

MITIGATION OF SALINITY STRESS OF PEPPER (*CAPSICUM ANNUUM* L.) BY ARBUSCULAR MYCORRHIZAL FUNGUS, *GLOMUS CONSTRICTUM*

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Abstract. A pot experiment was investigated to study the effect of arbuscular mycorrhizal (AM), *Glomus constrictum* (Trappe) on growth response, photosynthetic pigments, gas exchange parameters, antioxidant enzymes and nutrition of pepper plants irrigated with different concentrations of sea water. Four concentrations (tap water, 10%, 20% and 40% of sea water) were used. Salinity stress markedly reduced all studied parameters, except antioxidant enzymes, of pepper plants. Under saline conditions, AM fungi significantly increased growth parameters, photosynthetic pigments, nutrient contents (except Na⁺), gas exchange rate and antioxidant enzymes of pepper plants when compared to non-mycorrhizal ones. Those improvements were linked to mycorrhizal infection degree in pepper. Generally, growth, nutrition, photosynthetic pigments and antioxidant enzymes of pepper appeared to be highly dependent on the AM in salinized soil compared to non-salinized soil. This study suggested that mycorrhizal colonization could be used to mitigate the detrimental effects of salinity, particularly in soils irrigated with high concentration of sea water.

Keywords: salinity, mycorrhiza, growth, pepper, antioxidant enzymes

Abbreviations: *P_N*: net photosynthetic rate; AM: arbuscula mycorrhizal; AMF: arbuscular mycorrhizal fungi; AMR: arbuscula mycorrhizal response; Dwt: dry weight; Fwt: fresh weight; *E*: transpiration rate; *g_s*: stomatal conductance; LA: leaf area; Non-AMF: non-arbuscular mycorrhizal fungi; N: nitrogen; P: phosphorus; K: potassium; Na: sodium; Mg: magnesium; Ca: calcium; CAT: catalase; POX: peroxidase; APX: ascorbate peroxidase.

Introduction

Water salinity is a very important environmental problem, particularly in Saudi Arabia, decreasing plant growth and yields worldwide, and affecting about 10% of the world's global land (Abdel-Ghani, 2009; Al-Amri et al., 2013; Abdel-Fattah et al., 2016). The saline area is still extending due to irrigation and land clearing (Abdel-Fattah et al., 2013; Sheng et al., 2013). Increasing salt concentration in soil suppresses the plant ability to absorb and uptake water, negatively decreases metabolic processes, nutrient absorbance, root hydraulic conductivity and all photosynthetic rates, all of these results in negatively affecting the plant ability to grow (Al-Karaki et al., 2001; Abdel-Fattah and Asrar, 2012).

Pepper (*Capsicum annuum* L.) is an important vegetable plant in Saudi Arabia. Raising growth of pepper plant through improving the productivity area as increasing the cultivated area in salinized newly reclaimed lands is the major important national target. Increasing salt tolerance of vegetables, horticultures and economic plants is required to tolerate the increase in food production in many regions of the world in general and Saudi Arabia in particular (Al-Amri et al., 2013). Expansion of salt-tolerant vegetables or desalinization of soil by washing excessive salts is not enough to remove this problem (Kumar et al., 2010; Abdel-Fattah et al., 2016). Recently, application of biological agents like mycorrhizal symbiosis as a practical applied technology to

mitigate salt stress effects on plant growth and development has customary a great attention (Poss et al., 1985; Abdel-Fattah and Asrar et al., 2012; Al-Amri et al., 2013).

Several investigators have concluded that the existence of the arbuscular mycorrhizal plants grown in saline stress conditions (Pond et al., 1984; Kumar et al., 2010; Wu et al., 2010; Asrar et al., 2014). Furthermore, the biotechnological application of arbuscular mycorrhizal fungi in salinized soil may increase plant tolerance and growth via mitigating the adverse effect of salt stress in various plants (Juniper and Abbott, 1993; Daei et al., 2009; Abdel-Fattah and Asrar, 2012). Under salinity stress conditions, plant tolerance and production are complicated different mechanisms. In this connection, arbuscular mycorrhizal fungi has several mechanisms to increase salt tolerance of associated plants through stimulating nutrient uptake (Evelin et al., 2009; Abdel-Fattah et al., 2013); decreasing the uptake of Na^+ and preventing their movement to aerial parts of plant (Daei et al., 2009); increasing water uptake (Al-Karaki, 2006; Asrar et al., 2012); maintaining ionic balance by increasing and/or selective uptake of nutrients (Kumar et al., 2010); increasing of some antioxidant defense enzyme activities (catalase, peroxidase, superoxide dismutase and ascorbate peroxidase) and molecules like carotenoids, glutathione and tocopherols (Ibrahim et al., 2011); accumulating of polyamines like spermine and spermidine in associated host plants and regulating the osmotic status of plants through maintaining the turgor pressure of the leaves, and stimulating the photosynthetic and transpiration rate, and water use efficiency in the host plants (Juniper and Abbott, 2006; Auge et al., 2008).

