The effect of salt-pretreated *Glomus fasciculatum* on salinity tolerance induction of barley plants

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Abstract

The present study reports a factorial greenhouse experiment using a randomized complete block design in 2014 which investigated the effects of salt-pretreated *Glomus fasciculatum* fungus, salinity, and the interaction effect of these variables on the physiological properties of barley. The first factor was mycorrhiza salinity pretreated with different levels of salt (0, 25, 50 and 100 mM) and the second factor included application of salinity to barley plant at 0, 25, 50, 100 and 200 mM. Mycorrhiza pretreatment with 25 mM salt increased fresh and dry weight in the absence of salinity treatment in the plant but in the presence of salinity, it reduced the dry weight of the plant. Also mycorrhiza pretreatment with salt reduced the leaf area and increased peroxidase, catalase, polyphenols peroxidase enzymes activities and malondialdehyde content. Also by increasing salinity, fresh and dry weight and leaf area decreased and antioxidant enzyme activities, malondialdehyde and proline contents increased.

Keywords: antioxidatives; barley; mycorrhiza; salinity pretreatment; salt

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Introduction

Among the environmental stresses, salinity is a serious problem that has harmfully affected about two million square kilometers of lands used in agriculture. Salt stress is in fact a major limiting factor in plants production around the world. On the other hand, an increase is expected in the salinity of the agricultural lands so that 30 percent of the lands in the next 25 years and over 50 percent by 2050 is predicted to be withdrawn from agricultural practices (Wang et al., 2003). On the other hand, the earth is considered as a salty planet because most of the waters on this planet contain about 30 gr of salt (sodium chloride) per liter. Water salinity affects agricultural practices the land. There are about 900 million hectares of lands around the world affected by salinity and the development of salinity stricken lands is considered a serious threat to the agriculture (Munns and Tester, 2008).

Salinity in the soil or water is one of the main stresses in arid and semi-arid regions and can severely limit plant growth and production (Koca et al., 2007; **Allakhverdiev** et al., 2007). Huang and Redman (1995) mentioned the reduction of the chlorophyll content in the leaves of barley plants in the salinity stress conditions. Na^+ and Cl^- ions are the most common ions found in soil and salty

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water and both of them can have unfavorable effects on plants, because by increasing the osmotic pressure, the soil solution as it creates ion toxicity in plant, disturbs the balance of ions such as potassium needed by plants.

Selection of salinity tolerant plants in all stages of plant life, especially at the germination stage is very important. In general, uniformity in the emergence depends on the germination percent and both of them are affected by the salinity concentration, soil, water potential, nutrients, environmental temperature, and interaction effect of these factors (Kaya et al., 2001). In addition to osmotic imbalances, ionic and nutritional factors in the plants, salinity also disrupts the water absorption and biosynthesis of abscisic acid (ABA) in leaves that in turn affect the osmotic conductivity. This will then influences the photosynthetic electron displacement and enzyme activities (Parida and Das, 2005). Physiological and metabolic changes that may occur in response to salinity include ROS, such as Superoxide radicals (O_2^-), Single Oxygen (O_1^-), hydroxyl radicals (OH^-), and the formation of H_2O_2 (Misra and Gupta, 2006).

Plants have complex antioxidant systems to avoid the harmful effects of ROS (Ghorbanli et al. 2015). The environmental stresses that cause an increase in ROS production lead to oxidizing the photosynthetic pigments, membrane lipid, proteins, and nucleic acids (Smirnoff, 1993). Plant cells are equipped with a broom of free radicals including catalase antioxidant, peroxidase, and polyphenols peroxidase enzymes to protect against oxidative damages (Beltagi, 2008). Adaptation to the salt by increasing the antioxidant content to eliminate ROS is reported by previous studies (Hernandez et al., 1993; Sehmer et al., 1995).

The research showed that mycorrhiza fungus with extensive hyphal network and increasing the level and root rate absorption, the plants efficiency in the water absorption and nutrients, particularly sedentary elements such as phosphorus, zinc, copper will increase and cause the improving of them (Marschner and Dell, 1994). Studies have shown that the efficiency of microorganisms resistant to salinity can be effective in restoration and production of resistant materials (Rodriguez and Redman, 2008). The existence of mycorrhiza fungus in the saline soils and creation of the symbiosis with the plant roots in these circumstances indicate that probably some of these fungi are resistant to salinity stress and in their symbiotic relationship with plants improves plant growth and increases their tolerance to salinity (Sharifi et al. 2007).

