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Arbuscular Mycorrhizal Fungi and Spermine Alleviate the Adverse Effects of Salinity Stress on Electrolyte Leakage and Productivity of Wheat Plants

By

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With 3 Figures

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Summary

IBRAHIM A. H., ABDEL-FATTAH G. M., EMAM F. M., ABD EL-AZIZ M. H. & SHOKR A. E. 2011. Arbuscular mycorrhizal fungi and spermine alleviate the adverse effects of salinity stress on electrolyte leakage and productivity of wheat plants. – Phyton (Horn, Austria) 51 (2): 261–276, with 3 figures.

A pot experiment was conducted to investigate the possible role of arbuscular mycorrhizal (AM) fungi, spermine and their combination in alleviating the adverse effects of salinity stress on electrolyte leakage (EL), root colonization and productivity of wheat plants. Two levels of salinized underground water were used (6.09 dSm^{-1} and 10.63 dSm^{-1}). At three studied growth stages, salinity stress mark-

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edly increased the electrolyte leakage of wheat leaves and the effect was increased with increasing the salinity level. Although the application of low salinity level did not decrease shoot dry weight and grains number of wheat plants, it significantly decreased grain yield and harvest index of these plants. The highest salinity level markedly reduced all yield parameters. The imposed salinity stress significantly decreased total carbohydrates, protein and moisture level of the developed wheat grains. Conversely, the level of ash and fibers was increased in response to the applied stress. The inoculation with arbuscular mycorrhizal fungi mitigated the detrimental effects of salinity on EL, yield parameters and grains quality of wheat plants when compared to non-mycorrhizal treatments. The highest root colonization with AM fungi was observed at the booting stage, whereas the lowest one was reported at the tillering phase. Exogenous application of spermine reduced EL and improved the productivity of wheat plants grown in the stress conditions. Interestingly, the dual treatment with AM fungi and spermine added more enhancement of wheat yield in both control and salt stress conditions via reduction of EL and increase of mycorrhizal colonization.

Zusammenfassung

IBRAHIM A. H., ABDEL-FATTAH G. M., EMAM F. M., ABD EL-AZIZ M. H. & SHOKR A. E. 2011. Arbuscular mycorrhizal fungi and spermine alleviate the adverse effects of salinity stress on electrolyte leakage and productivity of wheat plants. [Negative Auswirkungen von Salzstress auf Elektrolyt-Leakage und Produktivität von Weizen werden durch arbusculäre Mycorrhiza-Pilze und Spermin verbessert]. – Phyton (Horn, Austria) 51 (2): 261–276, mit 3 Abbildungen.

Mit einem Topfexperiment wurde geprüft, ob durch arbuscularere Mycorrhiza-Pilze (AM), durch Spermin und durch eine Kombination beider die Negativ-Effekte von Salzstress auf Elektrolyt-Leakage (EL), Wurzelbesiedelung und Produktivität von Weizenpflanzen verbessert werden kann. Zwei unterschiedliche Salinitätsstufen (6,09 dSm^{-1} und 10,63 dSm^{-1}) wurden untersucht. Eine positive Korrelation zwischen Salinitätsstufen und einer merklich erhöhten EL durch den Salzstress wurde in allen drei geprüften Wachstumsphasen bei den Weizenblättern gefunden. Durch den Einfluss von geringem Salzstress nahm das Trockengewicht der Triebe und die Kornanzahl der Weizenpflanzen ab, Kornertrag und Ertragsindex waren signifikant verringert. Die höchste Salinitätsstufe reduzierte alle Ertragsparameter merklich. Durch den künstlich herbeigeführten Salzstress nahm der Gesamt-Kohlenhdrat-, Eiweiß- und Feuchtigkeitsgehalt der Weizenkörner ab. Genau umgekehrt verhielt es sich beim Asche- und Fasergehalt: als Stressantwort erhöhten sich diese. Ein Vergleich mit arbusculären Mycorrhiza-Pilzen inokulierten Pflanzen mit Pflanzen ohne Mykorrhiza zeigte eine Verringerung der schädigenden Auswirkungen auf EL, Ernte-Parameter und Qualität der Weizenkörner durch die Inokulation. Am besten mit AM besiedelt wurden die Pflanzen in der Phase des Ährenschiebens, während in der Bestockungsphase die Pilzbesiedelung am geringsten war. Exogen appliziertes Spermin reduzierte die EL und verbesserte die Produktivität der Weizenpflanzen unter Stressbedingungen. Interessanterweise hatten die Weizenpflanzen durch eine Doppelbehandlung mit AM und Spermin einen besseren Ertrag dank einer Reduktion der EL und einer Vermehrung der Mykorrhizabesiedelung, sowohl bei der Kontrolle, als auch bei den Pflanzen unter Salzstress.

Introduction

Salinity is a global problem reducing plant growth and productivity worldwide, and affects about 7 % of the world's total land area (FLOWERS & al. 1997, ABDEL-GHANI 2009). The area is still increasing as a result of irrigation and land clearing. Hence increased salt tolerance of crops and horticultural species is needed to sustain increases in food production in many regions in the world (MUNNS & al. 2006).

Membranes are important in regulating uptake and transport of ions and differences in response to salt stress exist at plasma membrane level between the salt tolerant and salt sensitive cultivar (KHATKAR & KUHAD 2000). Under salt stress conditions, plant tolerance and production are complex mechanisms. Many studies point to cell membranes as an initial site of stress injury and drastically damaged by an environmental stress. Commonly, changes in electrolyte leakage have been measured to detect stress injury of cell membrane. Leakage will vary in relation to the membrane's abilities to take up and retain solutes and therefore, will reflect stress induced changes in both membrane potentials and membrane permeability (HOQUE & ARIMA 2000, KHATKAR & KUHAD 2000, LIU & al. 2006).