Due to the detrimental effects of salt stress on different plants species and the function of arbuscular mycorrhizal fungi in improving the salinity tolerance, the present study was evaluated to study the efficiency of arbuscular mycorrhizal fungus (*Glomus constrictum*, Trappe) for enhancing plant growth, photosynthetic pigments, nutrition, gas exchange and some antioxidant enzymes of Pepper plants grown in soil irrigated with different concentrations of sea water.

Materials and methods

Experimental design

The experiment with a (2×4) factorial design was done. The treatments consisted of two arbuscular mycorrhizal ones [*Glomus constrictum* (AMF) and non-inoculated (--AMF)] and four levels {0.0% (control), 15%, 30% and 60% of sea water} g Kg^{-1} and 0.5 g kg^{-1} soil]. The sea water was obtained from the Red sea, Gadda, Saudi Arabia. Each of the eight treatments was replicated ten times (one plant per each pot) to give 90 pots as total.

Inoculum preparation of arbuscular mycorrhizal fungus

The inoculums mycorrhizal fungus, *Glomus constrictum* (Trappe), was isolated from saline site of Shaqra region using the wet sieving method of Gerdmann and Nicolson (1963). The spores of AM were left to multiply for 4 monthes in greenhouse under controlled environmental conditions. The mycorrhizal inoculums consisting of 10 g of rhizoplane soil (approx. 600 spores) and 0.5 g of colonized onion root pieces with a colonized level of 80% were inoculated to each pot. The mycorrhizal inoculums were added at 5 cm depth the pepper seedlings upon planting.

Plant and growth conditions

Seeds of Pepper (*Capsicum annuum* L.) were surface disinfected by soaking in 7% sodium hypochlorite for 10 min then rewashed with deionized sterilized water and left to germinate for 48 h on wetted sterilized filter paper in dark at 25 °C. Similar sterilized germinated seedlings were transplanted (one plant/pot) into 20 cm diameter plastic pots containing 3 kg of autoclaved (120 °C for 3 separate consequent time) sandy loam soil. Soil characteristics were: pH (water) = 7.31; available nitrogen (15.0 mg kg⁻¹); available phosphorus (5.12 mg kg⁻¹); potassium (40 mg kg⁻¹); magnesium (53 mg kg⁻¹) and organic matter (0.33%). All pots were irrigated by tap water (control) until the second week, and then the pots were divided into four groups for salinity treatments. The 1st group irrigated with tap water (control), the 2nd, 3rd and 4th groups were irrigated with 15, 30 and 50% sea water respectively. Chemical analysis of sea water are: Cl⁻, 21.5 g/l; Na⁺, 12.5 g/l; K⁺, 0.49 g/l; salinity, 38.2 g/kg; pH 8.2 and its electrical conductivity was 48 mmhos/cm. Half of all pots treated or non-treated with different concentrations of sea water were inoculated with the mycorrhizal fungus, *Glomus constrictum* (as described before). Arbuscular mycorrhizal inoculums were added 3 cm below the seedlings at planting time. The non-mycorrhizal treatments (-AMF) were provided with filtered washings of an equal amount of the mycorrhizal soil inoculum to provide the same associated microorganisms without AM spores. All pots were arranged in a complete randomized blocks design in a glasshouse of Experimental Station of Plant Production Department, College of Food and Agriculture Sciences, King Saud University under controlled conditions of 550 µmol m⁻² s⁻¹ light intensity, 23/18 °C day/night temperatures, 70–80% relative humidity and 16-h photoperiod. Plants for all treatments received 32 mg sulfur (K₂SO₄) pot⁻¹ as a nutrient solution after four weeks of planting. All plants were watered regularly with the corresponding irrigation sea water level. Ten plants for each treatment were harvested 10 weeks after planting.

Growth parameters

At harvest, the plants for each harvest were carefully uprooted with minimum disturbance of roots. The roots were washed with tap water to remove adherent soil particles and the shoot height per plant was measured. Roots and shoots fresh and dry weights (after drying the samples at 80 °C for 48 h in oven) were recorded. Leaf area was determined using a leaf area meter (Li-Cor, Lincoln, NE). Arbuscular mycorrhizal growth responses (AMR) for each estimated parameter was calculated following Menge et al. (1978) using the following formula: (parameter value of mycorrhizal treatment (AMF) - parameter value of non-mycorrhizal treatment (-AMF)) / {parameter value of non-mycorrhizal treatment (-AMF)} × 100.

Estimation of photosynthetic pigments

Photosynthetic pigments content (chlorophyll 'a', and 'b', carotenoids and total pigments) of the fully mature third leaves for each treatment was determined by extraction in 85% aqueous acetone for 5 min. The homogenate samples were centrifuged at 8000 rpm for 10 min. The supernatant was made up to 10 ml with acetone and it measured spectrophotometrically against a blank of aqueous acetone. The concentrations of the pigments were determined using the equations of Abdel-Fattah et al. (2013).

Measurement of photosynthesis, transpiration and stomatal conductance

Net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) of the mature third leaves were measured by using Li-Cor, 8400XT, Lincoln, NE, USA. Leaf was fitted into leaf chamber. Gas exchange measurements were made in the growth chamber under saturated light conditions and adjusted to an intensity of $480 \mu\text{mol m}^{-2} \text{s}^{-1}$. Four replicates for each treatment were used.

