



Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions

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aestivum* L.)

Summary

We conducted this study because of the importance of salinity in many parts of the world, and because almost all research regarding the effects of arbuscular mycorrhiza (AM) on plant growth under salinity have been conducted under greenhouse conditions. The hypothesis was that, with respect to the great abilities of AM fungi under stress, they are able to alleviate salinity stress under field conditions. Hence, the objectives were to evaluate the effects of different species of arbuscular mycorrhizal fungi on: (1) the yield and yield components of different wheat cultivars, and (2) nutrient uptake of different wheat genotypes under field saline conditions. The soil salinity of 7.41 dS/m and three AM species including *Glomus etunicatum*, *G. mosseae* and *G. intraradices* and wheat genotypes of Roshan, Kavir and a mutated line of Tabasi were tested. The electrical conductivity of the irrigation water was 13.87 dS/m. Under salinity, AM species significantly increased the growth and nutrient uptake of the mutated Tabasi line compared with the other genotypes, especially in comparison to the Kavir genotype. The mycorrhizal Tabasi genotype resulted in the lowest concentrations of Na⁺ and Cl⁻. The influence of different species of AM on enhancing plant growth under salinity was observed in the following order: *Glomus etunicatum* > *G. mosseae* > *G. intraradices*. The symbiosis of *Glomus etunicatum* and *G. intraradices* with the Tabasi mutated line resulted in the highest (42.08%) and the lowest (7.55%) increases in wheat dry weight, respectively. The highest (38.3%) and the lowest (4.5%) grain yield increases were related to the symbiosis of Tabasi mutated line with *Glomus etunicatum* and *G. intraradices*, respectively. Although different species of AM can be very beneficial to wheat plants under salinity stress, it is obvious from the results of this research that *Glomus*

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etunicatum can perform more efficiently under such conditions compared with other AM species. This indicates the great importance of selecting the right combination of AM species and host plant to make cultivation under salinity even more likely.
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Introduction

Soil salinity decreases crop yield through increasing osmotic stress on the plant. Under saline conditions, nutrient imbalance, reduced nutrient uptake including P, and iontoxicity are resulted because of high Na⁺ and Cl⁻ concentrations (Alam, 1994; Jacoby, 1994; Cornillon and Palloix, 1997; Miransari and Smith, 2007). Under arid and semi-arid conditions, the unfavorable effects of salinity are intensified due to practices such as fertilization and irrigation with saline ground water (Villa-Astoria et al., 2003). New methods are necessary to increase plant tolerance under salinity. This is particularly important when subsoil salinity exists (Munns and James, 2003).

One of the ways to decrease the unfavorable effects of salinity on plant growth is to produce inbred crop plants, which are highly tolerant to salinity stress (Miransari and Smith, 2007). In addition, in recent years, the use of biological methods as a practical way to alleviate soil stresses, including salinity, on plant growth has received increased attention (Al-Karaki and Hammad, 2001; Giri and Mukerji, 2004; Al-Karaki, 2006; Miransari and Smith, 2007, 2008; Miransari et al., 2007, 2008).

The symbiosis of arbuscular mycorrhiza (AM) with the host plant and hence, the production of a very extensive network of hypha, improves plant nutrient uptake and photosynthesis in the host plant (Mukerji and Chamol, 2003; Al-Karaki, 2006). Mycorrhizal symbiosis is actually a specialized network of hypha, enhancing the uptake and translocation of nutrients to the plant, compared with plant roots (Marschner and Dell, 1994). Hence, under soil stresses such as compaction and moisture tension, mycorrhizal plants are, due to the higher soil exploration by hypha, able to adsorb greater amounts of water and nutrients and hence increase their tolerance to the stresses (Ruiz-Lozano et al., 1995; Miransari et al., 2007, 2008).