The introduction of arbuscular-mycorrhizal (AM) fungi to site with saline soil may improve early plant tolerance and growth (POSS & al. 1985, RUIZ-LOZANO & al. 1996, GIRI & MUKERJI 2004). Although improved salt tolerance of mycorrhizal plants can be related to enhanced mineral nutrition, particularly phosphorus (ABDEL-FATTAH 1997), the effect of these fungi on salt tolerance may not only be limited to this mechanism. Arbuscular-mycorrhizal fungi may influence plant hormones (DANNEBERG & al. 1992) or improve water uptake (RUIZ-LOZANO & AZCON 2000). Other mechanisms may include osmotic adjustment, which assists in the maintenance of leaf turgor, and effects on physiological processes such as photosynthesis, transpiration, conductance and water use efficiency (JUNIPER & ABBOTT 1993).

AM colonization have been shown to decrease yield losses of plants in saline soils (POSS & al. 1985). This improvement of AM plants productivity has been attributed especially to enhanced acquisition of low mobility nutrients such as P, Zn, Cu (ABDEL-FATTAH 2001). As a result, endomycorrhizal plants are often more competitive and better able to tolerate environmental stresses than are non-mycorrhizal plants (PARADI & al. 2002).

Spermine is a polyamine (tetramine) and has been suggested to afford protection against a large variety of environmental stresses including salinity and potassium deficiency (CHATTOPADHAYAY & al. 2002, IBRAHIM 2004, ZHAO & al. 2007). The protective function of polyamines is mainly due to their cationic nature at cellular pH. By binding to proteins and lipids, polyamines can stabilize cellular structures such as thylakoid membranes (TIBURCIO & al. 1994). Polyamines have also been proposed to act as radical scavengers and as regulators of K^+ channels in stomata (ZHAO & al. 2007). In this respect, IBRAHIM 2004 found that seeds soaking in 0.03 mM spermine mitigated the adverse effects of seawater salinity stress on growth, chlorophyll content, K/Na ratio and yield of broad bean (*Vicia faba* L.) plants.

Recently, the involvement of polyamines (PAs) in the interaction between hosts and mycorrhizal fungi has been studied (NIEMI & al. 2006, SANNAZZARO & al. 2007). Vitro cultivation system showed that ectomycorrhizal fungi and specific polyamines have a synergistic effect on adventitious root formation of *P. sylvestris* (NIEMI & al. 2002). However, up to now, no attempt has been made to evaluate the dual effect of spermine and AM fungi on performance of crop plants grown under salinity stress conditions. This study aimed to investigate the possible role of mycorrhizal inoculation, spermine and their combination treatments in mitigating the adverse effects of salinity stress on electrolyte leakage, mycorrhizal colonization and productivity of wheat plants.

Material and Methods

Plant and Growth Conditions

Grains of wheat (*Triticum aestivum* L. cv. GM9.) were surface sterilized in 0.005M $HgCl_2$ solution for three minutes and subsequently rinsed with sterilized distilled water. Some of the seeds were soaked in distilled water and others were soaked in 20 ppm spermine (SIGMA) for 20 hours. The choice of this concentration is based on a preliminary experiment. The soaked grains were germinated in Petri dishes for three days on moist filter paper under dark conditions. Four uniform germinated seedlings were planted into sterilized plastic pots (15 cm width x 20 cm height) containing autoclaved sterilized sandy soil (at 121 °C, 1.5 air pressure and for 2 separate one-hour periods). This soil was taken from the experimental farm of the Faculty of environmental agricultural sciences at Al-Arish. For mycorrhizal inoculation, each pot was inoculated with 1 gm of chopped fresh onion roots colonized by stock culture of *Glomus mosseae* and *Acaulospora laevis*. The inoculum was placed in wells below the seedlings before sowing. These mycorrhizal fungi were originally isolated from salinized Egyptian soil, purified, multiplied in a greenhouse and identified by the authors. Each non-mycorrhizal pot received 1 gm of autoclaved mycorrhizal onion roots and 1 ml of filtered (Whatman No. 1) washings of the fungal inoculum to provide the same microflora without mycorrhizal fungi. All pots were irrigated by tap water (control) until the third week, then the pots were allocated into twelve treatments: two mycorrhizal treatments (non-mycorrhizal and mycorrhizal) × three salinity levels (control, 6.09 dSm^{-1} and 10.63 dSm^{-1}) and two spermine levels (0 and 20 ppm). The saline water was obtained from 2 different natural underground water wells at Al Arish area (North Sinai, Egypt). These twelve treatments were technically replicated 10 times to give a total of 120 pots. Plants were grown in a glasshouse at the experimental farm of the Faculty of Environmental Agricultural Sciences at Al-Arish under natural day/night conditions (minimum/maximum temperature, relative humidity and day length were 14/18 °C, 55/65% and 10/11h respectively) and watered regularly with the corresponding irrigation water until grains maturity (150 days from planting). Two weeks from planting, each pot received 1g KNO_3 as inorganic fertilizer. Irrigation to field capacity was carried out when soil water content had fallen to 60 % of its initial value.

Analysis of Irrigation Water

Electrical conductivity and Cl-ions were determined according to the methods of RICHARDS 1954. Ca^{2+} and Mg^{2+} ions were evaluated following the procedure of JACKSON 1967. The concentration of Na, K was determined by a Perkin Elmer (Germany) flame photometer (model 2100).

Measurement of Electrolyte Leakage

Electrolyte leakage (EL) was measured by an electrical conductivity meter using the following formula:

$EL = EC_1/EC_2 \times 100$ (DIONISIO-SESE & TOBITA 1998). Where EC_1 is the initial electrical conductivity (fresh tissues); EC_2 is final electrical conductivity (121 °C killed tissues).