The aim of this study was to investigate the effect of salinity pre-treated mycorrhiza which forms symbiotic relation with barley, on the barley plants under various levels of salinity.

Materials and Methods

This research was conducted in a greenhouse at Islamic Azad University, Saveh branch. The factorial experiment was based on a randomized complete block design with three replications. The first factor included mycorrhiza pretreatment with 0, 10, 50 and 100 mM salt and the second factor included application of salinity to the plant at 0, 25, 50, 100 and 200 mM.

Seeds were obtained from Seed and Plant Institute in Karaj under scientific name of (Hordeum vulgar) Reyhan cultivar. Before planting they were disinfected with five percent sodium hypochlorite for 10 minutes and then sequentially washed with distilled water three times and rinsed. Mycorrhiza *Glomus fasciculatum* fungi variety was procured from the Research Institute of Environmental Fanavaran Turan Shahrood. 40 grams fungus was applied to each pot. One kg pots with a height of 15 cm and a bottom diameter of 10 cm and a head diameter of 16 cm were used and the pots were lined with 0.4 mm Leca (Light Expanded Clay Aggregate) up to 10 cm of their height. After washing the seeds, they were soaked for 24 hours. Mycorrhiza was also pre-treated with sodium chloride salt (NaCl) for 24 hours under concentrations of 0, 25, 50, and 100 mM. Then, salt residue was removed by washing and the mycorrhiza was mixed with swelled seeds of barley plant and placed at a depth of 2 to 3 cm in the pots containing leca. In each plots 10 seeds were placed and covered with a thin layer of leca. Pots were irrigated with Hoagland solution with a pH about 5.8 to 6 on a daily basis and according to need of the plant for 10. The pots were kept under natural light condition and a temperature of 28 to 32 °C.

NaCl was used to prepare 0, 25, 50, 100, and 200 mM lit^{-1} of sodium chloride solution. Seven liters of Hoagland were poured into 7 glasses, one liter each, and sodium chloride solution (0, 25, 50, 100, and 200 $mM.lit^{-1}$) was added to the Hoagland solution. Pots were then treated with this solution for 10 days, twice a day and each time 40 ml for each pot. After 10 days the applying of treatment, plants were harvested for experiments. First each plant was slowly removed from the Leca not to damage roots. Then roots and small portion of the stems were washed with distilled water. The fresh weight was measured using a Sartorius digital scale with 0.001 precision. Then, plants were wrapped with aluminum foil and put in the oven at the 70 °C for 72 h and their dry weight was measured using a digital scale with a precision of 0.001. To calculate plant leaf area ($mm^{2)}$, Canon 210 vide scanner and Leaf Area Meter software were used. Chlorophyll content was measured based on the method suggested by Amon (1949). Catalase activity was measured based on the method explained by Cakmak and Horst (1991) and peroxidase enzyme activity was measured based on the method of Pandolfini et al. (1992). Moreover, polyphenols peroxidase enzyme activity was measured based on Kahn (1975). Finally, MDA was measured based on (Janero ,1990)method and proline based on Bates (1973) method.

ANOVA was used as statistical tool to analyze the obtained data by using SPSS software. Also the measured mean index by Means were compared using Duncan's test ($p \leq 0.05$).

Results

Plant fresh weight

As Table (1) shows, salt-pretreated mycorrhiza, salinity treatment ($p \leq 0.01$), and the interaction effect ($p \leq 0.05$) had a significant effect on plant fresh weight. Comparison of mean plant fresh weight under the influence of mycorrhiza pretreatment factors with salt along with the salinity in the plant showed that application of 25 mM salt in the plant in the absence of mycorrhiza pretreatment increased plant fresh weight in comparison with control. But, by increasing the salinity level, plant fresh weight decreased.

Applying various levels of salinity decreased the plant fresh weight. Mean comparison of the plant fresh weight under the influence of mycorrhiza pretreatment with the different amounts of salt in the presence of different levels of salt indicated the highest plant fresh weight (0.62 g) at 25 mM salt treatment in the absence of mycorrhiza pretreatment while the lowest plant fresh weight (0.17 g) was observed in the mycorrhiza pretreatment with 100 mM salt treatment along with application of 200 mM salt (Table 2).