Other researchers have noted that AM can alleviate the stress of salinity on plant growth through inhibiting high uptake of Na⁺ and Cl⁻ and their transfer to the plant shoots (Giri and Mukerji, 2004; Scheloske et al., 2004; Al-Karaki, 2006). In cotton plants under saline conditions, AM increased

plant dry weight by 68% through avoiding increased uptake of Na⁺ and Cl⁻ (Tian et al., 2004). In addition, the dry weight of mycorrhizal lettuce plants increased at salinity levels of 2, 4, 8 and 12 dS/m by 3.4%, 8.2%, 11.7% and 29.3%, respectively, compared with non-mycorrhizal plants (Cantrell and Linderman, 2001).

The inoculation of two tomato varieties under greenhouse conditions with AM under salinity stress increased plant dry matter and the uptake of P, K, Zn, Cu and Fe (Al-Karaki et al., 2001, 2006). Also, experiments under greenhouse conditions using saline water (2.4 dS/m) to irrigate tomato seedlings inoculated with *G. mosseae* indicated that the fresh weight of mycorrhizal plants increased by 60% and 29% under saline and non-saline conditions, respectively, compared with non-mycorrhizal seedlings (Al-Karaki, 2006). These results all indicate the greater effectiveness of AM under higher levels of stress or their significant interaction with the stress level (Miransari et al., 2007, 2008).

Almost all research regarding the role of mycorrhizal symbiosis in enhancing plant tolerance, growth and yield under salinity stress has been conducted under greenhouse or growth chamber conditions using sterilized soil, in which salty water or salty soil have been used. Data regarding the effects of AM on plant growth under field saline conditions are rare. With respect to the abilities of AM under stress, the hypothesis was that AM preinoculation of wheat plants grown under field conditions and irrigated with saline ground water could alleviate salt stress on wheat growth and yield. The objectives were to evaluate the effects of different species of arbuscular mycorrhizal fungi on: (1) the yield and yield components of different wheat cultivars, and (2) nutrient uptake of different wheat genotypes under field saline conditions.

Materials and methods

Inoculum preparation of arbuscular mycorrhizal species

The inoculum of arbuscular mycorrhizal species including *Glomus etunicatum*, *G. mosseae*

and *G. intraradices* isolated from saline soils (Aliasgharzadeh et al., 2001) were produced over a four-month period on sorghum plants under greenhouse conditions using sterilized sand (Miransari et al., 2007, 2008). The nutrient requirements of plants were supplied. Plants were irrigated until flowering, and after two weeks plant shoots were harvested from the soil surface. The mixture of plant roots and sand were used as the inoculum. The inoculation potential of different isolates was tested using the MPN method (Feldman and Idczak, 1992; Mahaveer et al., 2000). Sterilized soil concentrations of 0.1, 0.01 and 0.001 of AM were used for sorghum plantation, which were grown for 1 month. The plants were then harvested and the inoculation percentage on the sorghum roots was determined (Mahaveer et al., 2000).

Wheat varieties

Two wheat varieties, a local and an inbred one, and a mutated line were used. The Roshan variety is a spring cultivar, semi-early maturing, relatively tolerant to lodging, tolerant to drought and grain senescence, with a 46 g weight for the one thousand grains. The Kavir variety is a spring cultivar, early maturing, tolerant to lodging, grain senescence and salinity, with 36–40 g weight for the one thousand grains. The Tabasi mutated line (T-65-7-1) was produced by the Institute of Agricultural, Medical and Industrial Research, Tehran, Iran. This line is tolerant to drought and salinity stresses and also completely standing and tolerant to lodging. The Tabasi mutated line (T-65-7-1) was produced using preliminary experiments. After determination of the related amounts of radiation and seed moisture adjustment (11–13%) using Cobalt (Co^{60})

source, seeds were radiated with γ radiation of 300 g at 55 rad/min. The experiment was conducted in Shahryar, Karaj, Iran in 2006–2007 in a field with a 1500 m³ area.