Determination of Mycorrhizal Root Colonization

Immediately after harvest, the root systems for each treatment were washed carefully with tap water to remove adhering soil particles. Randomly sampled roots were cut into 0.5 to 1 cm pieces for estimation of mycorrhizal colonization after clearing and staining with trypan blue (PHILLIPS & HAYMAN 1970). Stained root pieces were examined under a compound microscope (Nikon, Japan) at 40x magnification. The rate of mycorrhizal colonization (F %) was determined microscopically as described by TROUVELOT & al. 1986.

Estimation of Harvest Index

Harvest index = [Economic yield (grain yield)/ biological yield (above ground dry matter)] $\times$ 100 (BEADLE 1993)

Grain Biochemical Analysis

Protein, carbohydrates, fibers and ash content of grains powder were determined according to A.O.A.C. methods 1995.

Statistical Analysis

The experiment was a completely random and factorial design. Values were expressed as means and the significant differences between means at $P < 0.05$ were given by LSD test using SPSS software (version 10). In addition, the correlation coefficients between the economic yield and the other evaluated parameters were estimated.

Results

Saline Water Analysis

Main chemical characteristics of the used underground water are presented in Table 1. From this table, it is clear that the used irrigation water was rich in Na^+ and Cl^- ions and poor in K^+ ions.

Table 1. Chemical analysis of the used underground water.
All ions are expressed as mM.

Constituents	Salinity level		
	Control (S0)	Low (S1)	High (S2)
EC (dSm^{-1})	1.24	6.09	10.63
Ca^{2+}	8.0	24.0	50.0
Mg^{2+}	8.0	30.4	42
Na^{+}	2.5	44.5	201
K^{+}	0.4	0.47	0.84
Cl^{-}	4.0	41.0	57.0

Changes of Electrolyte Leakage (EL)

The results for electrolyte leakage are shown in Fig. 1. At all studies growth stages, salinity stress markedly increased the electrolyte leakage of wheat leaves as compared with control plants. This effect was increased with increasing the salinity level. It is also obvious from the results that the electrolyte leakage of the leaves increased with time. Mycorrhizal inoculation, spermine and their combination treatments, over all conditions, significantly reduced the EL of wheat leaves in relation with untreated (non mycorrhizal non spermine) plants. In the tillering phas, at low salinity level, the spermine treatment slightly decreased the EL of wheat leaves while the fungal infection either alone or in combination with spermine significantly reduced it in relation with the untreated plants. In the anthesis stage, at high salinity level, the fungal colonization treatment caused a non significant decrease in the EL which contrasts a significant decrease in case of the spermine treatment either alone or in combination with mycorrhizae.

Changes in Intensity of Mycorrhizal Colonization

The results presented in Table 2 indicate that mycorrhizal inoculation caused a marked root colonization at the three studied growth stages. Inversely, no mycorrhizal colonization was observed in the uninoculated wheat plants. The imposed salinity stress significantly decreased the mycorrhizal colonization of wheat roots and the effect was more elicited with the highest salinity level (10.63 dSm^{-1}). The dual treatment with M and spermine significantly increased root infection as compared with mycorrhizal plants only. The highest root colonization was observed at the booting stage, whereas the lowest one was reported at the tillering phase.

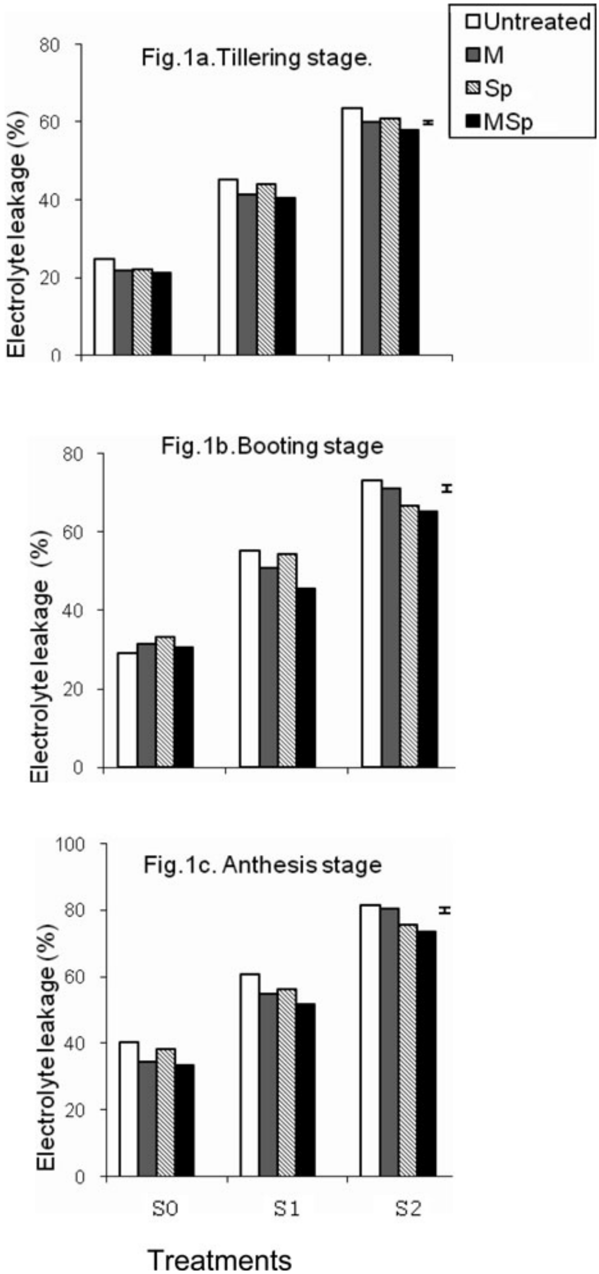

Fig. 1. Effect of arbuscular mycorrhizal and spermine on electrolyte leakage of wheat plants grown under salinity stress. Where, M, Mycorrhiza; Sp, spermine; S0, control; S1, 6.09 dSm^{-1}; S2, 10.63 dSm^{-1}.The vertical bars represent LSD value at $P < 0.05$.