Nutrient analysis

Root and shoot samples for each treatment were oven-dried, grounded. A known weight of the dried material was extracted in a digestion flask containing 10 ml of three acid mixture ($\text{HNO}_3:\text{H}_2\text{SO}_4$: 60% HCL, at the ratio of 3:2:1 (v/v/v), respectively) in kjeldahed digestion flask. Phosphorus (P) was determined by the vanadate-molybdate colorimetric method (Jackson, 1973). Calcium (Ca^{++}), potassium (K^+), magnesium (Mg^{++}) and sodium (Na^+) in digested samples by using atomic absorption (model unicom 969). Total nitrogen (N) was analyzed using Kjeldahl method (Nelson and Sommers 1973).

Estimation the activities of antioxidant enzymes (Catalase, peroxidase and ascorbate peroxidase)

Antioxidant enzymes were analyzed by homogenization of a known leaf fresh weight in 50 mM cold phosphate buffer (pH 7.0) containing 1 mM EDTA (Ethylene Diamine tetra acetic acid) and 5 mM ascorbic acid in case of ascorbate peroxidase assay (Gajewska and Sklodowska, 2008). Peroxidase (POX) and Catalase activities (CAT) were estimated according to the method of Aebi (1983). The specific enzyme activity of CAT and POX was expressed as μmol of H_2O_2 consumed/min. Ascorbate peroxidase (APX) activity was assayed using the method of Nakano and Asada (1981) and the specific activity of this enzyme was expressed as μmol oxidized ascorbate/min.

Mycorrhizal colonization

After harvest, the root systems for each treatment were washed carefully with tap water to remove all the adhering soil particles. Randomly sampled roots from five plants per treatment were cut into 0.5 to 1 cm pieces, then cleared with 10% KOH solution and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970) for estimated of mycorrhizal colonization. 40 root segments of each treatment were putted on slides and were examined with a light microscope (Carl Zeiss, Japan) at 40x magnification, where visually were allocated six classes of mycorrhizal colonization (from 0 to 5, depending on the occurrence of mycorrhizal structure in the root segment) and four levels of arbuscular abundance (from A0 to A3). With this values, mycorrhizal colonization levels [Frequency of mycorrhizal colonization (F %), the intensity of mycorrhizal colonization (M %) and arbuscular frequency (A %)] of the stained roots were estimated according to Trouvelot et al. (1986) using the "Mycocalc" software (<http://www.dijon.inra.fr/mychintec/mycocalcprg/download.html>).

Statistical analysis

All results were statistical analyzed using two-factor analysis of variance (ANOVA). Means of all treatments were divided by Duncan's multiple range tests by the least

significant difference (LSD, $P \leq 0.05$) method using the Costat software. All of the measurements were performed five times for each treatment. The means and calculated standard error (SE) are recorded.

Results

Growth responses

Sea water stress significantly reduced biomass of shoots and roots, leaf area and shoot length of both mycorrhizal (AMF) and nonmycorrhizal (-AMF) pepper plants compared with control treatment (irrigated with tap water) and the rate of reduction increased with increasing sea water concentration in the soil. However, the reduction in most growth criteria was more pronounced in non-mycorrhizal pepper plants (Table 1). In this connection; Leaves of mycorrhizal pepper plants grown at higher concentrations of sea water were highly greener and less senescent than those in the non-AM plants (Fig. 1). Values of mycorrhizal dependency of pepper plants (AMR) in responding to AMF colonization were significantly increased with increasing sea water concentration in soil.

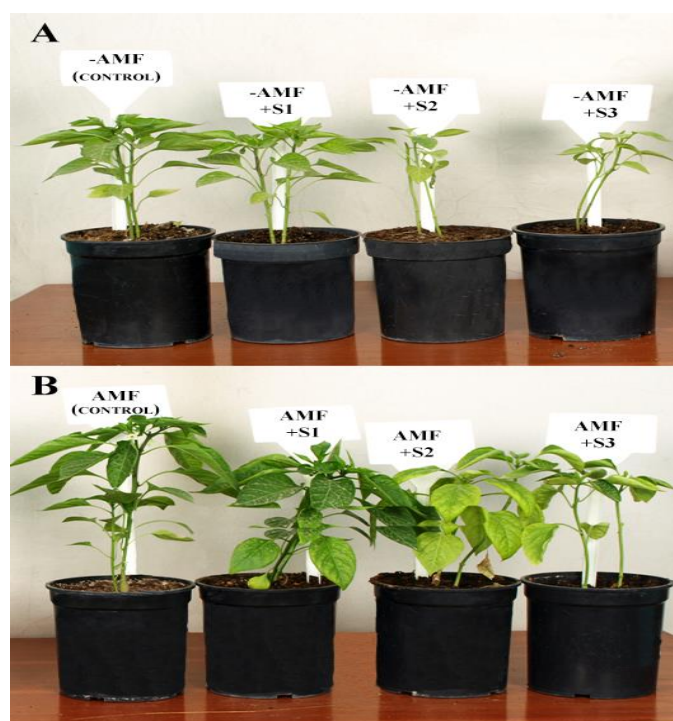


Figure 1. Growth of non-mycorrhizal (-AMF) [A] and Mycorrhizal (AMF) [B] Pepper plants grown in soil irrigated with different concentrations of sea water [Control (0.0), S1 (10%), S2 (20%) and S3 (40%)]. The leaves of non-mycorrhizal pepper plants were more senescent (less green) than those of mycorrhizal plants

Photosynthetic pigments

The photosynthetic pigments content (chlorophyll a, chlorophyll b and carotenoids) in mycorrhizal leaves of pepper plants were significantly greater than those of non-mycorrhizal plants grown either with or without sea water irrigated in soil (Table 2).