Soil and water physical and chemical properties

Before planting, combined soil samples to a 30-cm depth were collected and their physical and chemical properties tested. Specifically, our tests included determination of soil texture using the hydrometry method (Gee and Bauder, 1986), pH and salinity of a saturated paste (Rhoades, 1982), organic C (wet oxidation method, Nelson and Sommers, 1982), total N (Kjeldahl method, Nelson and Sommers, 1973), and the concentration of available P (sodium bicarbonate extraction method, Olsen, 1954), available K (flame photometer method, emission spectrophotometry, Knudsen et al., 1982), Ca and Mg (using titration method). In addition, the concentrations of Cl (using chloridimeter), Fe and Mn (diethyenetriaminepentaacetic acid (DTPA) method, Baker and Amachar, 1982, using atomic absorption spectrometer, Model Perkin Elmer 3110) were determined (Table 1).

Water chemical properties including pH, electrical conductivity, sodium absorption ratio (SAR) and the concentrations of HCO_3^- , SO_4^{2-} , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , and Na^+ were also measured (Table 2).

Experimental design

The experiment was a factorial design (including two factors) on the basis of a completely randomized design in four replicates including 12 treatments,

Table 1. Soil physical and chemical properties.

Sand (%)	Silt (%)	Clay (%)	Soil texture	pH	E.C. (dS/m)	O.C. (%)	TN (%)
46	24	30	S-C-L/L	7.66	7.41	0.45	0.05
Ava.P (mg/kg)	Ava.K (mg/kg)	Ca (mg/L)	Mg (mg/L)	Cl^- (mg/L)	Fe (mg/kg)	Mn (mg/L)	
12	194	645	115	674	(3.76)	8.86	

E.C.: electrical conductivity, O.C. organic carbon, TN; total nitrogen, Ava.P: available P, Ava.K: available K.

Table 2. Water chemical properties.

pH	Salinity (dS/m)	Sodium absorption ratio (%)	HCO_3^- (mg/L)	SO_4^{2-} (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Cl^- (mg/L)	Na^+ (mg/L)
7.44	13.87	15.5	183	2684	3	698	256	2852	1893

which were randomly assigned to each replicate (making a total of 48 plots). The first factor was the species of AM including control (M0), *Glomus etunicatum* (M1), *G. mosseae* (M2), *G. intraradices* (M3), and the second factor was the wheat genotype, including the mutated Tabasi line (M), the inbred Kavir cultivar (K), and the local Roshan cultivar.

Field preparation and application of experimental treatments

N and P fertilizer were surface broadcast at 300 and 45 kg/ha using urea and triple superphosphate, respectively. The field was fertilized with 50% of the urea (150 kg/ha) and all of the triple superphosphate before seeding. The remaining part of the N fertilizer was applied at stemming (75 kg/ha) and flowering (75 kg/ha). Each plot measured 6 × 2 m and included 6 rows spaced at 25 cm. To avoid interaction between different plots, the two side rows were not planted. The spacing between plots in each replicate and the spacing between replicates were 1.5 and 3 m, respectively. The tillage practices, including cultivation and disking,

and were then milled. Plant P was measured spectrophotometrically by acetic acid extraction (Prokopy, 1995). K and Na were determined using a flame photometer, and Zn and Cl were measured using an atomic absorption spectrometer and chloridometer, respectively.

To measure yield components, 10 plants were harvested from the middle rows and plant height, ear length, number of grains per ear, number of ear per square meter, number of branches per plant, and root dry weight were determined. After drying the seeds at 75 °C, the weight of a thousand grains was determined. Grain protein was also measured using the wet oxidation method to measure the grain total N.

Root colonization by AM was determined by preparing root samples at 1 g in each experimental unit according to the method of Philips and Hayman (1970), and roots were stained using the Gridline-Intersect Method (Giovannetti and Mosse, 1980). The harvest index and AM symbiosis percentage for grain yield and shoot total dry weight were also measured.

For shoot dry weight and grain yield (Figure 1), the AM benefit (%) was calculated using the following equations (Raju et al., 1990):

AM benefit on shoot dry weight

$$= \frac{\text{Shoot dry weight for control plants} - \text{shoot dry weight for inoculated plants}}{\text{Shoot dry weight for control plants}} \times 100$$

AM benefit on grain yield

$$= \frac{\text{Grain yield for control plants} - \text{grain yield for inoculated plants}}{\text{Grain yield for control plants}} \times 100$$

were the common conventional practices in the region. Rows in the plots were created using a rower. The field was surface irrigated with the saline ground water using a water pump.

