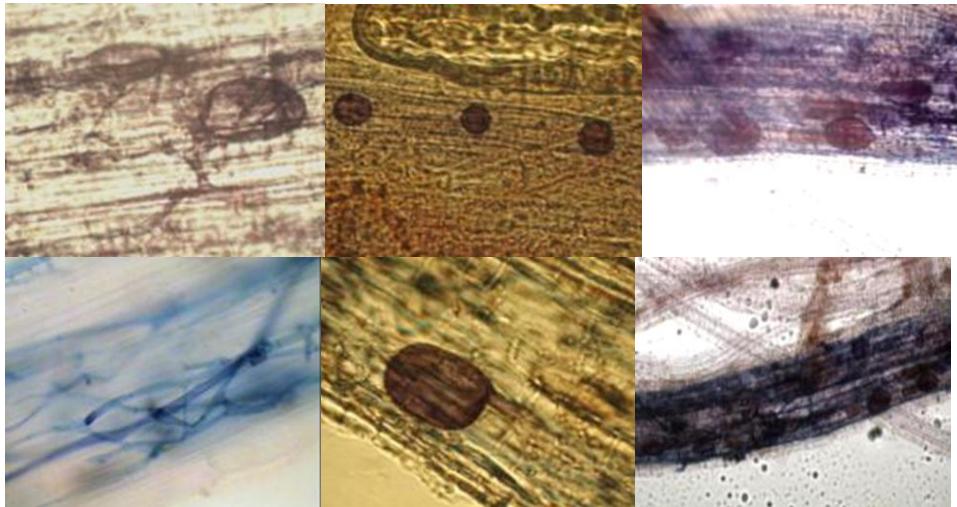
\*significant at P< 0.05, \*\* significant at P< 0.01, NS Non significant

**Table 5.** Some macro- and microelements of rice grains under different salinity and MLF levels

Treatments		P	N	Fe	Zn	P	N	Fe	Zn
MLF inoculation	Salt stress	mg/kg				mg/plant grain		μg/plant grain	
- MLF	control	1193	14513	72.4	34.0	5.49	66.8	332.7	155.3
	S1	1359	14729	71.8	32.3	5.72	61.1	301.4	135.3
	S2	1537	14684	77.6	35.5	5.06	48.1	256.9	116.5
	S3	1715	17054	67.8	33.5	4.65	52.9	202.9	98.9
	control	1169	14591	71.1	37.1	5.76	69.9	349.8	181.3
	S1	1344	14826	63.6	32.7	6.27	69.1	314.7	152.4
+MLF	S2	1535	15919	75.7	38.2	5.61	58.8	278.0	142.3
	S3	1880	16936	67.1	33.1	5.55	46.7	184.5	91.1
LSD (0.05)		267.7	538	19.8	2.58	1.08	7.15	80.54	20.02
Significance									
Salt stress (SS)		**	**	NS	**	NS	**	**	**
MLF		NS	*	NS	*	*	*	NS	**
SS × MLF		NS	**	NS	NS	NS	**	NS	NS

\*significant at P<0.05, \*\* significant at P<0.01, NS Non significant

**Fig. 1.** Tolerance index (Ti) of MLF and non-MLF plants at different salinity levels.



**Fig. 2.** Detection of chlamydospores of *Sebacina vermicifera* in root cells of *Oryza sativa*.

## Discussion

Salt stress adversely affected the growth parameters of rice plants, however, a more pronounced reduction in plant leaf area was observed in non-MLF plants. Similarly, the beneficial effect of mycorrhizae like endophytes, *Sebacina vermicifera* and *Piriformospora indica*, on plant growth and increasing of root and shoot biomass such as *Nicotiana attenuata* (Barazani *et al.*, 2007), maize and tobacco (Varma *et al.*, 1999) and tomato (Wang *et al.*, 2011) were reported. Additionally, previous studies demonstrated that inoculation of mycorrhizae like endophyte at moderate or high salt stress (Waller *et al.*, 2005; Baltruschat *et al.*, 2008) increased barley tolerance.

In this study, N uptake was higher in rice plants as a result of MLF inoculation which suggest that MLF as well as mycorrhizal fungi (Sing and Varma, 2005; Kaya *et al.*, 2009) can reduce the antagonistic effect of NaCl on N uptake. Micronutrients concentrations were declined with increasing salinity levels overall. However, this decline was less in MLF treated plants compared to non-treated plants. The previous studies have demonstrated that inoculation with AM fungi improves growth of plants under salt stress (Cho *et al.*, 2006; Kohler *et al.*, 2009) which mainly has been attributed to enhanced nutrient uptake, particularly of N and P and subsequent increased growth (Jeffries *et al.*, 2003; Kohler *et al.*, 2009; Al-Khalil, 2010; Dolatabadi *et al.*, 2011). There were no significant interaction between salt stress and MLF indicating that *Sebacina vermicifera* fungi in salt- stress and control plants could improve growth and yield components.

## Conclusion

It is concluded that salt treatments significantly affected plant biomass, leaf area, tiller number, grain yield and yield components, root parameters and some macro and micronutrients accumulation in Tarom Hashemi cultivar. Rice plants under both control and salt stress conditions seemed to be superior when MLF was supplied. Generally, the results of this experiment indicated a positive and ameliorate effect of MLF fungi on rice performance under salinity stress. It is also concluded that application of MLF is considered to be one of major effective ways to reduce salt stress disadvantageous but that further studies are needed to confirm the current results and determine long-term effects of MLF and its practicability on salt stress amelioration in rice cultivation.

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