Table 1. Variance analysis of the effects of salt-pretreated *Glomus fasciculatum* fungus, salinity in the plant, and their interaction effect on the quantitative and qualitative characteristics of barley

S.O.V	df	Plant fresh weight	Plant dry weight	LA	POD Activity	CAT Activity	PPO Activity	MDA	Proline
Block	2	0.05**	0.0000002**	72.8**	31846.98**	136.45**	19073.31*	51.25**	0.46**
Mycorrhiza pretreatment with salt	3	0.11**	0.00001**	13.59**	483468.17**	7399.79**	445116.57**	33.89**	646.21**
Salinity treatment in plant	4	0.11**	0.0001**	29.07**	243579.75**	10565.03**	413542.53**	30.73**	282.43**
a*b	12	0.01*	0.00003*	2.01*	9967.82**	357.87**	24088.53**	2.03**	18.66**
Error	38	0.004	0.0001	0.79	1094.78	64.39	4338.93	0.55	2.71
Cv%	df	16.97	10.38	8.1	8.49	15.03	9.98	9.01	13.4

Table 2
The mean comparison of the effects of salt-pretreated *Glomus fasciculatum* fungus, salinity in the plant, and their interaction effect on the quantitative and qualitative characteristics of barley

Mycorrhiza pretreatment with salt **(mM)**	Salinity treatment in plant (mM)	**CAT Activity (Δ Abs mg^{-1} protein)**	**PPO Activity (Δ Abs mg^{-1} protein)**	**MDA (n mol g^{-1} FW)**	**Proline (u mol g^{-1} FW)**
0	**0**	11.18±2.52 ^{k}	291.96±14.59 ^{j}	5.18±0.42 ^{f}	3.44±0.51 ^{i}
0	**25**	13.5±3.24 ^{k}	395.36±16.99 ^{i}	5.78±0.52 ^{def}	2.77±0.45 ^{i}
0	**50**	28.43±1.35 ^{i}	421.76±14.02 ^{i}	6.63±0.61 ^{def}	4.29±0.69 ^{hi}
0	**100**	41.72±1.19 ^{gh}	440.08±18.4 ^{i}	7.38±0.51 ^{cdef}	7.55±0.51 ^{fg}
0	**200**	67.43±3.19 ^{de}	718.88±18.51 ^{e}	8.48±0.69 ^{bcdef}	9.77±0.42 ^{f}
25	**0**	13.93±1.14 ^{k}	398.32±21.43 ^{i}	5.63±0.43 ^{ef}	4.29±0.58 ^{hi}
25	**25**	18.55±0.92 ^{jk}	470.09±15.27 ^{i}	6.65±0.76 ^{cdef}	3.76±0.58 ^{hi}
25	**50**	38.9±1.89 ^{h}	558.13±10.58 ^{h}	7.77±1.08 ^{bcdef}	6.06±0.59 ^{gh}
25	**100**	60.9±4.06 ^{e}	637.62±17.95 ^{fg}	8.49±1.21 ^{bcdef}	13.01±0.43 ^{e}
25	**200**	90.53±2.92 ^{c}	1025.67±24.79 ^{b}	9.38±1.09 ^{bc}	17.63±1.02 ^{d}
50	**0**	18.72±0.15 ^{jk}	411.43±13.15 ^{i}	6.57±0.82 ^{cdef}	7.87±0.45 ^{fg}
50	**25**	26.92±2.06 ^{ij}	601.44±18.25 ^{gh}	7.11±0.92 ^{cdef}	8.92±0.19 ^{f}
50	**50**	49.84±1.61 ^{fg}	643.83±12.56 ^{fg}	7.97±1.1 ^{bcdef}	13.58±0.98 ^{e}
50	**100**	69.91±2.41 ^{d}	804.99±82.82 ^{d}	9.17±1.05 ^{bcd}	17.4±1.27 ^{d}
50	**200**	95.14±2.91 ^{c}	1050.9±12.14 ^{b}	10.79±1.67 ^{b}	21.92±1.25 ^{c}
100	**0**	40.97±1.17 ^{h}	711.28±18.53 ^{ef}	7.69±1.12 ^{bcdef}	12.13±0.51 ^{e}
100	**25**	51.74±0.98 ^{f}	775.03±12.48 ^{de}	8.95±1.08 ^{bcde}	17.59±0.96 ^{d}
100	**50**	86.34±3.56 ^{c}	795.12±12.78 ^{d}	9.68±1.38 ^{bc}	22.06±0.96 ^{c}
100	**100**	109.6±6.97 ^{b}	938.13±9.61 ^{c}	10.76±1.53 ^{b}	24.46±0.94 ^{b}
100	**200**	133.38±5.3 ^{a}	1135.47±28.15 ^{a}	14.03±0.75 ^{a}	27.09±0.94 ^{a}

