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Synergistic effect of arbuscular mycorrhizal fungi and spermine on amelioration of salinity stress of wheat (*Triticum aestivum* L. cv. gimiza 9)

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Abstract

The previous studies about the interactive effect of spermine (Spm) and arbuscular mycorrhizal (AM) fungi on physiological aspects and root colonization in wheat under salinity stress are insufficient. In this regard, a pot experiment was conducted to investigate the effect of *Glomus mosseae* (Nicol.& Gred.) Gred. and Trappe and Spm on growth and some physiological aspects of wheat (*Triticum aestivum*L. cv. gimiza 9) plants grown under three levels of saline underground water (1.2 dsm^{-1} "control", 6.09 dsm^{-1} and 10.63 dsm^{-1}). Salinity stress significantly decreased shoot mass, total and specific leaf area, photosynthetic pigments, spermine and spermidine concentration of wheat plants in comparison with control plants and this effect was more pronounced with the highest level of salinity. The application of mycorrhizal inoculations or spermine mitigated the adverse effects of salinity stress on shoot biomass and specific leaf area of wheat when compared with non-mycorrhizal ones. These treatments appeared to enhance photosynthetic pigments, proline, protein, spermine and spermidine, and lowered putrescine contents of the salt-stressed plants. Interestingly, the dual treatments with AM fungi and spermineh have been considered as additional positive effect on growth, salt tolerance and mycorrhizal root colonization in wheat plants. This effect was accompanied with more enhancements in chlorophylls content at both salinity levels, and proline and protein content at the highest salinity level only. Furthermore, this combination treatment, AM fungi + Spm, caused more reduction in putrescine content than AM inoculation and spermine treatments each alone. A negative correlation appeared between the salinity tolerance index and putrescine level in the used cultivar at low and high salinity. The dual application of AM fungi with Spm could be important for salt alleviation in wheat plants growing in saline soils.

Keywords: Arbuscular mycorrhizal fungi, Chlorophyll, Proline, Protein, Polyamines, Salt tolerance index, Soil salinity, Wheat.
Abbreviations: AM_arbuscular mycorrhiza; Pas_polyamines; Put_putrescine; Spd_spermidine; Spm_spermine; S0_control; S1_6.09 dsm^{-1}; S2_10.63 dsm^{-1}; Chl a_chlorophyll a; Chl b_chlorophyll b.

Introduction

Soil salinity is a worldwide problem, restricting plant growth and production especially in arid, semiarid and tropical regions through reducing nutrients uptake and increasing osmotic stress of plants (Abdel-Ghani, 2009; Abdel-Fattah and Asrar, 2011). Those regions are still increasing as a result of salt water irrigation and land degradation. Hence, increase in salt tolerance of crops and horticultural species is required to sustain the increase in food production in many regions of the world (Munns et al., 2006; Nagaz et al., 2012).The most efficient strategy to solve the problem of salinity in developing countries is to improve the salt tolerance of crop plants because increasing the salt tolerance is much less expensive for poor farmers than using other management practices (e.g. leaching salt from the soil surface etc.) (Qureshiand Barrett-Lennart, 1998). In recent years, the use of the biological application (mycorrhizal symbiosis) is a practical method to alleviate soil stresses like salinity on plant growth has received a greater attention (Daei et al., 2009; Wu et al., 2010; Ibrahim et al., 2011; Abdel-Fattah and Asrar, 2012). Arbuscular mycorrhizal (AM) fungi as an integral part of terrestrial ecosystems, since they form symbiotic association with more than 90% of plant species (Harley and Smith, 1983; Mankarios and Abdel-Fattah, 1994; Van der Heijdenand Sanders, 2002). Many studies have reported the presence of the AM association in salt stress conditions (Pond et al., 1984; Ruiz-Lozano and Azcon, 2000; Agwa and Abdel-Fattah, 2001; Sheng et al., 2009; Kumar et al., 2010; Ibrahim et al., 2011). Several studies have reported the protective effect of AM fungi against salt stress and improved plant tolerance and growth (Al-Karaki et al., 2001; Sheng et al., 2009; Garg and Chandel, 2011; Abdel-Fattah and Asrar, 2012). Under salt stress conditions, plant tolerance and production are complicated mechanisms. Possible mechanisms for improving salt resistance of the mycorrhizal plants could be due to an increased in root hydraulic conductivity (Robert et al., 2008), stomatal regulation or transpiration rate (Allen and Cunningham, 1983), enhanced water uptake at low soil moisture levels as a result of extraradical hyphae (Fagbola et al., 2001), osmotic adjustment which promotes turgor maintenance even at low tissue water potential (Auge´ et al., 1986), increased photosynthetic activity, proline and carbohydrate accumulation, increased polyamines content (Niemi et al., 2006; Wu et al., 2010) and increased nutritional status in

Table 1. Interactive effect of spermine and mycorrhizal fungi on growth parameters and tolerance index of wheat plants grown under S0, S1 and S2 salinity level, where S_0, 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 $P \leq 0.05$.

