The effect of salinity pretreatment of *Glomus mosseae* on induction of salinity tolerance in *Lycopersicum esculentum* L.

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Abstract

Tomato (*Lycpoersicum esculemtum*) belongs to the Solanaceae potato family and is an important crop plant. It is relatively resistant to salinity, but in the saline environment growth and production of the plant significantly reduces. On the other hand, the presence of mycorrhiza fungus can improve the adverse effects of salinity. A factorial experiment in a completely randomized design with three replications was conducted on tomato plants in the Islamic Azad University. The first factor was mycorrhiza treatment with NaCl at 0 (control), 50, and 100 mM, and the second factor included salinity stress at 0 (control), 50, 100, and 200 mM. Based on the obtained results from variance analysis, the effects of mycorrhiza pretreatment with NaCl were significant on the root length. Also, the level of salinity pretreatment of mycorrhiza had a significant effect on proline content ($p \leq 0.05$). Moreover, salinity treatment had a significant effect on stem length, leaf area, the inoculation percentage of mycorrhiza, and proline content ($p \leq 0.01$) and on stem length ($p \leq 0.05$). Moreover, salinity pretreatment of mycorrhiza and salinity treatment reduced root length, the inoculation percentage of mycorrhiza, and proline accumulation. Salinity treatment reduced root length, leaf area, stem length, and the inoculation percentage of mycorrhiza while it increased proline content. Finally, it was found that salinity stress reduced the root length, stem length, leaf area, leaf water content, the inoculation percentage of mycorrhiza, potassium, phosphorous, and nitrogen while it increased the proline content. Finally, mycorrhiza pretreatment with NaCl was found to reduce the negative effects of salinity stress.

Key words: proline; salinity pretreatment; tomatoes; salinity; mycorrhiza

Anoshe, M. and M. Farzami Sepehr. 2016. 'The effect of salinity pretreatment of *Glomus mosseae* on induction of salinity tolerance in *Lycopersicum esculentum* L.'. *Iranian Journal of Plant Physiology* 6 (4), 1815-1824.

Introduction

Soil salinity is an increasing problem for agriculture that results in decrease in the rate of plant growth and production especially in dry and semidry regions (Apse *et al*., 1999). High levels of salt would remain in those agriculture lands which need to be irrigated regularly though some salt would be washed out in the process of evaporation and it results in increase in salt density near the root (Pond *et al*., 1984).

Salinity stress is an important abiotic stress. Reducing the restrictive effects of salinity has positive influence on agriculture products

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Received: October, 2015
Accepted: March, 2016

(Huang *et al*., 2009). Some researchers stated that salinity influences plant growth through disturbing the balance of soluble nutrition in the soil and also poisoning the soil (Hajer *et al*., 2006). Soil or water salinity is a limiting abiotic factors which causes disorder in normal plant growth in dry and semidry regions around the world (Mansour, 2000).

Tomatoes are one of the most important greenhouse plants in semidry regions with saline underground water. In order to obtain optimum product, there is a need to conduct research on the effect of salinity on growth and chemical composition of this crop as salinity has negative effects on plant function through decreasing the weight of fruit and its marketability.

Studies have shown that fresh weight of tomato decreases by salinity treatment (Sato *et al* ., 2006). Reduction in the fresh and dry weight of shoots and roots were also reported under salinity condition (Hajer *et al*., 2006). Salinity influences the concentration of nutrients and their transfer into the root, shoots, and fruits. Anjum *et al*. (2008) reported that introduction of salt treatment changes the distribution of trace elements in root and shoots in comparison with no salt conditions. Overusing chemical fertilizer and also irrigation with saline water, by increasing osmotic stress negatively affects growth and causes some changes in the chemical compounds in various plants such as tomatoes (Petersen *et al*., 1998). The level of salinity in the soil and water is a major stress in dry and semidry regions and could restrict plant growth and its productivity greatly (Koca *et al*., 2007). High level of salinity is one of the growth inhibiting factors for agriculture products (Katkafi *et al*., 1982). As a result, increasing the soil salinity is considered as a determining factor and increasing the density of salt causes ionic imbalance in cells and results in ionic toxicity and osmotic stress (Mandhania *et al*., 2006).