Table 2. Effect of arbuscular mycorrhizal fungi and spermine on levels of mycorrhizalroot colonization of wheat plants grown under salinity stress. Where, S0, control; S1, 6.09 dSm^{-1}; S2, 10.63 dSm^{-1}. Values in each column with the same letter (s) are not significantly different at $P < 0.05$.

Treatments			Mycorrhizal colonization (%)		
Salinity	Spermine	Fungal status	Tillering stage	Booting stage	Anthesis stage
S0	–	+	43^{b}	76^{b}	51^{b}
	+	+	47^{a}	81^{a}	55^{a}
S1	–	+	37^{d}	67^{c}	49^{c}
	+	+	40^{c}	70^{c}	52^{b}
S2	–	+	34^{e}	61^{d}	41^{e}
	+	+	36^{d}	63^{d}	44^{d}

Changes in Yield Parameters

It can be seen from Fig. 2 that the administration of low salinity level (6.09 dSm^{-1}) non significantly decreased shoot dry weight and number of grains of wheat plants in comparison with control plants. The application of high salinity level (10.63 dSm^{-1}) markedly reduced theses parameters. Additionally, the two salinity levels greatly reduced 100 grains weight, grain yield and harvest index of wheat plants and the degree of reduction appeared to depend on the salinity level (Fig. 2). There was about 90% reduction in grain yield in case of the highest salinity level and about 50% with the lowest one.

The application of mycorrhizae and spermine mitigated the adverse effects of salinity stress on yield parameters of wheat plants. Again, the dual treatments with mycorrhizal fungi and spermine added more increase in grain yield in both control and stress conditions.

Changes in Biochemical Aspects of the Developed Grains

The imposed salinity stress significantly decreased total carbohydrates and protein content of the developed wheat grains and the effect was increased with increasing the salinity level (Fig. 3). Mycorrhizal colonization, spermine and their combination treatments alleviated the adverse effect of salinity stress on these metabolites. On some occasions, the combination treatment induced more increase than mycorrhizal and spermine treatment each alone.

It can be seen also that ash and fibers content of wheat grains was significantly increased in response to salinity stress. Moreover, the highest salinity level (10.63 dSm^{-1}) induced more increase than the lowest one (6.09 dSm^{-1}). Over all conditions mycorrhizal infection and spermine application significantly decreased the level of these substances as compared