Treatments salinity level	Spermine	Mycorrhizae	Shoot length (cm)	Shoot fw (g)	Shoot dw (g)	Specific leaf area ($cm^2.g^{-1}dw$)	Tolerance Index
S0	−	−	69.00^{d}	12.61^{d}	5.25^{d}	313^{d}	-
	−	+	77.33^{b}	14.04^{b}	6.99^{b}	343^{b}	-
	+	−	74.33^{c}	13.74^{c}	6.35^{c}	328^{c}	-
	+	+	83.30^{a}	14.45^{a}	7.59^{a}	371^{a}	-
S1	−	−	27.83^{f}	5.34^{g}	2.26^{g}	193^{g}	0.43^{c}
	−	+	28.67^{ef}	6.09^{f}	2.62^{ef}	234^{e}	0.50^{ab}
	+	−	28.20^{ef}	5.94^{f}	2.56^{f}	220^{f}	0.49^{b}
	+	+	29.93^{e}	6.41^{e}	2.70^{e}	243^{e}	0.51^{a}
S2	−	−	22.00^{h}	3.36^{j}	1.14^{j}	129^{i}	0.22^{f}
	−	+	23.50^{gh}	3.99^{i}	1.47^{hi}	143^{h}	0.28^{e}
	+	−	22.67^{gh}	3.88^{i}	1.41^{i}	135^{h}	0.27^{e}
	+	+	24.00^{g}	4.30^{h}	1.55^{h}	147^{h}	0.30^{d}

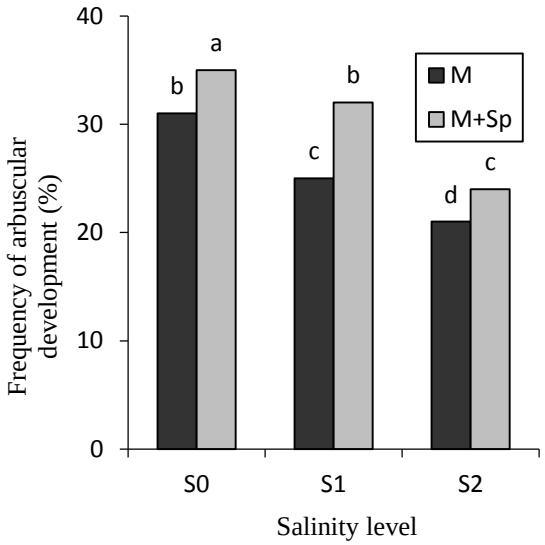

Fig 1. Interactive effect of spermine and mycorrhizal fungi on levels of mycorrhizal root colonization in wheat plants grown under salinity stress. Values with the same letter (s) are not significantly different at $P \leq 0.05$

mycorrhizal plants (Scheilenbaum et al., 1999). These mechanisms may be important in adaptation of the mycorrhizal plants to salt stress conditions. Polyamines (PAs) such as putrescine (Put), spermidine (Spd) and spermine (Spm) are small biologically active molecules involved in various physiological processes and play an integral role under different environmental stress conditions (Zapata et al., 2008; Goyaland Asthir, 2010). Some investigators suggested that PAs are either essential plant growth regulators (PGRs) or secondary messengers of PGRs (El Meskaouiland Trembaly, 2009). As compared with stress intolerant plants, stress-tolerant plants generally have a large capacity to enhance PAs biosynthesis in responses to stress, resulting in a 2- to 3-fold increase of endogenous PA levels over those in unstressed plants (Kasukabe et al., 2004). Additionally, exogenous spermine was found to alleviate the adverse effects of salinity stress on some plants (Ibrahim, 2004;Yamaguchiet al., 2006; Ali et al., 2009). The protective function of polyamines is mainly due to their cationic nature at cellular. By binding to proteins and lipids, polyamines can stabilize cellular structures such as thylakoid membranes (Tiburcio et al., 1994). Polyamines have also been proposed to act as radical scavengers, as regulators of K^+ channels in stomata (Zhao et al., 2007) and ethylene antagonist (El Meskaoui1 and Trembaly, 2009). Some recent studies have concerned the effect of exogenous polyamines on root mycorrhizal development and plant growth (Wue et al., 2010; Ibrahim et al., 2011). However, the interactive effect of spermine and mycorrhizal fungi on physiological aspects and root colonization in wheat under salinity stress is lacking. Thus, the objective of the present study was to evaluate the effect of exogenous spermine, arbuscular mycorrhizal fungi and their combination on growth and frequency of arbuscular development in wheat plants grown under two levels of salinity. In addition, changes in photosynthetic pigments, proline, protein, and polyamine contents were assessed in these plants.

Results

Changes in growth criteria

The results presented in Table 1 indicate that S1 (6.09 dSm^{-1}) salinity significantly decreased shoot mass, shoot length and specific leaf area of wheat plants in relation with control

Table 2. Correlation coefficients between the evaluated physiological parameters and mycorrhizal colonization, and the salt tolerance index of wheat plants, where *, ** significant at $P \leq 0.05$ and 0.01, respectively.

Parameters	Correlation coefficient at S1	Correlation coefficient at S2
Chl a	0.89**	0.78**
Chl b	0.88**	0.78**
Carotenoids	0.86**	0.93**
Proline	0.91**	0.93**
Protein	0.95**	0. 83**
Putrescine	− 0.97**	− 0.66*
Spermidine	0.54	0.95**
Spermine	0.48	0.50
Putrescine/Spermine + Spermidine ratio	− 0.99**	− 0.94**
Arbuscular development	0.75**	0.78**

plants. Application of S2 (10.63 dSm^{-1}) salinity added more reduction in these parameters. Exogenous application of spermine and mycorrhizae, each alone, mitigated the adverse effects of salt stress on these growth parameters. The dual treatment with mycorrhiza and spermine induced more enhancements in wheat growth than the sole treatments. The application of S2 caused 50% reduction in salt tolerance index (ST index) of wheat plants in comparison with S1. The mycorrhiza and spermine treatments enhanced ST index and the effect was more elicited with the dual treatment (mycorrhizae + spermine) than each one alone.