Plants' response to salinity increase is complicated and involves some morphological, physiological, and metabolic changes (Parida and Das, 2005). Loss of the balance of necessary anions and cations, change in the capacity to keep water, and toxicity resulted from the high density of salt ions are the main reasons of salinity induced damage in plants. It is found that absorbance of soluble nutritional ingredients in the soil is influenced by osmotic potential manipulation (Azcon *et al*., 1997). High concentration of Na^+ in saline soil disturbs the cationic absorbance balance, and causes a reduction in absorption of potassium and to some extent calcium by plants (Halperi and lynch, 2003). Salinity damages occur through osmotic effect that just as much as water shortage has the toxic effects of ions and disturbance in nutrient absorption (Shabala *et al*., 2000). Under salinity stress plants face two processes: dehydration and ionic toxicity (Munns and Tester, 2008).

Beside genetic engineering, some biological solutions are suggested such as using resistant cultivars and mycorrhiza (Dixon *et al*., 1993). Inappropriate environmental conditions such as salinity could have negative effects on inoculation and life of mycorrhiza from a root development period to the next. It is reported that adding various kinds of salt has negative effects on mycorrhiza inoculation. Also it is shown that the salts containing Na and Cl have negative effects on germination and life of the spores of symbiotic fungi (Estaun, 1989). Mycorrhiza fungus probably causes growth improvement through different mechanisms under salinity condition. One of these mechanisms is the improvement in mineral nourishment particularly that of phosphorous and low usage elements (Al Karaka and Al Raddad, 1997). Studies show that application of mycorrhiza resistant to salinity could be influential in revival and production of resistant cultivars (Rodriguez Rosales *et al*., 1999). The presence of mycorrhiza in saline soil and its symbiotic relationship with plant roots under salinity conditions show that some of these fungi are probably resistant to salinity stress and by symbiotic relationship they will increase the tolerance of plants through improvement of their growth (Yano-melo *et al*., 2003). Symbiosis with mycorrhiza leads to salinity resistance and physiological changes under salinity stress (Zhongqunlle *et al*., 2007).

This research aimed at investigating the effect of salinity on tomato plant growth indexes, the effect of salinity on the absorption of nutritional substances, and mycorrhiza pretreatment using salt in order to determine

mycorrhiza resistance in the environment. The aims of this study are in fact, research into growth, resistance, and physiological parameters in tomato plants under symbiotic relationship with *Glomus mosseae* fungus pretreated with various levels of salinity and also investigating the possibility of growing tomato in saline conditions after inoculation with salt pretreated *Glomus mosseae* and introducing salinity treated Glomus as a stimulating factor for developing tolerance to salinity stress.

Materials and Methods

This study was conducted in Agriculture Faculty of the Islamic Azad University, Saveh Branch. The experiment was a factorial study using completely randomized design with three replications. The first factor was mycorrihza pretreatment with NaCl at 0, 50, and 100 mM and the second factor included salinity stress at 0, 50, 100, and 200 mM. Tomato seeds (*Solanum lycopersicum*) obtained from Pakan Bazr Isfahan Co. were disinfected with hypochlorite 5% for ten minutes before planting and then were washed three times. Mycorrhiza fungi serovar *Glomus mosseae* were prepared from Shahrood Tooran Zist Fanavaran and for each pot with the capacity of one kilogram, 40 grams of fungus was used. Pots of 15 cm height and 16 cm diameter were used and up to 10 cm height of pots were filled with licka 0.4 mm. After washing, seeds were primed with water. Mycorrhiza was pretreated with 0, 50, and 100 mM NaCl for 24 hours. Then, in order to remove residual salty substances, mycorrhiza was washed and inoculated with *Glomus mosseae* and cultivated in pots filled with 2-3 cm of licka. Ten seeds were sown in each pot and were covered with a thin layer of licka.