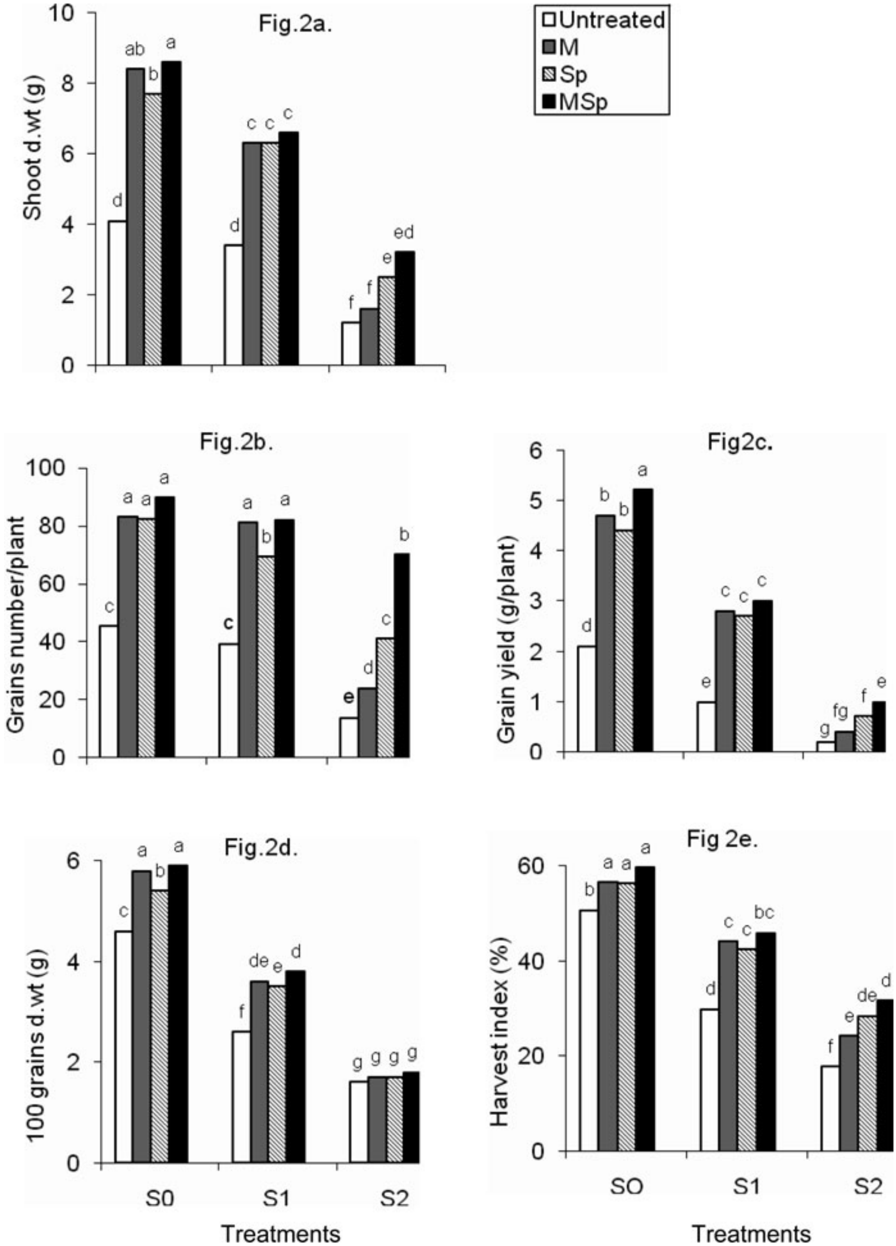

Fig. 2. Effect of arbuscular mycorrhizal fungi and spermine on yield parameters of wheat plants grown under salinity stress. Where, M, Mycorrhiza; Sp, spermine; S0, control; S1, 6.09 dSm^{-1}; S2, 10.63 dSm^{-1}. Values with the same letter (s) are not significantly different at $P < 0.05$.

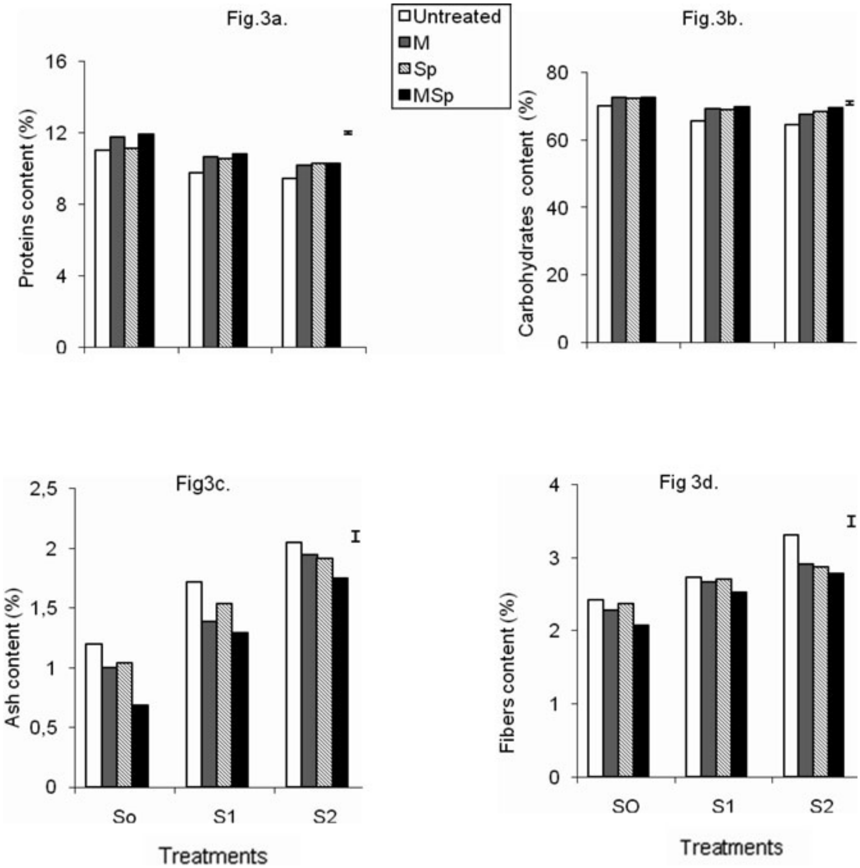

Fig. 3. Effect of arbuscular mycorrhizal fungi and spermine on biochemical aspects of grains of wheat plants grown under salinity stress. Where, M, Mycorrhiza; Sp, spermine; S0,control; S1, 6.09 dSm^{-1}; S2, 10.63 dSm^{-1}. The vertical bars represent LSD value at $P < 0.05$.

with non M non Sp treatment. The dual treatment with mycorrhizal fungi and spermine added more reduction in these substances than mycorrhizal or spermine treatment each alone.

Correlation Coefficients Between Grain Yield and the Other Evaluated Parameters

It is clear from Table 3 that the economic yield (grain yield) was not significantly correlated with the electrolyte leakage of wheat plants grown under control conditions. However, this correlation was significantly negative ($r = -0.74$; $p = 0.01$) under salinity stress conditions. This may indicate that reducing the leaf electrolyte leakage under salinity stress would improve wheat grain yield. A modest positive correlation appeared between grain yield and mycorrhizal root colonization. In control as well as stress conditions, shoot dry weight and grain biomass appeared to have the highest correlation coefficients with grain yield.

Table 3. Correlation coefficients (r) between grains yield and the other evaluated parameters of wheat plants grown under control and salinity conditions. Abbreviations: *, ** significant at $P < 0.05$ and 0.01 respectively.

Parameters	Correlation coefficients	
	Control	Salt stress
Electrolyte leakage	–0.14	–0.74**
Root colonization	0.63**	0.56*
Grains number	0.95**	0.89**
Shoot dry weight	0.98**	0.97**
Grain biomass	0.96**	0.98**
Harvest index	0.56**	0.86**
Protein content	0.73**	0.93**
Carbohydrates	0.85**	0.74**
Fibers content	–0.58**	–0.77**
Ash content	–0.83**	–0.97**

Discussion

The application of 6.09 dSm^{-1} and 10.63 dSm^{-1} salinity stress markedly increased the electrolyte leakage of wheat leaves at all studied growth stages. These results are in confirmation with those of HOQUE & ARIMA 2000 and KHATKAR & KUHAD 2000. This increase appeared to be due to plant cell injury and membrane damage with salinity stress (HOQUE & ARIMA 2000, SHIA & SHENGB 2005). Mycorrhizal inoculation either alone or in combination with spermine treatments mitigated the adverse effect of salinity stress on the electrolyte leakage of wheat leaves. This effect of mycorrhiza may be attributed to its improvement of nutrient uptake and reduction of specific ion effect (DIONISIO-SESE & TOBITA 1998). This supports the previous findings that AM-inoculated plants grow better than non-inoculated plants under salt stress conditions (RUIZ-LOZANO & al. 1996, GIRI & MUKERJI 2004).