In each plot, the AM inoculum was applied underneath each seed at 200 active propagules (Most Probable Number method, Feldman and Idczak, 1992). Surface irrigation was used to irrigate the field with respect to the soil and climatic parameters and plant water requirements. Weeds were removed manually. At harvest, except for side lines which were not harvested, the complete plot yield was weighed and, using a combine, the straw was removed and grain total weight was determined.

To measure plant nutrient concentrations, 10 plants were randomly selected from each plot and washed with tap and distilled water. The plant samples were dried at 70 °C for 72 h using an oven

Statistical analysis

Data were analyzed using SAS (SAS Institute Inc., 1988). Analysis of variance was used to examine different experimental factors and their interactions. Treatment means were also compared (Steel and Torrie, 1980).

Results

Wheat cultivars and AM species significantly affected different parameters related to wheat growth and yield. Additionally, various AM species colonized wheat roots at different intensities. The significant interaction effects of AM and wheat cultivars on root colonization indicate that the response of wheat cultivars to root colonization by

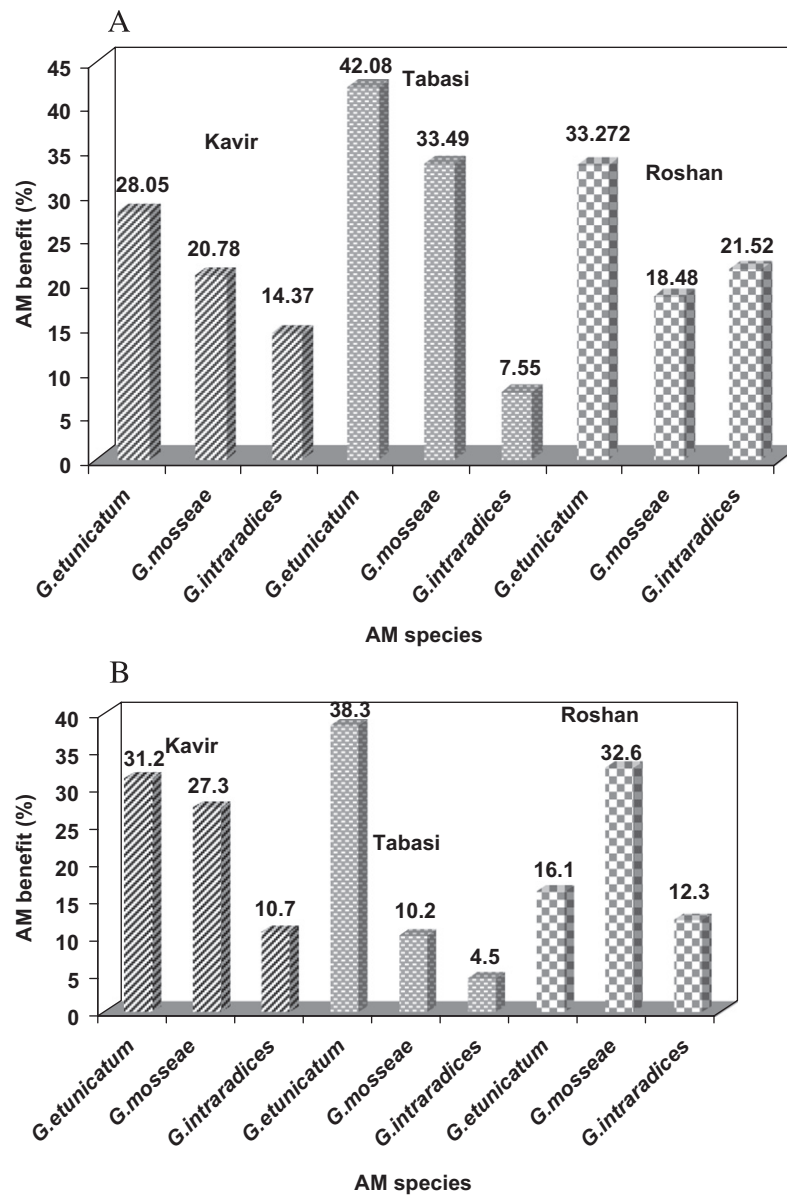


Figure 1. Arbuscular mycorrhizal benefit (%) on shoot dry weight (A) and grain yield (B).

different AM species differs among different cultivars (Tables 3 and 4).