After preparation, pots were irrigated with Hoagland solution on a daily basis for 10 days. Pots were kept at 28-32 °C under natural lighting conditions. NaCl was used to prepare a solution containing 0, 50, 100, 200 mM/l salinity. To this end, 4 liters of Hoagland solution was prepared and distributed into 4 glasses equally and 0, 50, 100, and 200 Mm/l NaCl was calculated and added into the Hoagland solution. Then treatments were applied to the pots for 15 days. These were 200 ml of treatment solutions added into each pot every day. After 15 days of treatment, plants were harvested for analyses. First, each plant was weeded out of licka carefully in such a way that the roots were not damaged; then, roots and a small part of stems were rinsed with distilled water and the remaining water was removed. The whole plant fresh weight was then calculated using a digital scale (Sartorius) with the error of measurement of 0.001. After recording the fresh weight, plants were wrapped in aluminum foil and put in an oven with set at 70 °C for 72 hours. Dry weight of plants was then calculated by the digital scale with the measurement error of 0.001. The leaf area was measured in mm using Canon scanner (Lide 210) and Leaf Area Meter software.

Phosphorous measurement

To measure the concentration of mineral elements (phosphorous), the samples were dried in the oven at 100 °C for 24 hours. Plant material was homogenized in 17.5 mM 2-(N-morpholino) ethane sulfonic acid buffer at pH=5.6 and then was centrifuged at 2000 rpm and 4°C. 140 microliter of the supernatant solution and 30 microliter of molybdate solution were mixed and 30 microliter of malachite was added after 10 minutes. Phosphate was measured using spectrophotometer method at 610 nanometer after 2 hours (Tabatabaei, 2009).

Potassium measurement

Potassium was measured by Film Photometer method. The Film Photometer was turned on and the potassium filter was put in the device. The plant extract that was already prepared using acid digestion was fed into the pipe and all samples were adjusted in flame diffusion equipment at 548 nm (Benton, 2001).

Nitrogen measurement

After acid digestion of dried samples, all samples were transferred into a warmer device set at minimum 200 °C for full digestion. It took 30 - 45 min for complete digestion. After that, samples were transferred into the room condition and were cooled and adjusted to 100 ml capacity. Ten ml of acid boric 4 % including

two indicators, namely, green bromo-creosol and red methyl were used. After adding solution, funnel was washed with deionizer and 10 milliliter of NaOH 40% was added. Five milliliter of solution was instilled to a flask containing 10 milliliter of boric acid. After that, H_2SO_4 was titrated from 1 to 200 (Benton, 2001). The area of plants under study was calculated in mm^2 using Canon scanner (Lide 210) and Leaf Area Meter.

Mycorrhiza inoculation percentage

Roots were colored using Philips and Hayman (1970) method and then Crossed Lines method was used (Tenant, 1975). To measure proline Bates (1973) method was used. Data collected through measurements were analyzed by ANOVA through SPSS software using Duncan test at $p \leq 0.05$.

Results

Root length

As results of variance analysis show, mycorrhiza pretreatment with salt and pretreatment for plant has a significant effect with the probability of 5 percent on root length but in their interaction effect no meaningful influence was observed. Mean root length under mycorrhiza pretreatment showed reduction compared to the control. It was also found that root length was reduced in the process of pretreatment. Results of comparison of mean root length under influence of mycorrhiza pretreated with various levels of salinity showed that with an increase in salinity, the root length is reduced in comparison with the control (Fig. I).

Leaf area

Analysis of variance showed that salinity treatment has a significant effect on the leaf area of the pants under study ($p \leq 0.01$) but mycorrhiza pretreatment with salt and its interaction effect on leaf area had no significant effect on leaf area. Results of comparing of mean of leaf area under influence of salinity stress suggests that mycorrhiza pretreatment with salt leads to leaf area decrease; Also, the leaf area is reduced by salinity stress at 100, 200 mM concentrations as compared with the control. Results of interaction effect of mycorrhiza pretreatment with salt and salinity stress showed that the maximum leaf area is related to mycorrhiza pretreatment with 50 mM salt in the absence of salinity stress and the minimum area is related to presence of 200 mM salinity in mycorrhiza pretreated with 100 mM salinity (Fig. II).