Continue :

Mycorrhiza pretreatment with salt **(mM)**	Salinity treatment in plant (mM)	**Plant fresh weight (gr)**	**Plant dry weight (gr)**	**LA**	**POD Activity (Δ Abs mg^{-1} protein)**
0	**0**	0.58±0.07 ^{ab}	0.0439±0.005 ^{ab}	14.94±1.44 ^{a}	103.53±6.5 ^{l}
0	**25**	0.62±0.06 ^{a}	0.0474±0.004 ^{a}	14.14±1.87 ^{ab}	129.15±4.81 ^{kl}±
0	**50**	0.49±0.04 ^{abc}	0.0377±0.007 ^{bcd}	12.71±1.78 ^{abcd}	208.66±17.07 ^{jk}
0	**100**	0.39±0.03 ^{cdefg}	0.0366±0.002 ^{bcd}	9.86±0.81 ^{cde}	28.41±23.8 ^{hij}
0	**200**	0.28±0.03 ^{ghijk}	0.002 ^{def}±0.0316	9.12±0.87 ^{de}	338.53±23.7 ^{ghi}
25	**0**	0.53±0.08 ^{abc}	0.0424±0.003 ^{abc}	13.64±1.22 ^{abc}	131.68±15.41 ^{kl}
25	**25**	0.49±0.06 ^{abcd}	0.0424±0.003 ^{abc}	12.45±1.37 ^{abcd}	211.78±21.21 ^{jk}
25	**50**	0.43±0.05 ^{cdef}	0.0359±0.003 ^{bcd}	10.87±0.83 ^{bcde}	300.11±28.63 ^{hi}
25	**100**	0.34±0.02 ^{efghi}	0.0311±0.003 ^{def}	9.87±1.04 ^{cde}	388.22±24.08 ^{fg}
25	**200**	0.22±0.01 ^{ijk}	0.0248±0.001 ^{fgh}	9.23±1.04 ^{de}	522.95±25.8 ^{d}
50	**0**	0.46±0.05 ^{bcde}	0.0344±0.003 ^{cde}	11.81±1.04 ^{abcde}	254.91±22.5 ^{ij}
50	**25**	0.41±0.02 ^{cdefg}	0.0354±0.003 ^{bc}±	11.59±1.04 ^{abcde}	284.58±16.0 ^{hij}
50	**50**	0.34±0.02 ^{efghi}	0.0309±0.002 ^{def}	10.83±1.04 ^{bcde}	349.06±20.3 ^{fgh}
50	**100**	0.29±0.04 ^{ghijk}	0.0256±0.001 ^{efgh}	9.55±1.04 ^{de}	512.05±19.66 ^{d}
50	**200**	0.2±0.02 ^{jk}	0.0187±0.001 ^{h}	9.12±1.04 ^{de}	640.17±30.61 ^{c}
100	**0**	0.36±0.04 ^{defgh}	0.0328±0.001 ^{def}	11.27±1.04 ^{abcde}	429.78±28.91 ^{ef}
100	**25**	0.31±0.04 ^{fghij}	0.0288±0.001 ^{defg}	10.94±1.04 ^{bcde}	490.51±33.4 ^{de}
100	**50**	0.29±0.02 ^{ghijk}	0.0254±0.001 ^{efgh}	9.66±1.04 ^{de}	617.02±28.66 ^{d}
100	**100**	0.25±0.01 ^{hijk}	0.0213±0.001 ^{gh}	9.33±0.61 ^{de}	732.28±57.49 ^{b}
100	**200**	0.17±0.03 ^{k}	0.0185±0.001 ^{h}	8.4±1.3 ^{e}	880.33±42.36 ^{a}

Each value is the mean of three replicates. Values followed by different letters in each column are significantly different at

$P \leq 0.05$

Plant dry weight

Analysis of variance showed that mycorrhiza pretreatment with salt and salinity treatment in plant ($p \leq 0.01$) and their interaction effect ($p \leq 0.05$) had a significant effect on plant fresh weight (Table 1). Comparison of the mean dry weight of the plants under the influence of mycorrhiza pretreatment with salt salinity, the plant dry weight treated with 25 mM salt increased but by increasing the salinity, the plant dry weight decreased. Also, mycorrhiza pretreatment with different levels of salt decreased plant dry weight so that the highest dry weight (0.0474 g) was observed at 25 mM salinity and in the absence of mycorrhiza pretreatment. On the other hand, the lowest plant dry weight (0.0185 g) was observed in the mycorrhiza pretreatment with 100 mM salt along with 200 mM salinity (Table 2).