Changes in frequency of arbuscular development

The imposed salinity stress significantly decreased the frequency of arbuscular development of AM inoculated plants (Fig.1). There was about 20% and 30% reduction in response to S1 and S2 respectively. No mycorrhizal colonization was observed in the un-inoculated wheat plants. Over all conditions, the dual treatment with mycorrhizae and spermine significantly increased the frequency of arbuscular development in wheat roots as compared with mycorrhizal treatment only. The stimulation effect of exogenous spermine on arbuscular development was 17%, 28% and 15% in control, S1and S2, respectively.

Changes in photosynthetic pigments

It can be seen from Fig 2 that salinity stress significantly decreases chlorophyll "a", chlorophyll "b" and carotenoids concentration in wheat leaves in comparison with control plants. S1 and S2 salinity caused about 54% and 63% reduction in chl "a" levels respectively. The reduction in chl "a" was higher than chl "b" or carotenoids in response to the applied salinity. The application of spermineor AM fungi partially alleviated the adverse effects of salinity stress on these pigments. The combination treatment, AM fungi + spermine, added more enhancement in chlorophylls level than AM fungi or spermine alone. Over all conditions, the values of carotenoids content were nearly similar in response to AM inoculation, spermine and their combination treatments (Fig. 2c).

Changes in proline and protein content

The application of salinity stress significantly increased free proline and protein concentrations in wheat leaves in comparison with control plants (Fig. 3). The application of S1and S2 salinity increased free proline by 130% and 200% respectively. It may be interesting to note that S1 induced more increase in protein concentration in wheat than S2 salinity. Over all conditions, mycorrhizal inoculation and spermine treatments increased proline and protein levels of wheat plants as compared with the untreated plants. The dual treatment AM + Spm, at the highest salinity level only, added more enhancement in proline and protein content than spermine and AM inoculation each alone.

Changes in polyamines concentration

Putrescine (Put) was at higher concentration than Spd and Spm in wheat leaves (Figure 4). Put was accumulated in wheat in response to salinity stress in comparison with control plants. About 8 and 9 folds increase was found in response to 6.09 dSm^{-1} and 10.63 dSm^{-1} salt stress, respectively. Conversely, the applied salinity significantly decreased spermidine (Spd) and spermine (Spm) concentration of wheat leaves in relation with control plants. Over all conditions, the administration of AM inoculation and Spm greatly reduced Put levels in wheat in comparison with the untreated plants (non-spermine, non-mycorrhizal) plants. The dual treatment AM+Spm added more reduction in Put content of wheat leaves. It can be seen from the results that this combination treatment induced 33%, 76% and 50% reduction in Put level under control, S1 and S2 respectively. However, the application of AM inoculation, spermine and their combination treatments significantly increased spermidine and spermine levels in wheat in relation with the untreated plants. As expected from the increase of Put and the decrease of Spm and Spd contents in wheat under salinity, the estimated Put/(Spd+Spm) ratio was significantly increased in response to salt stress. Exogenous spermine and mycorrhizal inoculation appeared to reduce this ratio in wheat plants and the effect was more pronounced with the combination treatment. It can be concluded from Fig. 4 that the changes in total polyamine of wheat leaves were similar to those of the Put content.

Correlation coefficients with the salt tolerance index (ST index)

A summary of the correlation coefficients between salt tolerance index, frequency of arbuscular development and the evaluated physiological parameters at S1 (6.09 dSm^{-1}) and S2 (10.63 dSm^{-1}) salt stress conditions is presented in Table 2. The ST index had a strong positive correlation ($r = 0.78$-0.95, $p \leq 0.01$) with the frequency of arbuscular development, photosynthetic pigments, protein, proline and spermidine content at 6.09 dSm^{-1} salinity stress. On the other hand, puterscine and the ratio of put /spm + spd had a strong

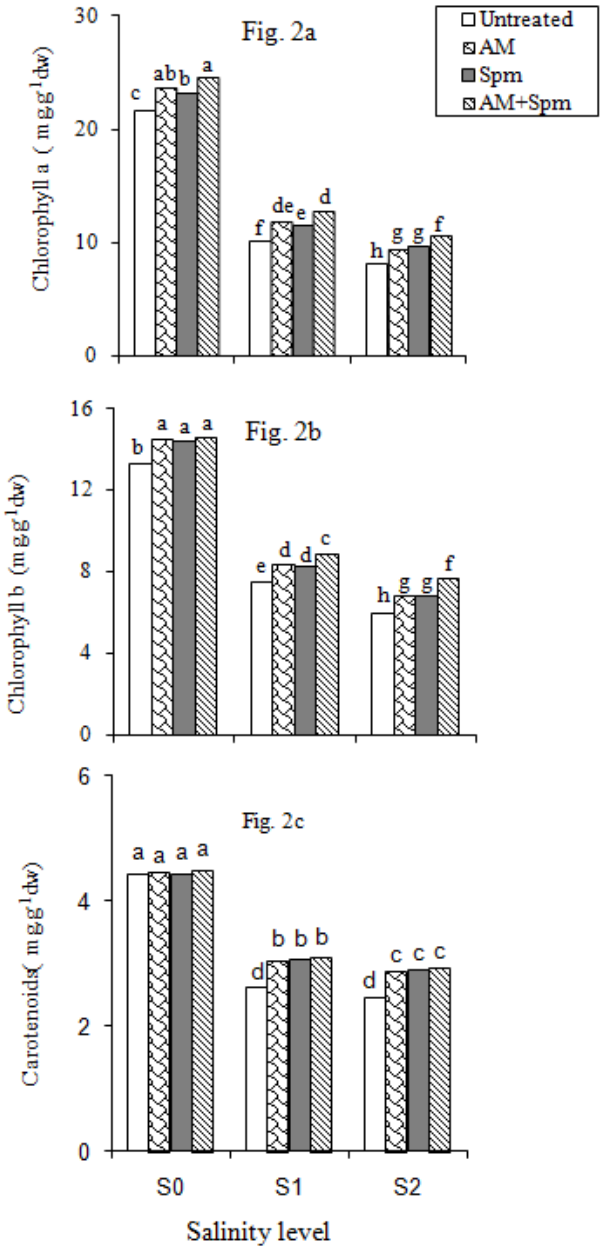

Fig 2. Interactive effect of spermine and mycorrhizal fungi on photosynthetic pigments content of wheat plants grown under salinity stress. Values with the same letter(s) are not significantly different at $P \leq 0.05$.