While cultivar significantly affected the concentration of P, K, Cl and the K/Na ratio, the AM species exerted a significant effect on concentrations of all nutrients. The significant interaction of cultivar and AM on the concentrations of P and K and the K/Na ratio indicated that the symbiosis between the two symbionts resulted in differential nutrient uptake efficiencies for different AM–host plant combinations (Tables 5 and 6).

Among the different AM species tested in this experiment *Glomus etunicatum* and *G. mosseae*, respectively, had the greatest effects on plant growth and yield parameters. For *G. intraradices* only grain

yield, root colonization, K/Na ratio and P and K concentrations were significantly higher compared with those of control plants (Tables 5 and 6).

The highest (42.08%) and the lowest (7.55%) AM benefit (with respect to native AM colonization) on shoot dry weight were observed in mycorrhizal Tabasi in symbiosis with *Glomus etunicatum* and *G. intraradices*, respectively. The highest AM benefit on shoot dry weight was in the following order: *Glomus etunicatum*, *G. mosseae* and *G. intraradices*. Tabasi in symbiosis with *Glomus etunicatum* and *G. intraradices* resulted in the highest (38.3%) and lowest (4.5%) AM benefit on grain yield, respectively (Figure 1). The symbiosis of *Glomus etunicatum* with Tabasi and Kavir and the symbiosis

Table 3. Analysis of variance for grain yield and yield components.

S.V.	DF	PH	EL	NGE	NEM	NBP	GY	WT	BY	HI	GP	RDW	RC
Rep.	3	45.325	0.408	15.909	3607.409	0.191	0.148	607.810	0.0888	71.0213	0.2834	0.01552	5.400
Cul.	2	1101.128**	1.269	478.187**	31857.062**	1.328**	3.660**	136.438**	7.456*	710.977**	3.702**	1.452*	801.674**
AM	3	37.035	0.353	152.298	6672.576*	0.124	2.707**	106.776**	0.536*	298.311*	0.407	0.629	2430.27**
AM × Cul.	6	65.534	0.358	126.631*	1600.451	0.262	0.205*	41.775	0.188	221.611	0.843*	0.306	29.409*
Error	33	61.810	0.516	27.465	1991.182	0.089	0.079	19.100	0.166	108.358	0.280	0.291	12.114
C.V.		13.01	9.79	15.67	11.16	24.17	12.85	12.55	8.63	17.70	3.61	14.34	10.38

PH: plant height, EL: ear length, NGE: number of grains per ear, NEM: number of ears per square meter, NBP: number of branches per plant, GY: grain yield, WT: weight of thousand grains,

BY: biomass, HI: harvest index, GP: grain protein, RDW: root dry weight, RC: root colonization.

*5% level of probability.

**1% level of probability.

of *G. mosseae* with Roshan were the most efficient associations in increasing wheat yield under saline conditions.

Most growth and yield parameters of the mutated Tabasi line including grain yield, shoot dry weight, ear length, number of grains per ear, number of ears per square meter, number of branches per plant and harvest index were significantly different from those of other genotypes. With respect to plant height and weight of a thousand grains, the Roshan cultivar was superior compared to the other genotypes, and the difference was significantly different from the Kavir cultivar. Root dry weight of Tabasi was significantly higher than that of the other genotypes (Table 5).

While the shoot concentrations of P, K and Zn for Tabasi were significantly higher than the other genotypes, Na and Cl concentrations were the lowest and the highest in the Tabasi and Kavir genotypes, respectively (Table 6). Tabasi showed the highest percentage of root colonization, which was significantly different from the other genotypes (Table 5). In addition, the K/Na ratio was also the highest in the Tabasi genotype and was significantly different from Kavir and the control (not-inoculated) plants. With respect to grain protein percentage, Tabasi contained the highest amount, significantly different from Kavir (Table 6).