Mycorrhiza inoculation percentage

Analysis of variance showed that mycorrhiza pretreatment with salt ($p<0.05$) and salinity treatment ($p<0.01$) have meaningful effect on mycorrhiza inoculation and also their interaction has no significant effect on percentage of mycorrhiza inoculation. Comparison of mean percentage of inoculation of mycorrhiza under influence of mycorrhiza pretreatment with salt showed that pretreatment with 100 mM salt decreased inoculation percentage in comparison with the control group. Interaction of effects of mycorrhiza pretreatment with salt and salinity treatment showed that maximum mycorrhiza inoculation percentage was observed in the control plants and the plants with 50 mM salinity pretreated mycorrhiza while the minimum inoculation percentage was observed in plants with 100 mM salinity pretreated mycorrhiza and 200 mM salinity (Fig. III).

Proline

Analysis of variance show that the mycorrhiza pretreatment with salt, salinity treatment in the plant, and their interaction effect on proline were significant ($p<0.01$). Based on the obtained results, proline accumulation increased with salinity pretreatment of mycorrhiza. Also salinity stress led to proline accumulation. Comparing the mean proline content under influence of mycorrhiza pretreatment with salt and salinity treatment showed that by increasing the level of salinity, the proline level increased.

Stem Length

Data obtained from variance analysis showed that the effects of salinity treatment

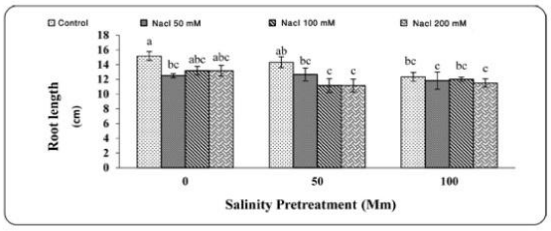

Fig. I. Interaction effect of different levels of salinity pretreatment of mycorrhiza and salinity treatment on tomato root length.

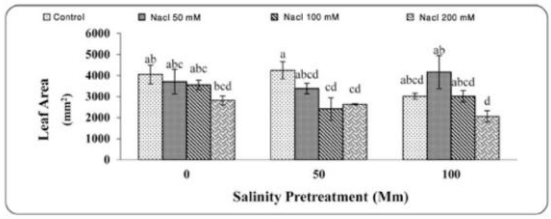

Fig. II. Interaction effect of different levels of salinity pretreatment of mycorrhiza and salinity treatment on tomato leaf area.

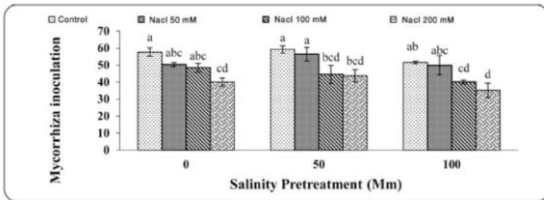

Fig. III. Interaction effect of different levels of salinity pretreatment of mycorrhiza and salinity treatment on percentage of mycorrhiza inoculation

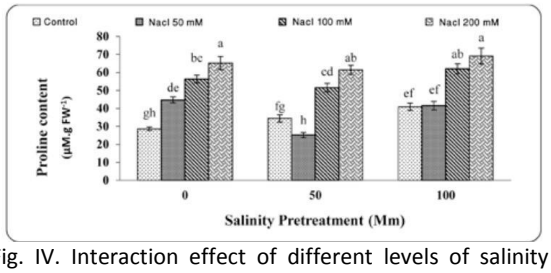

Fig. IV. Interaction effect of different levels of salinity pretreatment of mycorrhiza and salinity treatment on proline content of tomato.

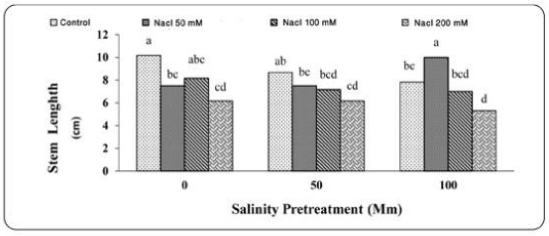

Fig. V. Interaction effect of different levels of salinity pretreatment of mycorrhiza and salinity treatment on stem length of tomato

($p<0.01$) and its interaction effect with salt pretreated mycorrhiza ($p<0.05$) were meaningful on the stem length but pretreating mycorrhiza with salt had no significant effect on stem length. Mean stem length was reduced under salinity stress. Also, mycorrhiza pretreatment with salt in the presence of salinity stress showed increase in the stem length compared with control (Fig. V).