negative correlation with the estimated tolerance index. A non-significant correlation was appeared between ST index and spermine content. In general, the results for the correlation coefficients at S2 are comparable with those obtained S1 salinity.

Discussion

Salinity stress, unlike drought, is an intricate phenomenon which includes osmotic stress, specific ion effect, nutrient deficiency etc. thereby affecting various physiological and biochemical mechanisms associated with plant growth and development (Sairam et al., 2002).The observed decrease of wheat growth parameters in response to 6.09 dSm^{-1} and 10.63 dSm^{-1} salinity stress is in confirmation of already reported results (Abdel Samad, 1993; Sairam et al., 2002). The applied salinity not only affected the host plant but also the AM fungi. This negative effect of salt stress on mycorrhizal colonization was also reported by many researchers and probably due to the direct effect of salinity on the fungi (Al-karaki et al., 2001; Evelin et al., 2009). Our data are consistent with the findings of Ibrahim (2004) and Yamaguchi et al. (2006) that exogenous spermine alleviates the adverse effects of salt stress plant growth. Ali et al. (2009) attributed this to the antioxidant effect of spermine, which partially or completely counteracted the suppressive effects of moderate or high salinity levels on plant growth. In addition, the enhancement effect of mycorrhizal inoculation on shoot growth and salt tolerance of wheat plants is in good conformity with those of Al-karaki et al.(2001) and Sheng et al.(2009). This effect could be related to better leaf water status, less leaf membrane damage, higher solutes (proline and sugars), and higher leaf chlorophyll concentrations of AM plants than non-AM plants (Kumar et al., 2010). Chlorophyll contents have been suggested as one of the parameters of salt tolerance in plants (Srivastava et al., 1998) and carotenoids are responsible for quenching of singlet oxygen (Knox and Dodge, 1985); hence, their levels may determine the relative salt tolerance of the plants. In the present study, the administration of spermine or mycorrhizal inoculation mitigated the adverse effects of salinity stress on photosynthetic pigments content of wheat plants. These results are compatible with those of Ibrahim (2004) and Al-karaki et al. (2001). The enhancement effect of spermine on chlorophyll content may be attributed to increased stability of chloroplast membranes (Tiburcio et al.,1994), whereas that of mycorrhizal inoculation could be related to enhanced Mg and N uptake (Evelin et al., 2009). The observed increase in proline and protein content of salt stressed wheat plants is in a good conformity with the results of many authors (Sairam et al., 2002; Amini and Ehsanpour, 2005; Goudarzi and Pakniyat, 2009). Some other researchers reported an increase in proline and decrease in protein content (Lutts et al., 1999; Ibrahim, 2004). This increment in proline may results from induction of enzymes of proline biosynthesis and/or decrease doxidation to glutamate (Stewart, 1981). Recently, puterscine degradation under salt stress was also found to contribute in proline formation (Su and Bai, 2008). Several roles have been attributed to this supra optimal levels of proline e.g. osmoregulation (Delauney and Verma, 1993) and detoxification free radicals (Kaul et al., 2008). Higher protein concentration may be due to higher efficiency of osmotic regulation mechanism in wheat plants which in turn prevent proteins reduction under salt stress (Flowers and Yeo, 1995), and synthesis of osmotin like protein or structural protein (Amini and Ehsanpour, 2005). Exogenous spermine and AM colonization each alone added more increase in proline and protein levels of the salt stressed plants and this is compatible with Evelin et al. (2009) and Roychoudhury et al. (2011). The obtained strong positive correlation between the salinity tolerance index, and proline and protein content manifested the important role of these organic molecules in wheat defense against salt stress. The accumulation of putrescine (Put), and reduction of spermidine (Spd) and spermine (Spm) levels of the salt-stressed wheat plants was similar to findings reported in maize (Xingyu, 1999) and bean (Ibrahim, 2004) plants. These changes could result from increased arginine decarboxylase (ADC) activity that causes putrescine accumulation and impairment of higher polyamine biosynthesis from putrescine (Palavan-Ünsal, 1995). The application of spermine or AM fungi enhanced the Spm and