Leaf area

Salt-pretreated mycorrhiza, salinity treatment in plants ($p \leq 0.01$), and their interaction effect ($p \leq 0.01$) had significant effects on leaf area (Table 1). Comparison of mean leaf area affected by the mycorrhiza pretreatment factor with different levels of salinity and application of salinity in plant (Table 2) suggests that mycorrhiza pretreatment with salt decreased leaf area. This was also the case when salinity was applied to plants. In fact, the highest leaf area in (14.94 mm^2) was observed in the control and while the lowest leaf area (8.4 mm^2) was observed in the presence of 200 mM salinity in plant together with the mycorrhiza pretreatment with 100 mM salt.

Peroxidase enzyme activity

Based on the variance analyze, mycorrhiza pretreatment with salt, salinity treatment in plant, and their interaction effects ($p \leq 0.01$) had significant effects on the peroxidase enzyme activity. Comparison of mean peroxidase enzyme activity influenced by mycorrhiza salt pretreatment and salinity treatment showed that with an increase in salinity level of mycorrhiza salt pretreatment and also salt levels in plant, so did the peroxidase enzyme activity. In fact, the maximum peroxidase enzyme activity, i.e., 880.33 (Δ Abs mg^{-1} protein) was observed at 200 mM salinity in plant along with mycorrhiza pretreatment with 100 mM salt. On the other hand, the minimum peroxidase enzyme activity i.e., 103.53 (Δ Abs mg^{-1} protein) was recorded in the control treatment (Table 2).

Catalase enzyme activity

The analysis of variance of the catalase enzyme activity in the barley plant showed that mycorrhiza pretreatment with salt, salinity treatment in plant, and also their interaction effects ($p \leq 0.01$) had significant effects on the catalase enzyme activity (Table 1). Comparison of mean catalase enzyme activity affected by salt pretreated mycorrhiza with salinity treatment in plant (Table 2) showed that by applying different levels of salt and also salinity in the plant catalase enzyme activity increased in comparison with control. In fact, the maximum catalase enzyme activity, i.e., 133.38 (Δ Abs mg^{-1} protein) was observed under 200 mM salinity in plants with mycorrhiza pretreatment with 200 mM salt. The minimum catalase enzyme activity, i.e., 11.18 (Δ Abs mg^{-1} protein) was observed in the control plants. Applying 25 mM salinity treatment in the plant in the absence of salt pretreated mycorrhiza resulted in the catalase activity on the order of 13.5 (Δ Abs mg^{-1} protein). Also in the absence of salinity in the plant, the mycorrhiza pretreatment with 25 mM salt resulted in the catalase activity on the order of 13.93 (Δ Abs mg-1 protein).

Polyphenol oxidase enzyme activity

As Table (1) shows that salt-pretreated mycorrhiza, salinity treatment in plant, and their interaction effect ($p \leq 0.01$) had significant effect on the polyphenol oxidase enzyme activity. Based on the obtained data, mean polyphenol oxidase enzyme activity was affected by salt-pretreated mycorrhiza and salinity treatment in plants (Table 2). Pretreatment of mycorrhiza with various levels of salt increased polyphenol oxidase enzyme activity. Polyphenol oxidase enzyme activity also increased by applying 200 mM salinity to the plant in the absence of salt-pretreated mycorrhiza. No statistically significant difference was observed

between other salinity levels. The obtained results showed that by applying salinity in plant in the presence of pretreatment of mycorrhiza with different levels of salt, polyphenol oxidase enzyme activity increased. The highest polyphenol oxidase enzyme activity i.e., 1135.47 (Δ Abs mg^{-1} protein) was observed at 200 mM salinity treatment in plant with mycorrhiza pretreated with 200 mM salt.

MDA

Analysis of variance analyze (Table 1) showed that mycorrhiza pretreatment with salt, salinity treatment in plant, and their interaction had a significant effect on the MDA ($p \leq 0.01$). Comparison of mean MDA showed that salinity treatment in the absence of salt pretreated mycorrhiza increased MDA in comparing with the control. Also salt-pretreated mycorrhiza and application of salinity stress in plant increased MDA.