Discussion

The differences between different genotypes subjected to salinity, are obvious from our analyses. The results indicate that the mutated Tabasi line is more tolerant under salinity, compared with other genotypes. This is in agreement with Hetrick et al. (1984), who noted that different wheat genotypes, irrespective of their AM symbiosis, could perform differently under salinity.

Due to higher root dry weight and higher concentrations of P, K and Zn, Tabasi showed higher growth, grain yield and yield components under salinity. In addition, more translocation of C to the roots under stress resulted in the greatest root dry weight (Miransari and Smith, 2007, 2008; Miransari et al., 2007, 2008) and colonization and hence, AM symbiosis in this cultivar. It should also be noted that AM plants are able to adsorb higher rates of P, which, among other important roles in the plant, can markedly enhance root growth (Miransari et al., 2007, 2008). This can be very favorable for higher water and nutrient uptake under salinity stress.

Different researchers have indicated that the growth and grain yield of mycorrhizal plants

Table 4. Analysis of variance for wheat nutrient concentration.

S.V.		Mean of sum squares					
		P	K	Zn	Na	Cl	K/Na ratio
Rep.	3	0.00026	0.0177	22.865	0.581	5.564	0.0722
Cul.	2	0.0030**	2.055**	13.646	0.294	1.320**	0.284**
AM	3	0.0035**	1.228**	20.929**	1.178**	1.612**	0.067
AM × Cul.	6	0.0017**	0.245**	1.995	0.176	0.216	29.409*
Error	33	0.00005	0.038	4.505	0.243	0.146	0.061
C.V.		5.71	8.07	12.36	14.54	15.11	25.20

*5% level of probability.

**1% level of probability.

Table 5. Mean comparison of different wheat growth and yield parameters as affected by wheat genotypes, averaged for AM species and AM species averaged for wheat genotypes.

Trt.	PH (cm)	EL (cm)	NGE	NEM	NBP	GY (t/ha)	WT (g)	BY (t/ha)	HI (%)	GP (%)	RDW (g)	RC (%)
K	51.72b	7.14	36.56a	355.8b	1.70b	1.77c	31.68b	3.82c	51.18b	14.04b	3.72b	29.17b
M	61.24a	7.66	36.62a	445.6a	1.72b	2.23a	35.1ab	5.31a	63.59a	15.13a	4.11a	41.01a
R	68.25a	7.22	27.12b	397.5b	1.76b	2.76b	37.9a	4.7b	62.59a	14.32a	3.09b	29.78b
M ₀	58.97a	7.21	29.66b	373.7b	1.29a	1.26c	31.62b	4.31b	52.64b	14.83a	3.64a	13.62c
M ₁	61.74a	7.56	35.3a	427.8a	1.38a	2.90a	38.83a	5.02a	62.35a	14.83a	4.24a	45.61a
M ₂	62.11a	7.39	37ab	388ab	1.78a	2.6ab	35.1ab	4.8ab	62.28a	14.61a	3.90a	42.61a
M ₃	58.81a	7.20	31ab	408ab	1.13a	2.87b	33.70b	4.86b	57.6ab	14.71a	3.80a	32.44a

Tre.: treatment, K: Kavir, M: mutated Tabasi line, R: Roshan, M₀: control, M₁: *Glomus etunicatum*, M₂: *G. mosseae*, M₃: *G. intraradices*, PH: plant height, EL: ear length, NGE: number of grains per ear, NEM, number of ears per square meter, NBP: number of branches per plant, GY: grain yield, WT: weight of thousand grains, BY: biomass, HI: harvest index, GP: grain protein, RDW: root dry weight, RC: root colonization. Values within the same column, followed by different letters are statistically significant at 5% level of probability.

Table 6. Mean comparison of wheat nutrient uptake for different wheat genotypes averaged for AM species and AM species averaged for wheat genotypes.