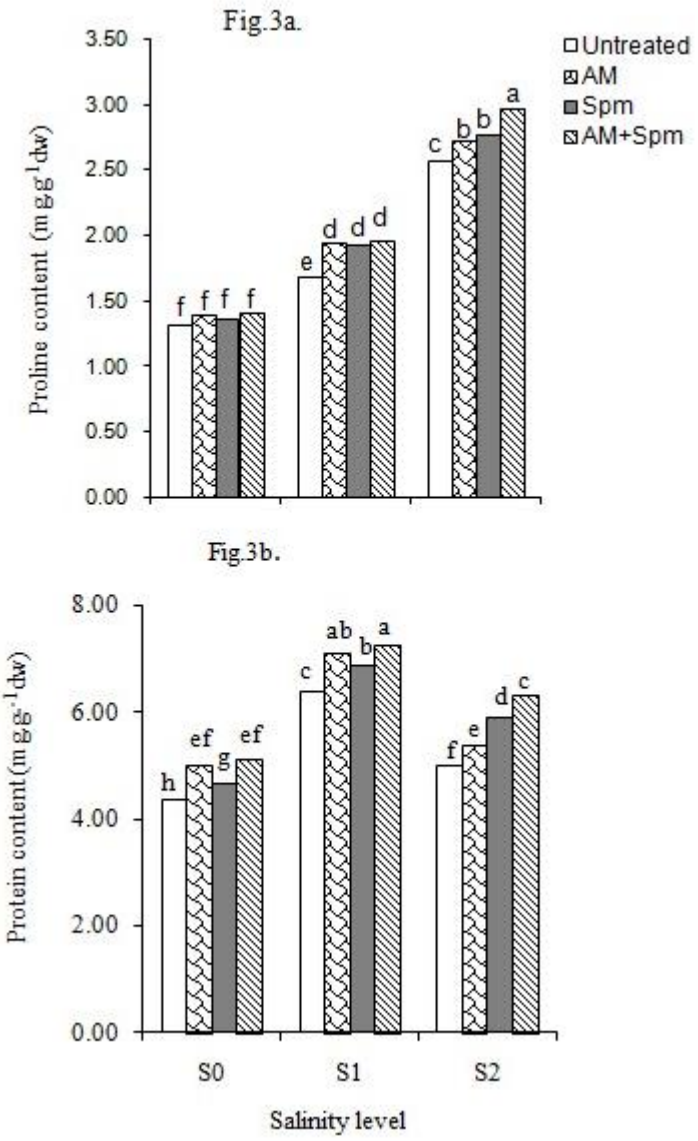

Fig 3. Interactive effect of spermine and mycorrhizal fungi on proline and protein content of wheat plants grown under salinity stress. Values with the same letter(s) are not significantly different at $P \leq 0.05$.

Spd level and lowered Put concentration of the salt –stressed plants. These results appeared torun in close parallelism with those of Goicoehea et al. (1998) for mycorrhizal plants and Ibrahim (2004) for spermine-treated plants. Interestingly, the decrease in Put content in response to exogenous spermine or mycorrhizal colonization was accompanied with an increase in free proline level. These changes could be attributed to the stimulation of proline biosynthesis from glutamate the common precursor of proline and putrescine or degradation of some putrescine to proline (Su and Bai, 2008). In this respect, Roychoudhury et al. (2011) found that the exogenous application of spermine elevated spermine and proline level, and lowered putrescine content of salt-stressed rice plants. The present study has revealed that the dual treatment with mycorrhiza and spermine induced more improvement in shoot growth and salt tolerance of wheat plants than mycorrhizal linoculation or spermine administration alone. This stimulatory effect appeared to be due to the enhanced AM root colonization that added more increase in proline and protein content, modulated chlorophylls, spermine and spermidine levels, and prevent over accumulation of putrescine in the salt- stressed plants. Our results support the concept that polyamines are involved in the interaction between host plants and mycorrhizal fungi (Niemi et al., 2006) and modulation of polyamine pools can be one of the mechanisms used by AM fungi to improve plant acclimation to saline soil (Evelin et al., 2009). In this respect, Wu et al. (2010) demonstrated that the dual spermine + AM fungi treatment enhanced growth parameters of trifoliate orange plants, root colonization and arbuscule numbers compared with the sole AM fungi treatment. The present investigation showed that arbuscular mycorrhizal inoculation and spermine alone or in combination significantly alleviated the harmful effects of salt stress on wheat plants grown in saline soil. These results may be of practical importance as they highlight the potential of using the dual treatment of spermine and mycorrhiza to improve plant tolerance and growth of plants growing under saline stress conditions, whereas the addition of spermine to mycorrhizal plants could strengthen the beneficial effect of the fungi.

Materials and methods

Plant material and growth conditions

Healthy and equalized Grains of wheat salinity sensitive cultivar (*Triticum aestivum* L. cv.gimiza9) were surface sterilized in 0.005M $HgCl_2$ solution for 3 min. and washed thoroughly with sterilized distilled water. Then the grains were divided into two sets according to the soaking solution. The 1st set was soaked in distilled water, whereas the 2nd soaked in 0.1 mM spermine (SIGMA) for 20 hours. The soaked grains were germinated in Petri dishes for three days on moist filter paper under dark conditions. Three uniform germinated seedlings were planted into sterilized plastic pots (15 cm width x 20 cm height) containing 4 kg of autoclaved (121ºC, 20 min; 1.5 air pressure for 3 separate time) sandy soil. For mycorrhizal inoculation, each pot was inoculated with 2 g of soil (approx. 120 spores/g soil) and 1 gm of chopped fresh onion roots colonized (M % = 85) by stock culture of *Glomus mosseae* (Nicol. &Gerd.) Gerd. and Trappe. The inoculum was placed in wells 3 cm below the seedlings before sowing to produce mycorrhizal plants. Each non-mycorrhizal pot received filter leaching (Whatman 1) from infected roots and sterilized equal amount of soil inoculum to provide the associated microorganisms other than mycorrhizal propagules. All pots were irrigated by tap water until the third week, then the pots were allocated into twelve treatments: two mycorrhizal treatments (non-mycorrhizal and mycorrhizal) × three salinity levels (control (1.2 dSm^{-1}), 6.09 dSm^{-1} and 10.63 dSm^{-1}) and two spermine levels (0 and 0.1 mM). The saline water was obtained from 2 different natural underground water wells at Al Arish area (North Sinai, Egypt). These twelve treatments were replicated 10 times to give a total of 120 pots. Plants were grown in a glasshouse under natural day/night conditions (minimum/ maximum temperature, relative humidity and day length were 14/18ºC, 55/65% and 10/11h, respectively) until anthesis stage (100 days from planting). Then the plants were harvest for growth, root colonization and biochemical analyses. During the course of the experiment, the plants were watered with the corresponding irrigation water when soil moisture content had fallen to 60 % of its initial value.