Plant dry weight

Pretreating mycorrhiza with salinity, salinity treatment, and the interaction of salinity and mycorrhiza pretreatment did not have a statistically significant effect on the plant dry weight. Comparison of mean dry weight of the mycorrhiza pretreated plants under salinity showed no significant difference between various levels of salinity. Also, no significant difference was seen between dry weights of the plants under salinity pretreatment. Moreover, comparison of the dry weights of the plants pretreated with mycorrhiza and under salinity treatment suggests no significant difference (Fig. VI).

Potassium

Salinity pretreated mycorrhiza and salinity treatment of the plant had a statistically significant on the plant dry weight ($p\leq0.01$) while the interaction of effects of salinity and mycorrhiza pretreatment did not have a statistically significant effect on the plant dry weight. Comparison of mean potassium content showed that salinity and pretreatment with mycorrhiza increased potassium compared with the control. On the other hand, potassium content decreased in the salt treated plants compared to the control. Interaction of salinity pretreated mycorrhiza and salinity treatment resulted in the highest potassium content in the treatment consisting of mycorrhiza pretreated with 100 mM salt and under no salinity treatment while the lowest potassium content was recorded in the plants without salt pretreated mycorrhiza and 100 and 200 mM salinity (Fig. VII).

Phosphorous

Results of ANOVA analysis showed that salinity pretreatment of mycorrhiza and salinity

treatment of the plant had significant effects on the phosphorous contents of the plants under study ($p \leq 0.01$) while no significant effect was observed in the interaction of effects of salinity pretreated mycorrhiza and salinity treatment. Comparison of mean phosphorous showed an increase in its content in the plants treated with salinity pretreated mycorrhiza compared to those pretreated with mycorrhiza. Also, salinity stress reduced phosphorous contents in the plants under study. Comparison of the mean phosphorous contents in the plants under salinity pretreated mycorrhiza and those treated with salinity showed that the highest phosphorous content belonged to the no salinity stressed plants and mycorrhiza pretreatment with 100 mM salt (Fig. VIII)

Nitrogen

Analysis of variance showed that pretreatment of mycorrhiza with salinity and salinity treatment had significant effects on the nitrogen contents of the plants under study ($p \leq 0.01$). However, interaction of effects of salt pretreated mycorrhiza and salinity treatment did not result in any significant effect on the plants. Pretreatment of mycorrhiza with salt increased nitrogen absorption. Also, salinity at 200 mM reduced nitrogen contents. Figure (VIII) shows the results of comparison of mean nitrogen contents in the plants under salinity pretreated mycorrhiza. The figure suggests that the highest nitrogen content belonged to zero salinity treatment in mycorrhiza pretreated with 100 mM salt and the lowest nitrogen was observed in the plants treated with mycorrhiza not pretreated with salinity and 200 mM salt.

Relative growth rate

The findings of ANOVA showed that the effects of pretreating mycorrhiza with salinity, salinity treatment, and the interaction effects of salinity pretreated mycorrhiza and salinity treatment did not have a statistically significant effect on the plants' relative growth rate. Comparison of mean relative growth rate in the plants under salinity pretreated mycorrhiza suggested no statistically significant difference. On the other hand, comparison of mean relative

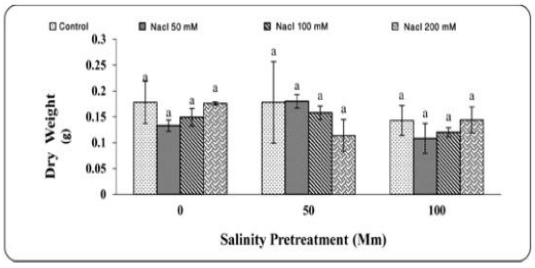

Fig. VI. Interaction effect of different levels of salinity pretreatment of mycorrhiza and salinity treatment on dry weight of tomato