Such increases in these contents were linked to the degree of mycorrhizal colonization. In most cases, this increase in carotenoides content in leaves of pepper plants in response to mycorrhizal effects was increased with increasing sea water concentration in the soil, particularly 40%. On contrary, no significant differences chlorophyll “b” was observed between mycorrhizal plants irrigated with 10% sea water and control treatment (Table 2).

Table 1. Growth parameters of mycorrhizal (AMF) and non-mycorrhizal (-AMF) pepper plants grown in soil irrigated with different concentrations of sea water

Treatments		Fresh weight (g plant ⁻¹)		Dry weight (g plant ⁻¹)		Shoot height (cm plant ⁻¹)	Leaf area (mm ² plant ⁻¹)
Sea water conc. (%)	AMF status	Shoot	Root	Shoot	Root		
Control (0.0)	-AMF	14.15 ± 0.96 ^D	1.58 ± 0.09 ^B	3.51 ± 0.44 ^{BC}	0.84 ± 0.05 ^B	28.5 ± 1.25 ^C	250 ± 8.11 ^C
	AMF	18.00 ± 0.98 ^A	2.01 ± 0.17 ^A	4.11 ± 0.57 ^{AB}	0.93 ± 0.09 ^A	40.1 ± 2.41 ^A	350 ± 9.21 ^A
	AMR (%)	27.2 ± 1.37	27.1 ± 2.09	17.1 ± 1.05 ^B	10.7 ± 0.96	40.7 ± 1.95	40.0 ± 1.38
10%	-AMF	14.00 ± 0.85 ^D	1.90 ± 0.13 ^{AB}	3.21 ± 0.45 ^C	0.77 ± 0.10 ^B	25.3 ± 1.05 ^{CD}	245 ± 7.99 ^C
	AMF	17.90 ± 0.54 ^A	2.11 ± 0.22 ^A	4.00 ± 0.53 ^A	0.88 ± 0.16 ^{AB}	39.1 ± 2.11 ^A	352 ± 9.01 ^A
	AMR (%)	27.90 ± 1.97	11.2 ± 1.98	24.6 ± 1.58	14.3 ± 0.93	59.5 ± 2.99	43.7 ± 1.37
20%	-AMF	13.38 ± 0.59 ^D	1.54 ± 0.19 ^B	2.99 ± 0.33 ^{CD}	0.70 ± 0.09 ^C	23.2 ± 1.08 ^D	238 ± 8.04 ^{CD}
	AMF	17.62 ± 0.98 ^B	1.93 ± 0.21 ^{AB}	3.87 ± 0.54 ^B	0.82 ± 0.08 ^{AB}	35.1 ± 2.11 ^B	340 ± 8.62 ^{AB}
	AMR (%)	30.90 ± 1.87	25.3 ± 1.99	29.4 ± 1.97	17.1 ± 0.83	51.3 ± 2.95	42.8 ± 1.88
40%	-AMF	12.10 ± 0.89 ^E	0.89 ± 0.08 ^D	2.11 ± 0.55 ^D	0.55 ± 0.03 ^D	18.8 ± 1.01 ^E	200 ± 6.25 ^D
	AMF	15.99 ± 0.54 ^C	1.11 ± 0.29 ^C	3.28 ± 0.58 ^{BC}	0.69 ± 0.04 ^C	29.6 ± 1.22 ^C	310 ± 8.11 ^B
	AMR (%)	32.20 ± 2.01	24.7 ± 1.89	55.4 ± 2.15	25.5 ± 1.34	57.4 ± 2.95	55.0 ± 2.08

Values in each column (except AMR) followed by the same letter(s) are not significantly different at $P \leq 0.05$ (Duncan's multiple range test). AMF: arbuscular mycorrhizal fungi, -AMF: non-mycorrhizal fungi, AMR: arbuscular mycorrhizal response. Each value represents the mean of five replicates ± standard error

Table 2. Photosynthetic pigments in leaves of mycorrhizal (AMF) and non-mycorrhizal (-AMF) pepper plants grown in soil irrigated with different concentrations of sea water