Mycorrhiza pretreatment with different levels of salt in the presence of different levels of salinity in plant resulted in the maximum MDA (14.03 nM per g fresh weight) under 200 mM salt treatment in plants together with mycorrhiza pretreatment with 100 mM salt. The minimum MDA (5.18 nM per g fresh weight) was observed in the control.

Proline

Analysis of variance analyze showed that salt-pretreated mycorrhiza, salinity treatment in plant, and their interaction had meaningful effect ($p \leq 0.01$) on proline content (Table 1). Comparison of mean, proline content affected by salt-pretreated mycorrhiza and salinity treatment in plant showed that by applying salinity proline content increased. Also the mycorrhiza pretreatment with different levels of salt increased proline. The highest proline in content (27.09 µmol.gr Fw^{-1}) was related to 200 mM salt treatment in plant and the mycorrhiza pretreatment with 100 mM salt. The lowest proline contents (3.44 and 2.77 µmol.gr Fw^{-1}) were observed in the control treatment and the absence of mycorrhiza pretreatment with salt in the presence of 25 mM salinity in plant, respectively (Table 2).

Discussion

Salinity by reducing the osmotic potential of soil makes difficult the water absorption by the plant (Prasad, 1997). Plant growth reduced in salinity stress condition, due to the reduction of water in root environment and specific effects of ions in the metabolic processes and this is in line with the findings of a study reported by Ghoulam et al. (2002). Plants need osmotic adjustment to tolerate salinity and one of the methods for osmotic adjustment is synthesis of organic material such as sorbitol, proline, and glycine in the tissues. Producing these materials needs energy consumption. Therefore, the energy consumed for osmotic adjustment reduced the growth and plant weight and this confirms the findings of Penuelas et al. (1997). Also the dry weight reduction of plant tissues is due to increasing of metabolic cost and reduction of using carbon by plant in its attempt to adapt to salinity (Netondo et al., 2004). Salt quickly reduces cell development speed and in fact, in high concentrations stopped this process. One of the plant adaptations to salinity is that salt is kept out of their cells and this causes the movement of water out of leaf cells and reduces its levels. Sometimes leaf area reduces the light absorption and dry matter production and this in turn reduces plant growth (Volkmar and Steppuha, 1998). Plant cells are equipped with a scavenging system for free radicals to protect against stress damages and these include the antioxidant enzymes such as catalase and peroxidase (Cho and Park 2000). Catalases, peroxidase, and polyphenol oxidase are very important enzymes that play a role in response to abiotic stresses such as salinity. Catalase is one of the most important H2O2 scavengers that performs this role by converting H_2O_2 to water and O_2 (Dixit et al., 2001). Peroxidase is responsible for the removal of hydrogen peroxide (Shalini and Duey, 2003). According to the obtained results in this study, by increasing salinity stress, antioxidant enzymes levels were increased. In fact, increasing the salinity level will increase the activity of antioxidant enzymes and this is similar to the

findings reported by Sairam et al. (2001) indicating that catalase, peroxidase, and polyphenol oxidase enzymes activities increased under salinity treatment. Possibly the increasing of these enzymes activity in plants in response to the increasing of stress shows that the studied plants use an antioxidant defense mechanism in order to resist against stress as this is also reported by Ajay et al. (2001). Stress can make changes in osmotic potential affect a wide range of metabolic activities in plants and through production of active oxygen radicals such as superoxides and hydrogen peroxide radicals lead to oxidative stress. Reactive oxygen species are the result of severe ionic and osmotic stresses that disrupt membrane structure and can lead to cell death (Bohnert and Jensen., 1996). In their attempt to confront these reactive oxygen species, plants increase production of specific anti-oxidative enzymes such as catalase, peroxidase, polyphenol oxidase, reductase glutathione, and superoxide dismutase (Ghorbanli et al., 2015).

Increase in malondialdehyde concentrations is already reported under salinity stress in corn (Gunes et al., 2007) as well as in rice seedlings (Bandeoglu et al., 2004). Destruction of cell membranes under salinity stress and the production of malondialdehyde which is the result of the decomposition of cell membrane lipids can be considered as a proper criterion to evaluate performance of tomato plants under salt stress. The increase in proline concentrations under salinity stress may be due to the reduction of proline oxidation into glutamate or conversion of protein to proline and this confirms the findings of Sannada et al. (1995). Proline also provides the required energy for placement of ions in the vacuole. In many halophytes plants there is enough proline or glycine betaine to create osmotic pressure in cells (Flower et al., 1977).

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