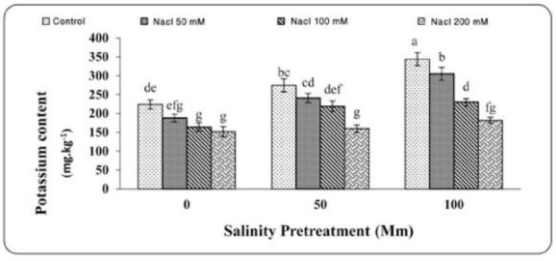

Fig. VII. Interaction effect of different levels of salinity pretreatment of mycorrhiza and salinity treatment on potassium content of tomato

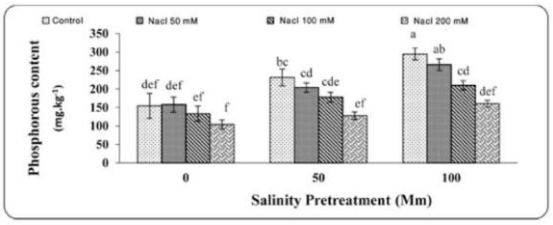

Fig. VIII. Interaction effect of different levels of salinity pretreatment of mycorrhiza and salinity treatment on phosphorous content of tomato

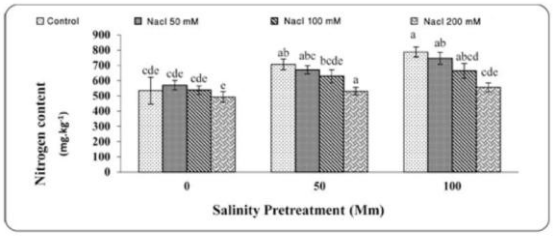

Fig. IX. Interaction effect of different levels of salinity pretreatment of mycorrhiza pretreatment and salinity treatment on nitrogen content of tomato

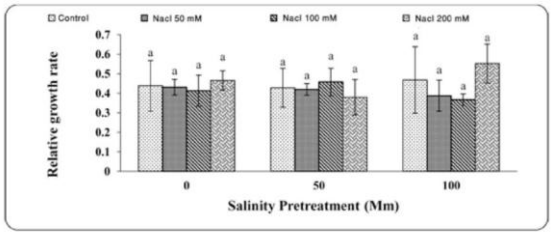

Fig. X. Interaction effect of different levels of salinity pretreatment of mycorrhiza pretreatment and salinity treatment on relative growth rate of tomato

growth rate showed that there was no statistically significant difference between various levels of salinity treatment. This was also the case with the salinity pretreated mycorrhiza and salinity treatment which had no significant effects on the relative growth rate in the plants under study (Fig. X).

Leaf water content

Analysis of variance showed no statistically significant difference between the effects of salinity pretreated mycorrhiza, salinity treatment, and the interaction of these treatments on the leaf water content of the plants. Analysis of variance showed no statistically significant difference between the effects of salinity pretreated mycorrhiza, salinity treatment, and the interaction of these treatments on the leaf water content of the plants. No significant difference was found between the effects of various levels of salinity pretreatment of mycorrhiza on the leaf water content. On the other hand, increase in salinity level reduced leaf water contents in the plants treated with salinity. As for the interaction of effects of salinity pretreated mycorrhiza and salt treatment, the maximum leaf water content was recorded under 100 mM salinity pretreated mycorrhiza and no salt treatment while the minimum leaf water content was observed under 50 mM salinity pretreated mycorrhiza along with200 mM salinity treatment (Fig. XI).

Salinity tolerance index

Salinity pretreated mycorrhiza, salinity treatment, and interaction of these two treatments did not have a significant effect the tolerance index of the plants in this study. Results of analysis of variance of mean tolerance index of the plants under salinity pretreated mycorrhiza showed no significant difference between various levels of salinity. There was no significant difference between mean relative tolerance indexes of the plants treated with various levels of salinity . This was also the case with the plants under salinity pretreated mycorrhiza and salinity treatment where there was no significant difference between the treatments in their tolerance index (Fig. XII).