Treatments		Chlorophyll content (µg g ⁻¹ fw.)		
Sea water conc. (%)	AMF status	Chlorophyll “a”	Chlorophyll “b”	Carotenoides
Control (0.0)	-AMF	830 ± 10.22 ^C	400 ± 4.22 ^B	680 ± 7.22 ^B
	AMF	1340 ± 18.22 ^A	570 ± 4.24 ^A	850 ± 9.54 ^A
	AMR (%)	61.4 ± 2.44	42.5 ± 0.87	25.0 ± 0.31
10%	-AMF	670 ± 9.25 ^D	290 ± 2.48 ^C	450 ± 3.99 ^{CD}
	AMF	1050 ± 11.47 ^B	480 ± 3.99 ^A	530 ± 2.41 ^C
	AMR (%)	56.7 ± 1.99	65.5 ± 1.49	17.8 ± 0.27
20%	-AMF	490 ± 7.22 ^E	225 ± 1.33 ^D	405 ± 4.14 ^D
	AMF	620 ± 8.71 ^D	290 ± 1.30 ^C	455 ± 4.00 ^{CD}
	AMR (%)	26.5 ± 1.05	28.9 ± 0.85	12.3 ± 0.19
40%	-AMF	360 ± 4.17 ^F	165 ± 0.73 ^E	215 ± 2.45 ^F
	AMF	400 ± 3.58 ^{EF}	195 ± 0.89 ^{CD}	338 ± 2.98 ^E
	AMR (%)	11.1 ± 0.84	18.8 ± 0.22	57.2 ± 1.97

Values in each column (except AMR) followed by the same letter(s) are not significantly different at $P \leq 0.05$ (Duncan's multiple range test). AMF: arbuscular mycorrhizal fungi, -AMF: non-mycorrhizal fungi, AMR: arbuscular mycorrhizal response. Each value represents the mean of five replicates ± standard error

Gas exchange parameters

Generally, g_s , A and E in mycorrhizal leaves of pepper plants were highly significant than those in non-mycorrhizal plants grown either in soil irrigated with or without sea water (Table 3), and the effect was more noticed in soil irrigated with higher concentrations of sea water. Such increases in gas exchange parameters were highly related to the degree of the mycorrhizal colonization for each treatment. Furthermore, the dependency of pepper plants on mycorrhizal fungi for improving gas exchange parameters was increased with increasing sea water concentration in soil. The differences in g_s , A and E in pepper leaves were not significant between mycorrhizal plants grown in soil irrigated with 10% sea water and control treatment.

Table 3. Net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) in leaves of mycorrhizal (AMF) and non-mycorrhizal (-AMF) pepper plants grown in soil irrigated with different concentrations of sea water

Treatments		Gas exchange parameters		
Sea water conc. (%)	AMF status	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)
Control (0.0)	-AMF	12.99 ± 0.80^C	$10.11 \pm 0.75B^C$	0.125 ± 0.021^C
	AMF	15.61 ± 0.88^A	12.24 ± 0.74^A	0.198 ± 0.023^A
	AMR (%)	20.17 ± 1.01	21.07 ± 1.41	58.40 ± 3.23
10%	-AMF	12.98 ± 0.69^C	09.88 ± 0.54^C	0.111 ± 0.020^C
	AMF	15.71 ± 0.71^A	12.20 ± 0.70^A	0.188 ± 0.035^A
	AMR (%)	21.03 ± 1.24	23.48 ± 1.92	69.40 ± 3.98
20%	AMF	10.11 ± 0.68^D	08.54 ± 0.57^{CD}	0.090 ± 0.019^D
	AMF	13.99 ± 0.66^B	11.88 ± 0.70^B	0.160 ± 0.027^B
	AMR (%)	38.38 ± 2.99	39.11 ± 1.98	77.80 ± 3.98
40%	AMF	08.65 ± 0.58^E	05.90 ± 0.44^D	0.055 ± 0.012^E
	AMF	12.11 ± 0.75^{CD}	09.24 ± 0.80^C	0.098 ± 0.017^D
	AMR (%)	40.00 ± 2.87	56.61 ± 2.60	78.20 ± 3.97

Values in each column (except AMR) followed by the same letter(s) are not significantly different at $P \leq 0.05$ (Duncan's multiple range test). AMF: arbuscular mycorrhizal fungi, -AMF: non-mycorrhizal fungi, AMR: arbuscular mycorrhizal response. Each value represents the mean of five replicates $\pm$ standard error

Antioxidant enzymes

Sea water stress significantly increased the antioxidant enzyme activities (CAT, POX and APX) in leaves of mycorrhizal and non-mycorrhizal pepper plants as compared to the control treatments (Table 4). However, mycorrhizal colonization significantly improved the contents of antioxidant enzyme activities than those in leaves of non-mycorrhizal plants, regardless of sea water treatments. This increase in antioxidant activities in leaves of pepper plants in response to mycorrhizal effects was increased with increasing sea water concentration in the soil, particularly in case of POX and APX. On the other hand, no significant differences in APX activity was observed between mycorrhizal and non-mycorrhizal plants grown in soil irrigated with normal water.