The administration of spermine decreased the detrimental effect of salinity stress on the electrolyte leakage of wheat leaves. This is compatible with the finding of CHATTOPADHAYAY & al. 2002 that the exogenous application of spermine significantly prevented the leakage of electrolytes and amino acids from roots and shoots of 12- day old rice seedlings induced by salinity stress. This protective function may be because spermine is positively charged and thus can interact electrostatically with negatively charged nucleic acids and proteins, and block ions channel activities of the plasma membrane (BRÜGGEMANN & al. 1998, ZHAO & al. 2007).

Inoculated wheat plants had a relatively high mycorrhizal root colonization in non-saline treatments, which decreased as soil salinity increased. This agrees with the results of AL-KARAKI & al. 2001 and TIAN & al. 2004. They found that plants of the non-inoculation treatments were not colonized by mycorrhizal fungi and the colonization rate of inoculated plants declined with increasing NaCl level. This indicate that salinity suppressed the formation of arbuscular mycorrhiza and decreased the hyphal growth and viability of *Glomus sp*. to a higher extent (RUIZ-LOZANO & AZCÓN 2000). The reduction of the infectivity of AM fungi under high saline conditions may be due to the osmotic effect or toxicity of specific ion effect on mycorrhizal spores during germination and the reduction of hyphal growth (ESTAUN 1991).

The combination treatment of AM fungi with spermine, in all conditions, added more increase in wheat root colonization. These results confirm the conclusion of EL GHACHTOULI & al. 1995 that externally added polyamines increase mycorrhizal frequency between *Pisum sativum* and the endomycorrhizal fungus *Glomus intraradices* L. This is inconsistent with the observation of SARJALA 1999 that no correlation was found between polyamine concentration and mycelium growth for ectomycorrhizal fungi. The highest values of mycorrhizal colonization was observed at the booting stage, whereas the lowest one was reported at the tillering phase. This is in conformity with the finding of MOHAMMAD & al. 1998 that the level of AM infection increased with time, peaking at stem elongation and then decline between stem elongation and midgrain-filling stage.

Salinity stress greatly reduced most studied yield parameters and the effect was more elicited with the highest level of salinity (10.63 dSm^{-1}). These devastating effects of salinity are known to result from ionic, osmotic and oxidative stresses of plants (MUNNS & al. 2006). Moreover, grain yield and grain biomass appeared to be the most affected yield parameters. It seemed that the reduction in grain weight under salinity might be due to the poor development of the seeds which might result from the limited supply of the essential metabolites (GORHAM & WYN JONES 1993, ABDEL-GHANI 2009). Grain yield was more correlated with grain biomass than the other yield parameters for plants grown under the stress conditions. This is in accordance with the results of IBRAHIM 2004 that reduction of seeds yield of salinity stressed *Vicia faba* L. plants was mainly due to reduction of seed biomass. On the other hand, MAAS & POSS 1989 found that salinity stress reduced grain yield by reducing seeds number more than seed weight. Additionally, the applied salinity stress disturbed the biochemical aspects of developing grains. The observed reduction of total protein and carbohydrates content may be attributed to impaired protein and carbohydrates metabolism during the vegetative growth and reduction of thickness of conductive canals between flag leaf and spike of wheat plants (ALDESUQUY & IBRAHIM 2000). Salinity administration increased the ash content of developed wheat grains. This is expected results due to increase in mineral fraction (mainly Na^+) during the vegetative growth which is widely documented in response to salt stress (IBRAHIM 2004, REDONDO-GOMEZ & al. 2007, ZHAO & al. 2007). Increased grain fibers content in response to the applied salinity is possibly due to increased passive bi-product, phenolic compounds, during plant growth (LEE & al. 2007).

Mycorrhizal inoculation improved yield parameters and grain quality of wheat plants grown under salinity stress as compared with non-inoculated plants. In agreement with our results MOHAMMAD & al. 2004 found that wheat plants inoculated with mycorrhizal fungi produced significantly more grains per spike and greater weight per 1000 grain than control plants. This can be attributed to early colonization of the roots by the inoculum which may improve efficiency of P absorption and hence crop productivity (GRANT & al. 2001). Similar results have also been reported by DUKE & al. 1986 who concluded that there are some other mechanisms such as induction of osmotica that lead to osmotic adjustment and improved salt tolerance in mycorrhizal plants.

Many studies suggested that the exogenous application of polyamines could improve plant performances under saline conditions (CHATTOPADHAYAY & al. 2002, ZHAO & al. 2007). Our results indicate that the application of spermine mitigated the adverse effects of salinity on wheat yield and quality. This is compatible with the findings of IBRAHIM 2004 that the exogenous application of spermine alleviated the devastating effects of 50% seawater salinity stress on yield parameters of *Vicia faba* L. plants. This beneficial effect of spermine appeared, at least partially, due to mitigation of the adverse effects of salt stress on the electrolytes leakage of wheat leaves during the vegetative growth. Others attributed this for maintenance of K/Na homeostasis (ZHAO & al. 2007), chlorophylls and protein level (PANDEY & al. 2000, CHATTOPADHAYAY & al. 2002), and regulation of chloroplast-encoded genes (CHATTOPADHAYAY & al. 2002).