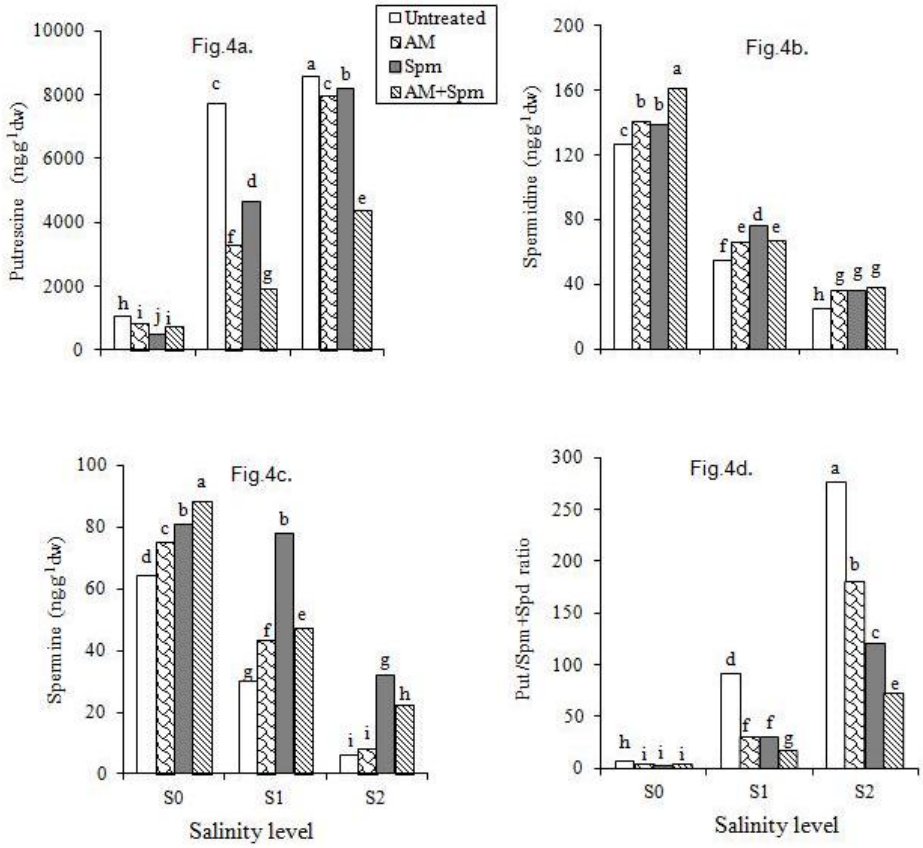

Fig 4. Interactive effect of spermine and mycorrhizal fungi on polyamines content of wheat plants grown under salinity stress. Values with the same letter(s) are not significantly different at $P \leq 0.05$.

Two weeks from planting, each pot received 1.5 g KNO_3 as inorganic fertilizer.

Measurements

Specific leaf area

The specific leaf area was estimated according to the following equation:

$$Specifiec\ leaf\ area = {}^{leaves\ area}/_{leaves\ dwt}$$

adopted by (Beadle, 1993).

Salt tolerance index (ST index)

Salt tolerance index of mycorrhizal and non-mycorrhizal wheat plants were determined according to (Shetty et al., 1995) as follows:

$$ST\ index = {}^{Dry\ mass\ of\ salt-stressed\ plants}/_{Dry\ mass\ of\ control\ plants}$$

Frequency of arbuscular development

Immediately after harvest, a part of the root systems for each treatment was 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 and Hayman, 1970). The frequency of arbuscular development (A%) was determined microscopically as described by Trouvelotet al. (1986).

Photosynthetic pigments concentration

Chlorophyll "a", chlorophyll "b" and total carotenoids (xanthophylls and β –carotene) were extracted rapidly from wheat flag leaf in acetone and their concentration were determined spectrophotometrically by the formulae of Lichtenthaler (1987).

Proline content

Proline was extracted by homogenization of a known leaf dry weight in 3% aqueous sulphosalicylic acid, and determined by the spectrophotometeric method according to Sadasivam and Manickam (1996) using pure proline (Merck) as a standard.

Total soluble proteins content

A known leaf fresh weight was macerated in phosphate-buffered saline (PBS) and the homogenate was centrifuged for 15 min. Protein was measured quantitatively in the supernatant by Commasie Brilliant blue method (Bradford 1976). The supernatant (100 µl) was mixed well with 5 ml of diluted dye binding solution. After at least 5 min but no longer than 30 min, the absorbency was read at 595 nm against a reagent blank.

Polyamines concentration

Dansylated polyamines were prepared from a trichloroacetic acid (TCA) extract of plant tissue by the direct dansylation technique as described by Greenland and Lewis (1984). Samples of the leaf tissue were pulverized in liquid nitrogen in a mortar with TCA (50 g/L). After centrifugation, the supernatant was removed and placed in glass centrifuge tubes on ice. The extract was mixed with 5 ml diethyl ether and the upper ether phase containing the TCA was removed and discarded. The resulting aqueous extract dried under a steam of air in water bath at 65 ^{o}C. Dansylated polyamines (putrescine, spermidine, and spermine) were estimated using thin layer chromatography (TLC) analysis (Vandemark et al., 1978).