Table 4. Antioxidant enzymes {catalase (CAT), Peroxidase (POX) and Ascorbate peroxidase (APX) in leaves of mycorrhizal (AMF) and non-mycorrhizal (-AMF) pepper plants grown in soil irrigated with different concentrations of sea water

Treatments		Antioxidant enzymes		
Sea water conc. (%)	AMF status	CAT ($\mu\text{mol H}_2\text{O}_2/\text{min}$)	POX ($\mu\text{mol H}_2\text{O}_2/\text{min}$)	APX ($\mu\text{mol oxidized ascorbate}/\text{min}$)
Control (0.0)	-AMF	140 $\pm$ 3.80 ^{E*}	0.93 $\pm$ 0.032 ^D	4.50 $\pm$ 0.78 ^D
	AMF	180 $\pm$ 4.41 ^D	1.11 $\pm$ 0.092 ^{CD}	4.68 $\pm$ 0.77 ^D
	AMR (%)	28.6 $\pm$ 1.07	19.3 $\pm$ 1.05	04.0 $\pm$ 0.42
10%	-AMF	145 $\pm$ 3.69 ^E	1.01 $\pm$ 0.040 ^{CD}	4.90 $\pm$ 0.76 ^C
	AMF	180 $\pm$ 4.05 ^F	1.26 $\pm$ 0.082 ^C	5.30 $\pm$ 0.79 ^{BC}
	AMR (%)	26.2 $\pm$ 1.13	24.7 $\pm$ 1.99	08.0 $\pm$ 0.55
20%	-AMF	189 $\pm$ 4.11 ^F	1.30 $\pm$ 0.18 ^C	5.25 $\pm$ 0.57 ^{BC}
	AMF	230 $\pm$ 4.65 ^C	1.88 $\pm$ 0.17 ^B	5.80 $\pm$ 0.57 ^B
	AMR (%)	21.7 $\pm$ 1.48	44.6 $\pm$ 2.55	10.4 $\pm$ 0.89
40%	-AMF	280 $\pm$ 5.88 ^B	1.55 $\pm$ 0.041 ^B	5.92 $\pm$ 0.88 ^B
	AMF	345 $\pm$ 5.96 ^A	2.30 $\pm$ 0.060 ^A	6.95 $\pm$ 0.89 ^A
	AMR (%)	23.2 $\pm$ 0.95	48.4 $\pm$ 2.22	17.4 $\pm$ 1.11

Values in each column (except AMR) followed by the same letter(s) are not significantly different at $P \leq 0.05$ (Duncan's multiple range test). AMF: arbuscular mycorrhizal fungi, -AMF: non-mycorrhizal fungi, AMR: arbuscular mycorrhizal response. Each value represents the mean of five replicates $\pm$ standard error

Mycorrhizal colonization levels

Mycorrhizal colonization levels (frequency (F%), intensity (M%) of mycorrhizal colonization and arbuscular development (A%)) in root tissues of pepper plants were significantly affected by sea water stress in soil. Both intensity of mycorrhizal colonization (M%) and arbuscular frequency (A%) were significantly decreased in pepper root tissues with increasing concentration of sea water in soil (Table 5). On the other hand, no significant differences were observed in the frequency of mycorrhizal colonization (F%) in root tissues between mycorrhizal plants irrigated with or without sea water concentration. On the contrary; no significant differences were observed in the intensity of mycorrhizal colonization (M%) between AMF plants grown either in soil irrigated with 10% sea water and control treatment. No mycorrhizal colonization was observed in the non-inoculated pepper plants.

Nutrient content

Increasing sea water concentration in soil significantly reduced N, P, Mg, Ca and K concentrations, but increased sodium in the shoots of mycorrhizal and non-mycorrhizal pepper plants. However, P, N, K, Ca and Mg contents in shoot tissues of the AM plants, in most cases, were significantly greater than those in non-mycorrhizal pepper plants grown in soil irrigated with or without sea water concentrations (Table 6). Such stimulations in nutrients content in response to the mycorrhizal effects were highly increased with increasing sea water concentration in the soil. AM-inoculated plants exhibited reduction in Na concentration in shoot tissues compared to non-mycorrhizal

control plants in either soil irrigated with different concentration of sea water and control treatment (Table 6). The rate of reduction in Na content in response to mycorrhizal effects (AMR) was increased highly with increasing sea water concentration in the soil. On the other hand, no significant differences in Mg content in shoot tissues were observed between mycorrhizal and non-mycorrhizal pepper plants at control and 20% sea water treatments

Table 5. Frequency of mycorrhizal infection (F %) Intensity of mycorrhizal colonization (M %) and arbuscular frequency (A %) in the root tissues of mycorrhizal (AMF) and non-mycorrhizal (-AMF) pepper plants grown in soil irrigated with different concentrations of sea water

Treatments		Level of mycorrhizal colonization (%)		
Sea water conc. (%)	AMF status	F	M	A
Control (0.0)	-AMF	0.0 ^C	0.0 ^{C*}	0.0 ^{C*}
	AMF	95.2 ± 4.11 ^A	80.3 ± 3.99 ^A	61.8 ± 2.63 ^A
10%	-AMF	0.0 ^{C*}	0.0 ^{C*}	0.0 ^{C*}
	AMF	93.7 ± 4.01 ^A	75.6 ± 3.54 ^A	57.0 ± 2.47 ^A
20%	-AMF	0.0 ^{C*}	0.0 ^{C*}	0.0 ^{C*}
	AMF	82.8 ± 3.58 ^A	71.3 ± 3.54 ^{AB}	48.7 ± 1.99 ^B
40%	-AMF	0.0 ^{C*}	0.0 ^{C*}	0.0 ^{C*}
	AMF	79.9 ± 3.65 ^A	66.5 ± 2.60 ^B	41.9 ± 1.037 ^B