Treatment	P (%)	K (%)	Zn (ppm)	Na (%)	Cl (%)
K	0.1263b	2.0324a	16.680b	3.491a	2.830a
M	0.1500a	2.7048a	18.224a	3.239a	2.258ab
R	0.1258b	2.5839a	16.574b	3.451a	2.505b
M ₀	0.1101c	1.9702b	15.7457b	3.660a	2.878a
M ₁	0.1445a	2.6759a	17.7913ab	3.209ab	2.489a
M ₂	0.1486a	2.5969a	18.6754a	3.048b	2.036b
M ₃	0.1330b	2.5185a	16.4272ab	3.658a	2.721a

K: Kavir, M: mutated Tabasi line, R: Roshan, M₀: control, M₁: *Glomus etunicatum*, M₂: *G. mosseae*, M₃: *G. intraradices*. Values within the same column, followed by different letters are statistically significant at 5% level of probability.

increase under salinity because of enhanced nutrient uptake and high root colonization in tolerant varieties (Subramanian and Charset, 1997; Al-Karaki et al., 2001; Cantrell and Linderman, 2001; Al-Karaki, 2006). This indicates that there are interaction effects between AM species and the stress. Accordingly, previous researchers have found that the efficiency of AM increases with increased level of stress (Hildebrandt et al., 1999; Tian et al., 2004; Audet and Charest, 2006; Subramanian et al., 2006; Miransari et al., 2007, 2008).

The significant effect of AM species on root colonization significantly affected the root colonization of different wheat cultivars. Higher root colonization by *Glomus etunicatum* and *G. mosseae* relative to *G. intraradices* resulted in increased nutrient uptake and less Na⁺ and Cl⁻ adsorption by plant, and hence, increased plant growth under salinity. This is very important under saline conditions, indicating the greater performance of such species and the reason for such superior abilities can be very applicable for cultivation in salty soils.

It is also very clear from the results that different combinations of wheat genotypes and AM species can perform differently under salinity stress. Their interaction was significant, emphasizing the great importance of selecting the right combination of AM species and host plant under stress to more efficiently alleviate the stress.

In addition to the enhanced uptake of P, K and Zn, through symbiosis with *G. mosseae* and *Glomus etunicatum*, the Tabasi genotype was also able to reduce the excess uptake of Na and Cl and hence their translocation to plant shoot. Thus, enhanced salt tolerance, and consequently, increased plant growth and yield, resulted. This is in agreement with the results of other scientists (Giri and Mukerji, 2004; Scheloske et al., 2004, Tian et al., 2004).

The K/Na ratio was the highest in the Tabasi genotype, and was significantly different from the Kavir genotype. In addition, there were significant differences between inoculated and control plants in this ratio, indicating the important role of AM in alleviating the stress. Rabie and Almadini (2005) also observed higher uptake of K by mycorrhizal plants compared with non-mycorrhizal plants under salinity. This can be very advantageous, as enhanced uptake of K under saline conditions can modify the unfavorable effects of Na on plant growth. This is also the reason for the higher application of K fertilizer under salinity.

Different species of AM differentially affected the growth and yield (van der Heijden et al., 1998a,b; Scheublin et al., 2004) of wheat genotypes under salinity. This is in agreement with the work of other researchers comparing AM species under different stresses (Feng et al., 2002; Mohammad et al., 2003; Miransari et al., 2007, 2008).

Conclusion

The results of this research work indicate the great significance of selecting more tolerant wheat genotypes (like Tabasi mutated line) and the right species of AM under stresses such as salinity. Accordingly, the symbiosis of Tabasi with more efficient AM species including *Glomus etunicatum* and *G. mosseae* under such conditions can be very beneficial in salty soils. According to the present results, Tabasi shows the important characteristics required for salt stress alleviation on plant growth under field saline conditions, including the high and efficient symbiosis ability with AM and morphological and physiological characters. The right combination of AM species and host plant can partially or

completely alleviate the stress of salinity and make the use of saline soil and water for cultivation of crop plants even more likely than before.

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