Statistical analysis

The experiment was designed as a factorial with three factors (salinity, spermine, and mycorrhiza). Results were based on six replicates for growth and mycorrhizal colonization, and three replicates for physiological parameters. The significant differences between means at $P \leq 0.05$ (LSD test), and the correlation coefficients between salt tolerance index and physiological parameters were given by SPSS software (version 15).

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References

Abdel-Fattah GM, Asrar, AA (2012) Arbuscular mycorrhizal fungal application to improve growth and tolerance of wheat (*Triticum aestivum* L.) plants grown in saline soil. Acta Physiol Plant. 34: 267-277

Abde-Ghani, AH (2009) Response of wheat varieties from semi-arid regions of Jordan to salt stress. J Agron Crop Sci. 195: 55-65

Abdel-Samad HM (1993) Counteraction of NaCl and $CaCl_2$ or KCl on pigment, saccharides and mineral contents in wheat. Biol Plant. 35: 555-560

Agwa HE, Abdel-Fattah GM (2001) Arbuscular mycorrhizal fungi (Glomales) in Egypt. II- An ecological view of some saline affected plants in the Deltatic Mediterranean Costal land. Acta Bot Hung. 28: 36-47

Ali RM, Abbas HM, Kamal RK (2009) The effects of treatment with polyamines on dry matter and some metabolites in salinity – stressed chamomile and sweet majoram seedlings. Plant Soil Environ. 55: 477-483

Al-Karaki GN, 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

Allen EB, Cunningham GL (1983) Effects of vesicular mycorrhiza on *Distichlis spicata* under three salinity levels. New Phytol. 93: 227-236

Amini F, Ehsanpour A (2005) Soluble proteins, proline, carbohydrates and Na+/K+ changes in two tomato (*Lycopersicon esculentum* Mill.) cultivars under in vitro Salt Stress. Amer J Bioch Biotech. 1:204-208

Auge RM, Schekel KA, Wample RL (1986) Osmotic adjustment in leaves of VA mycorrhizal rose plants in response to drought stress. Plant Physiol. 82: 765-770

Beadle CL (1993) Growth analysis. In:Halldo JMO, Bolhar-Nordenkamp HR, Leegood RC, Long SP (eds.) *Photosynthesis and production in a changing environment. A field and laboratory manual*: 36-46. Chapman and Hall, London.

Bradford MM (1976) A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem.72:248-254

Chen S, Natan G, Bruria H (2009) Proteomic analysis of salt-stressed tomato (*Solanum lycopersicum*) seedlings: effect of genotype and exogenous application of glycinebetaine. J Exp Bot. 60**:** 2005-2019

Delauney AJ, Verma DP (1993) Proline biosynthesis and osmoregulation in plants. Plant J. 4:215-223

Evelin H, Kapoor R. Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Annal Bot. 104: 1263-1280

El Meskaouii A, Trembaly FM (2009) Effects of exogenous polyamines and inhibitors of polyamine biosynthesis on endogenous free polyamine contents and the maturation of white spruce somatic embryos. Afr J Biotech. 8:6807-6816

Fagbola O, Osonubi O, Mulongox K, Odunfa SA (2001) Effects of drought stress and arbuscular mycorrhiza on the growth of *Gliricdia sepium* (Jacq). Walp, *Leucaenal leucocephala* (Lam). Dewit. In simulated eroded soil conditions. Mycorrhiza. 11: 215-223

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants–Where next. Aust J Plant Physiol. 22: 875-884

Garg N, Chandel S (2011) Effect of mycorrhizal inoculation on growth, nitrogen fixation, and nutrient uptake in *Cicerarietinum* (L.) under salt stress. Turk J Agric For. 35: 205-214

Goicoehea N, Szalai G, Antolin MC, Sachez-Diaz M, Palidi E (1998) Influence of arbuscular mycorrhizae and *Rhizobium* on free polyamines and proline levels in water-stressed alfalfa. Plant Physiol. 153: 706-711

Goudarzi M, Pakniyat, H (2009) Salinity causes increase in proline and protein contents and peroxidase activity in wheat cultivars. J Appl Sci. 9:348-353.

Goyal M, Asthir B (2010) Polyamine catabolism influences antioxidative defense mechanism in shoots and roots of five wheat genotypes under high. Plant Growth Regul. 60:13-25

Greenland AJ, Lewis DH (1984) Amines in barley infected by brown rust and their possible relevance to formation of "green island". New Phytol. 96:283-291

Harley JL, Smith SE (1983) Mycorrhizal Symbiosis. Academic Press, London, p 483.

Ibrahim AH (2004) Protective role of kinetin and spermine on seawater stressed broad bean (*Vicia faba*) plants. J Union Arab Biol.15: 61-79

Ibrahim AH, Abdel-Fattah GM, Eman FM, Abdel-Aziz MH, Shokr AE (2011) Arbuscular mycorrhizal fungi and spermine alleviate the adverse effects of salinity stress on electrolyte leakage and productivity of wheat plants. Phyton. 51: 261-276

Kasukabey HE, Nada K, Misawa S, Ihara I, Tachibana S (2004) Over expression of spermidine synthase enhances tolerance to multiple environmental stress and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol. 45: 712-722

Kaul S, Sharma SS, Mehta IK (2008) Free radical scavenging potential of L-proline: evidence from in vitro assays. Amino Acids. 34:315-320

Knox JP, Dodge AO (1985) Singlet oxygen and plants. Phytochemistry. 24: 889-896

Kumar A, Sharma S, Mishra S(2010) Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation, and mycorrhizal dependency of *Jatropha curcas* L. J Plant Growth Regul. 29:297-306

Lichtenthaler HK (1987) Chlorophyll and cartenoids: pigments of photosynthetic biomembranes. Method Enzymol. 148: 350-382

Lutts S, Majerus V, Kinet JM (1999) NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. Physiol Plant. 105: 450-458

Mankarios AT, Abdel-Fattah GM(1994) Ecology of VA-mycorrhizal in some Egyptian soils. Egypt J Bot. 34: 135-152.