The present study has revealed that the dual treatment with mycorrhiza and spermine induced more enhancement in wheat yield under salt stress than mycorrhizal inoculation or spermine administration alone through the enhancement of root mycorrhizal colonization and reduction of over increase in EL. Our results are compatible with the findings of WU & al. 2010 that the dual spermine + AM fungi treatment increased leaf area, root/shoot ratio, root biomass, root colonization and arbuscule numbers compared with the sole AM fungi treatment. This support the concept that polyamines are involved in the interaction between host plants and mycorrhizal fungi (NIEMI & al. 2006) and this would improve salt tolerance of the wheat plants. In this regard, SANNAZZARO & al. 2007 showed that mycorrhizal fungi modulate the levels of polyamine. Further, polyamine levels differ between salt sensitive and tolerant plant species. Therefore, the levels of polyamine could be the mechanism by which mycorrhizal fungi alleviate salt stress. The addition of spermine to plants colonized with mycorrhizal fungi could simply strengthen the beneficial effect of the fungi.

References

A.O.A.C. 1995. Official methods of analysis 17th ed. Association of analytical chemists. – Washington D.C., USA.

Abdel-Fattah G. M. 1997. Functional activity of VA-mycorrhiza (Glomus mosseae) in the growth and productivity of soybean plants grown in sterilized soil. – Folia Microbiol. 42: 495–502.

Abdel-Fattah G. M. 2001. Measurement of the viability of arbuscular-mycorrhizal fungi using three different stains; relation to growth and metabolic activities of soybean plants. – Microbiol Res. 156: 359–367.

Abdel-Ghani A. H. 2009. Response of Wheat Varieties from Semi-arid Regions of Jordan to Salt Stress. – J. Agron. & Crop Sci. 195: 55–65.

Aldesuquy H. S. & Ibrahim A. H. 2000. The role of shikimic acid in regulation of growth, transpiration, pigmentation, photosynthetic activity and productivity of *Vigna sinensis* plants. – Phyton (Austria) 40: 277–292.

Al-Karaki G. N., Hammad R. & Rusan M. 2001. Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. – Mycorrhiza 11: 43–47.

Beadle C. L. 1993. Growth analysis. – In: Photosynthesis and production in a changing environment. – In: Hall D. O., Scurlock J. M. O., Bolhar-Nordenkampf H. R., Leegood R. C. & Long S. P. (Eds.), A field and laboratory manual, pp. 36–46. – Chapman and Hall, London.

Brüggemann L. I., Pottosin II & Schönknecht G. 1998. Cytoplasmic polyamines block the fast-activating vacuolar cation channel. – Plant J. 16: 101–105

Chattopadhayay K. M., Tiwari B. S., Chattopadhyay G., Bose A., Sengupta D. N. & Ghosh B. 2002. Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants. – Physiol. Plant. 116 (2): 192–199.

Danneberg G., Latus C., Zimmer W., Hundeshagen B., Schneider-Poestsch H. J. & Bothe H. 1992. Influence of vesicular-arbuscular mycorrhiza on phytohormone balances in maize (*Zea mays* L.). – J. Plant Physiol. 141: 33–39.

Duke E. R., Johnson C. R. & Koch K. E. 1986. Accumulation of phosphorus, dry matter and betaine during NaCl stress of split-root citrus seedlings colonized with vesicular-arbuscular mycorrhizal fungi on zero, one or two halves. – New Phytol. 104: 583–590.

Dionisio-Sese M. L. & Tobita S. 1998. Antioxidant responses of rice seedlings to salinity stress. – Plant Sci. 135: 1–9.

El-Ghachtouli N., Paynot M., Morandi D., Martin-Tanguy J. & Gianinazzi S. 1995. The effect of Polyamines on endomycorrhizal infection of wild-type Pisum sativum. CV. Frisson (nod+myc+) and two mutants (nod-myc+and nod-myc-). – Mycorrhiza 5: 189–192.

Estaun M. V. 1991. Effect of NaCl and mannitol on the germination of two isolates of the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. Abstract, 3rd European symposium of mycorrhizas, University of Sheffield, U.K.

FLOWERS T. J., GARCIA A., KOYAMA M. & YEO A. R. 1997. Breeding for salt tolerance in crop plants – the role of molecular biology. – Acta Physiol. Plant. 19: 427–433.

GIRI B. & MUKERJI K. G. 2004. Mycorrhizal inoculant alleviates salt stress in Sesbania aegypyiaca and Sesbannia grandiflora under field conditions: evidence for reduce sodium and improved magnesium uptake. – Mycorrhiza 14: 307–312.

GORHAM J. & WYN JONES R. G. 1993. Utilization of Triticeae for improved salt tolerance in wheat. – In: Leih H. & Massoum A.A. (Eds.), Towards the rational use of high salinity tolerant plants, p.: 27–33. – Kluwer, Amsterdam, Netherlands.

GRANT C. A., FLATEN D. N., TORNASIEWICZ D. J. & SHEPPARD S. C. 2001. The importance of early season phosphorous nutrition. – Can. J. Plant Sci. 81: 211–224.

HOQUE M. D. A & ARIMA S. 2000. Evaluation of salt damage through cell memprane stability monitored by electrolyte leakage in water chestnut (Trapa sp.). – Bull.Fac. Agr. Saga Univ. No 85: 141–146.

IBRAHIM A. H. 2004. Protective role of kinetin and spermine on seawater stressed broad bean (*Vicia faba*) plants. – J. Union. Arab. Biol. Cairo 15 (B) Botany: 61–79.

JACKSON M. L. 1967. Soil Chemical Analysis. Constable Co. Ltd., London.