Discussion

It seems salinity stress through limiting absorption of the nutrients, usable water shortage, and toxicity of nutrients decreases cell development, leaf area, and photosynthesis. These led to a cut in produced carbohydrates and finally resulted in a reduction in the development of different parts of plant such as root length, stem length, plant dry weight, and leaf area, which are also reported n the previous studies

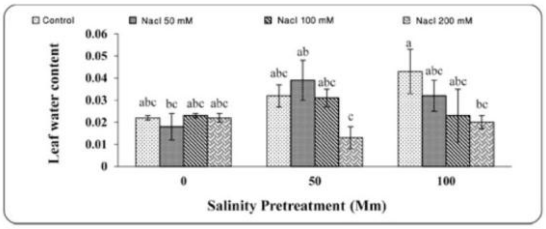

Fig. XI. Interaction effect of different levels of salinity pretreatment of mycorrhiza pretreatment and salinity treatment on leaf water content of tomato

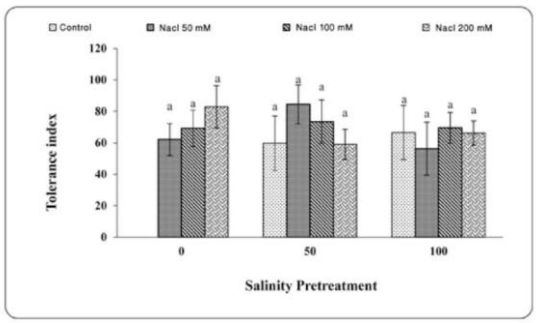

Fig. XII. Interaction effect of different levels of salinity pretreatment of mycorrhiza pretreatment and salinity treatment on tolerance index of tomato

(Ali et al ., 2004). Salt slows down the rate of cell development and stops it at high concentrations. One of the plants' adaptations to salinity is that they can keep the salt out of their cells and this causes water transport to outside of the leaf cells leading to reduction of leaf area. Sometimes reduction in leaf area results in a cut in light absorption and this leads to reduction in the production of the dry matter and also plant development (Volkmar and Steppuha, 1998) which is in line with the results of the present study.

Plants' development were decreased under salinity stress condition because of water shortage near root and specific effect of ions in

metabolic processes, which is similar to the findings of Ghoulant *et al*. (2002). When the plant grows in saline conditions, its photosynthesis activity will be decreased and as a result the rate of growing and leaf area are reduced, similar to the results obtained by Viera santos (2004). Growth decrease by salinity is because of NaCl competition in nutrient elements absorption. When the plant is put under salinity condition, balanced transportation of Na, Cl and other ions such as Cl and K that is disturbed (Niu, Xiaomu *et al* ., 1995).

Many agricultural plants are noticeably sensitive to light condition. This is due to accumulation of Na^+ inside the cell and its disturbing effect on ionic balance and osmotic adjustment, activities of many enzymes, cell metabolism, cellular metabolism and induction of debilitative toxicity. Abundance of Na in soils negatively affects K absorption (Turan *et al*., 2009). In sum, low level of sytosolic Na concentration and imbalance of K/Na ions are known as the most important issues in plants' tolerance to salinity. Salinity resistant agricultural cultivars show the high K to Na ratio (Summart *et al*., 2010). Absorption mechanism which distinguishes between similar ions such as K and Na could be considered as a useful index for selecting resistant cultivars in modification programs in order to improve and absorb nutrients (Khan *et al*., 2009). In saline soils, K absorbance is reduced because of the high density of Na. Na and K competition is reduced in the process of absorption, which is in line with the results of Bohra and Doerffling (1993). Decrease in soluble phosphorous action because of increase in its ionic power and decrease in the concentration of the soil soluble phosphorous as a result of formation of minerals of Ca and K, are the reasons why plants absorb less phosphorous in saline conditions (Grattan *et al* ., 1992).

There are lots of influential factors in decreasing N absorption by plant in saline conditions; these include the reduction in root permeability, the reduction in soil microbial activities followed by reduction in mineralization of the organic matters, and reduction in the absorption of nitrate as a result of supplying too much Cl anions around the roots as was reported by Kafkafi *et al*. (1982).

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