Munns R, James RA, Lauchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. J Exp Bot. 57: 1025-1043

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annal Review Plant Biol. 59: 651-681.

Nagaz K, Masmoudi MM, Ben Mechila N (2012) Impacts of irrigation regimes with saline water on carrot productivity and soil salinity. J Saudi Soc Agric Sci. 11: 19-27

Niemi K, Sutela S, Haggman 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

Palavan-Unsal N (1995) Stress and polyamine metabolism. Bulgar J Plant Physiol. 21:3-14

Philips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Brit Myco Soci. 55: 158-161

Pond EC, Meneg JA, Jarrell WM (1984) Improved growth of tomato in salinized soil by vesicular arbuscular mycorrhizal fungi collected from saline sites. Mycologia. 76: 74-84

Qureshi RH, Barrett L(1998) Three approaches for managing saline, sodic and waterlogged soils. In: Elbasam, N., Damborth, M. and Laugham, B.C. (eds.), *Saline agriculture for irrigated land in Pakistan*: 19-28. Kluwer Academic Publisher, Netherlands.

Robert M, Augé RM, Heather D, Carl F, Sams EA, Ghazala N (2008) Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza-induced increases in stomatal conductance. Mycorrhiza. 18: 115-121

Roychoudhury A, Basu S, Engupta DN (2011) Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of indica rice differing in their level of salt tolerance. J Plant Physiolol. 168:317-328

Ruiz-Lozano M, Azcon R (2000) Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity. Mycorrhiza. 10: 137-143

Sadasivam S, Manickam A (1996) *Biochemical methods*,p. 291. New age international limited publishers, 2nd edition, New Delhi.

Sairam RK, Rao KV, Srivastava GC (2002) Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci.163:1037-1046

Scheilenbaum L, Sprenger N, Schuepp H, Wiemken A, Boller E (1999) Effect of drought transgenic expression of a fructan synthesizing enzyme and of mycorrhizal symbiosis on growth and soluble carbohydrate pools in tobacco plants. New Phytol. 142: 67-77

Sheng M, Tang M, Chen H, Yang B, Zhang F, Huang Y (2009) Influence of arbuscular mycorrhizae on the root system of maize plants under salt stress. Canadi J Microbiol. 55:879-886

Shetty KG, Hetrick, BAD, Schwat AP (1995) Effect of mycorrhizae fertilizers amendaments on Zinc tolerance of plants. Envirn Pollut. 88: 308-314

Srivastava TP, Gupta SC, Lal P, Muralla N, Kumar A (1998) Effect of salt stress on physiological and biochemical parameters of wheat. Annal Arid Zone. 27:197-204

Stewart CR (1981) Proline accumulation: Biochemical aspects. In: Paleg LG, Aspinal LD (eds.), *Physiology and biochemistry of drought resistance in plants*: 243-259. Academic Press, Sydney.

Su GX, Bai X (2008) Contribution of putrescine degradation to proline accumulation in soybean leaves under salinity. Biol Plant. 52: 796-799

Tiburcio AF, Besford RT, Capell T, Bornell A, Testillano PS, Risueno MC (1994) Mechanism of polyamine action during senescence responses induced by osmotic stress. J Exp Bot. 45:1789-1800

Trouvelot A, Kough JL, Gianinazzi-pearson V (1986) Mesure du taux de mycorhization VA d'un systeme radiculaire recherché de methods d'estimation ayantune signification fonctionnelle. In: Gianinazzi-PearsonVand Gianinazzi S (eds.) Physiological and genetical aspects of mycoorrhizae:217-222. INRA publications, Paris.

Vandemark FL, Schmidt GJ, Slavin W (1978) Determination of polyamines by liquid chromatography and precolumn labelling for fluorescence detection. J Chromat Sci. 16: 465-469

Van der heijden MGA, Sanders IR (2002) Mycorrhizal ecology. Ecological studies. New York, USA: Springer-Verlag.

Wu QS, Zou YN, He XH (2010) Exogenous putrescine, not spermine or spermidine, enhances root mycorrhizal development and plant growth of trifoliate orange (*Ponciru strifoliata*) seedlings. Inter J Agric Biol. 12: 576-580

Xingyu J, Kefu Z, Junxia D (1999) The effect of NaCl on endogenous polyamine content in leaves and growth of maize and *Artiplex* seedlings. Plant Physiol Com. 35:188-190

Yamaguchi K, Takahashi Y, Berberich T, Imaia MA, Takahashi T, Michalel A, Kusano T, (2006) The polyamine spermine protects against high salt stress in *Arabidopsis thaliana*. FEBS Letters. 580:6783-6788

Zapatap J, Serrano M, Pretel MT, Botella MA (2008) Changes in free polyamine concentration induced by salt stress in seedlings of different species. Plant Growth Regul. 56:167-177

Zhao F, Song C, He J, Zhu H (2007)Polyamines improve K^+/Na^+ homeostasis in barleyseedlings by regulating root ion channel activities. Plant Physiol.145:1061-1072

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci. 6:66-71