Values in each column (except AMR) followed by the same letter(s) are not significantly different at $P \leq 0.05$ (Duncan's multiple range test). AMF: arbuscular mycorrhizal fungi, -AMF: non-mycorrhizal fungi. Each value represents the mean of five replicates ± standard error

Table 6. Nutrients (N, P, K) and minerals (Ca, Mg, Na) content in leaves of mycorrhizal (AMF) and non-mycorrhizal (-AMF) pepper plants grown in soil irrigated with different concentrations of sea water

Treatments		Nutrients and minerals content [mg g ⁻¹ (Dwt)]					
Water status	AMF status	N	P	K	Ca	Mg	Na
Control (0.0)	-AMF	2.80 ± 0.17 ^C	0.17 ± 0.052 ^B	38.9 ± 1.55 ^B	16.2 ± 0.88 ^{AB}	11.1 ± 0.55 ^A	12.0 ± 0.111 ^E
	AMF	3.18 ± 0.18 ^A	0.30 ± 0.081 ^A	42.2 ± 1.68 ^A	17.4 ± 0.89 ^A	11.5 ± 0.55 ^A	11.3 ± 0.17 ^E
	AMR (%)	13.6 ± 1.11	82.4 ± 3.22	9.65 ± 0.44	7.40 ± 0.54	3.60 ± 0.042	- 05.83 ± 0.44
10%	-AMF	2.81 ± 0.15 ^C	0.16 ± 0.045 ^B	34.2 ± 1.32 ^C	13.7 ± 0.77 ^C	10.8 ± 0.59 ^B	19.6 ± 0.98 ^C
	AMF	3.20 ± 0.15 ^A	0.30 ± 0.084 ^A	39.5 ± 1.44 ^{AB}	15.1 ± 0.71 ^B	11.5 ± 0.61 ^A	16.6 ± 0.88 ^D
	AMR (%)	13.9 ± 1.051	87.5 ± 3.85	15.5 ± 0.88	9.56 ± 0.52	6.48 ± 0.098	- 15.31 ± 1.04
20%	-AMF	2.45 ± 0.14 ^D	0.12 ± 0.034 ^C	26.6 ± 0.99 ^E	12.05 ± 0.68 ^C	08.7 ± 0.33 ^C	22.2 ± 1.05 ^B
	AMF	3.01 ± 0.15 ^B	0.27 ± 0.077 ^{AB}	32.2 ± 1.01 ^C	13.75 ± 0.66 ^C	09.6 ± 0.37 ^C	18.5 ± 1.55 ^{CD}
	AMR (%)	22.9 ± 1.55	125 ± 5.14	21.1 ± 0.98	14.1 ± 1.01	10.3 ± 0.99	- 16.67 ± 1.85
40%	-AMF	2.05 ± 0.091 ^E	0.09 ± 0.004 ^D	22.7 ± 0.57 ^E	7.02 ± 0.33 ^F	06.5 ± 0.12 ^D	28.9 ± 1.74 ^A
	AMF	2.92 ± 0.10 ^{BC}	0.25 ± 0.014 ^B	29.9 ± 0.59 ^D	9.00 ± 0.571 ^D	08.1 ± 0.15 ^C	21.3 ± 1.88 ^B
	AMR (%)	42.4 ± 2.05	178 ± 7.40	31.7 ± 1.41	28.2 ± 1.33	24.6 ± 1.99	-26.30 ± 2.11

Values in each column (except AMR) followed by the same letter(s) are not significantly different at $P \leq 0.05$ (Duncan's multiple range test). AMF: arbuscular mycorrhizal fungi, -AMF: non-mycorrhizal fungi, AMR: arbuscular mycorrhizal response. Each value represents the mean of five replicates ± standard error

Discussion

Salinity of soil is one of the severe abiotic stress problems particularly in arid and semi-arid area like Saudi Arabia. It directly negatively affecting the majority of arable lands worldwide, limiting the growth and production of most of the economically important crop, vegetable and horticulture plants (Giri et al., 2003; Al-Karaki, 2006; Kaya et al., 2009; Ibrahim et al., 2011; Abdel-Fattah and Asrar, 2012; Asrar et al., 2014; Elhindi et al., 2017). Augmenting salt concentration in soil decreases the plant ability to absorb water, negatively affect all metabolic processes and osmotic balance, nutrient absorption, water relations and net photosynthetic rate. All of these results adversely affecting plant growth and productivity (Ojala et al., 1983; Zuccarini, 2007; Scharnagl et al., 2018).