JUNIPER S. & ABBOTT L. 1993. Vesicular arbuscular mycorrhizas and soil salinity. – Mycorrhiza 4: 45–57.

KHATKAR D. & KUHAD M. S. 2000. Short-term salinity induced changes in two what cultivars at different growth stages. – Biol. Plant. 43 (4): 629–632.

LEE B. R., KIM K. Y., JUNG W. J., AVICE J. C., OURRY A. & KIM T. H. 2007. Peroxidases and lignification in relation to the intensity of water-deficit stress in white clover (*Trifolium repens* L.). – J. Exp. Bot. 58 (6): 1271–1279.

LIU J. H., NADA K., HONDA C., KITASHIBA H., WEN X., PANG X. & MORIGUCHI T. 2006. Polyamine biosynthesis of apple callus under salt stress: importance of the arginine decarboxylase pathway in stress response. – J. Exp. Bot. 57 (11): 2589–2599.

MAAS E. V. & POSS J. A. 1989. Salt sensitivity of wheat at various growth stages. – Irrigation Science (Historical Archive) 10, 29–40.

MOHAMMAD M. J., PAN W. L. & KENNEDY A. C. 1998. Seasonal mycorrhizal colonization of winter wheat and its effect on wheat growth under dry land field conditions. – Mycorrhiza 8: 139–144.

MOHAMMAD A., MITRA B. & KHAN A. G. 2004. Effects of sheared-root inoculum of Glomus intraradices on wheat grown at different phosphorous levels in the field. – Agri. Ecos. Envir. 103: 245–249.

MUNNS R., JAMES R. A. & LÄUCHLI A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. – J. Exp. Bot. 57 (5): 1025–1043.

NIEMI K., SARJALA T. & HÄGGMAN H. 2002. Effects of diamines on the interaction between ectomycorrhizal fungi and adventitious root formation on Scots pine in vitro. – Tree Physiol. 22: 373–381.

NIEMI K., SUTELA S., HÄGGMAN H., SCAGEL C., VUOSKU J., OKELA A. & SARJALA T. 2006. Changes in polyamine content and localization of *Pinus sylvestris* ADC and *Suillus variegatus* ODC mRNA transcripts during the formation of mycorrhizal interaction in an in vitro cultivation system. – J. Exp. Bot. 11: 2795–2804.

PANDEY S., RANADE S. A., NAGAR P. K., KUMAR N. 2000. Role of polyamines and ethylene as modulators of plant senescence. – J. Biosci. 25: 291–299.

PARADI I., BRATEK Z., BERECZ B. & HALASZ K. 2002. Influence of arbuscular mycorrhiza, P limitation and Cd-stress on polyamine contents of plants. – Acta Biologica Szegediensis 46: 59–60.

PHILIPS J. M. & HAYMAN D. S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. – Trans. Brit. Mycol. Soc. 55: 158–161.

POSS J. A., POND E., MENEG J. A. & JARRELL W. M. 1985. Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphate. – Plant and Soil 88: 307–322.

REDONDO-GÓMEZ S., MATEOS-NARANJO E., DAVY A. J,. FERNÁNDEZ-MUŃOZ F., CASTELLANOS E. M., LUQUE T. & FIGUEROA M. E. 2007. Growth and Photosynthetic Responses to salinity of the salt-marsh shrub *Atriplex portulacoides*. – Annals of Bot. 100: 555–563.

RICHARDS L. A. 1954. The Diagnosis and Improvement of Saline and Alkali Soils. USDA, Washington, DC., Handbook.

RUIZ-LOZANO J. M. & AZCÓN V. 2000. Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and Glomus deserticola under salinity. – Mycorrhiza 10: 137–143.

RUIZ-LOZANO J. M., AZCÓN R. & GOMEZ M. 1996. Alleviation of salt stress by arbuscular-mycorrhizal *Glomus* species in *Lactuca sativa* plants. – Physiol. Plant. 98: 767–772.

SANNAZZAROA A. I., ECHEVERRÍA M., ALBERTÓ E. O., RUIZ O. A. & MENÉNDEZ A. B. 2007. Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza. – Plant Physiol. and Biochem. 45: 39–46

SARJALA T. 1999. Effect of organic and inorganic nitrogen sources on endogenous polyamines and growth of ectomycorrhizal fungi in pure culture. – Mycorrhiza 8: 277–281.

SHIA D. & SHENGB Y. 2005. Effect of various salt-alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. – Environ. and Exp. Bot. 54: 8–21.

TIAN C. Y., FENG G., LI X. L. & ZHANG F. S. 2004. Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants. – Applied Soil Ecol. 26: 143–148.

TIBURCIO A. F., BESFORD R. T., CAPELL T., BORNELL A., TESTILLANO P. S. & RISUENO M. C. 1994. Mechanism of polyamine action during senescence responses induced by osmotic stress. – J. Exp. Bot. 45: 1789–1800.

TROUVELOT A., KOUGH J. L. & GIANINAZZI-PEARSON V. 1986. Mesure du taux de mycorhization VA d'un systeme radiculaire recherché de methods d'estimation ayant une signification fonctionnelle. – In: GIANINAZZI-PEARSON V, GIANINAZZI S. (Eds), Physiological and genetical aspects of mycoorrhizae, pp. 217–222. – INRA publications, Paris.

WU Q. S., ZOU Y. N. & HE X. H. 2010. Exogenous putrescine, not spermine or spermidine, enhances root mycorrhizal development and plant growth of trifoliate orange (Poncirus trifoliata) seedlings. – Int. J. Agric. Biol. 12: 576–580

ZHAO F., SONG C., HE J. & ZHU H. 2007. Polyamines improve K/Na homeostasis in barley seedlings by regulating root ion channel activities. – Plant Physiol. 145: 1061–1072.