It is evident from the present investigation that sea water stress significantly decreased of estimated growth parameters of mycorrhizal and non-mycorrhizal pepper plants compared to the control plants. However, mycorrhizal fungi improved growth and biomass of pepper plants grown in either sea water stressed or control soils when comparing with non-mycorrhizal plants. The dependency of pepper plants on mycorrhizal fungi was highly increased with increasing sea water concentration soil. These results are entirely consistent with earlier studies on other crop and vegetable plant species (Giri et al., 2007; Al-Amri et al., 2013; Sheng et al., 2013). Enhancement of growth in mycorrhizal pepper plants irrigated with different concentrations of sea water has been related partially to mycorrhizal enhancement of host plant P, N and K nutrition (Poss et al., 1985; Garg and Chandel, 2011; Evelin et al., 2012).

Interestingly, AM pepper plants had higher K, Ca, P and N contents than non-mycorrhizal plants grown at sea water stress treatment. In this connection, pepper plant tolerance to sea water stress was improved highly by mycorrhizal fungi. The obtained results suggested that improving growth of pepper plants duo to mycorrhizal colonization is a manifest of increased nutrients content and maintaining favorable ionic ratios than non-mycorrhizal pepper plants. These results are confirmed by the most previous studies (Kaya et al., 2009; Kumar et al., 2010; Scharnagl et al., 2018). Furthermore, improved P, N, K and Ca contents by mycorrhizal fungi in pepper plants grown under sea water stress conditions may contribute to the maintenance of vacuolar membrane integrity and facilitate the compartmentalization of Na ions with vacuoles (Cantrell and Linderman, 2001). Prevention of Na ion from interfering in metabolic pathways of growth and yield, thereby mitigated the toxicity of sea water stress (Poss et al., 1985; Abdel-Fattah and Asrar, 2012). Lower Na in the mycorrhizal plants than non-mycorrhizal plants may be explained by the dilution effect due to growth enhancement (Giri and Mukherjee, 2004; Al-Karaki, 2006). In addition, Mycorrhizal inoculation can increase K⁺ absorption under sea water stress conditions. The higher K⁺: Na⁺ ratio helps not only to prevent the disruption of various K-mediated enzymatic processes and inhibition of protein synthesis but also are beneficial in influencing the ionic balance of the cytoplasm or Na⁺ efflux from plants (Colla et al., 2008; Zuccarini and Okurowska, 2008).

Photosynthetic pigments like chlorophyll have been proposed as one of the most parameters of salt tolerance in plants (Sirivastava et al., 1998) and also carotenoids are responsible for reducing of single oxygen (Bourgou et al., 2012). In this connection, it is evident from the present investigation that the photosynthetic pigments in leaves of mycorrhizal pepper plants were significantly higher than those in non-mycorrhizal plants grown in soil irrigated with sea water. The dependency of pepper plants on AM

fungi was increased highly with increasing sea water concentration in soil. These results are in line with results of Elhindi et al. (2017) and Scharnagl et al. (2018). Furthermore, mycorrhiza improving Mg^{++} support a higher chlorophyll concentration. Effective Mg^{++} uptake helps by increasing the chlorophyll content and hence stimulating photosynthetic efficiency and plant growth (Camprubi et al., 2012; Metwally and Abdelhameed, 2018). Of particular interest in this study that g_s , P_N and E were highly significant in the AM pepper plants than in the non-mycorrhizal plants grown in soil irrigated with or without different concentrations of sea water. Such increased in the contents of gas water exchange were linked to the degree of mycorrhizal colonization. These results are in the same line of those obtained by Auge et al. (2008), Abdel-Fattah et al. (2013), Metwally and Abdelhameed (2018).

Sea water stress in soil not only affect the growth and production of pepper plant but also the levels of mycorrhizal colonization in associated pepper plants. These results were in agreement with the previous findings (Aroca et al., 2013; Abdel-Fattah et al., 2016; Elhindi et al., 2017). Based on the previous results of Jiang and Zhang (2002), who reported that antioxidant enzymes play an important role in scavenging of reactive oxygen species (ROS) and hence averting the oxidative stress induced damaging effects on several sensitive molecules like nucleic acids, proteins and lipids. The present study demonstrated that the activities of CAT, POX and APX in mycorrhizal plants were significantly higher than in non-mycorrhizal pepper plants grown either in soil irrigated with or without sea water. Such stimulation in antioxidant activities in response to the mycorrhizal effect were significantly increased with increasing sea water concentration in soil. In this connection, several investigators reported that mycorrhizal symbiosis helps plants to alleviate or reduced salt stress by enhancing the activities of antioxidant enzymes (Tang et al., 2009; Metwally and Abdelhameed, 2018).

Conclusion

Arbuscular mycorrhizal colonization has a great contribution from pepper plant to alleviate salinity stress imposed by irrigation with different concentrations of sea water through improving growth, increasing nutrient contents, stimulating gas exchange parameters, presentation of Na^+ content and transfer by the roots to the shoots and some metabolic contents of pepper plants. These benefits in response to mycorrhizal inoculation were greatly increased with increasing sea water concentration in soil, suggesting that pepper plants was highly depended on mycorrhizal fungi particularly at higher concentration of sea water. However, more investigations are required to explain in details the relationship between the molecular of AM efficiency for plant growth and other metabolic activates in different varieties of plants under salinity stress conditions.

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