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Full Length Research Paper

Role of mycorrhizal fungi and salicylic acid in salinity tolerance of *Ocimum basilicum* resistance to salinity

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Salinity is one of the common agricultural and biological problems. Most researchers showed that inoculation of plants with mycorrhizal fungi and using salicylic acid increase tolerance of plants due to salinity. In this study, the effect of mycorrhizal fungi, including *Glomus mosseae*, *Glomus intraradices*, and salicylic acid (0.2 mM) on tolerance of green basil (*Ocimum basilicum* L.) to salinity resulting from sodium chloride (75 and 150 mM) was compared and considered. The results show that salinity caused reduction of root inoculation of plants with *G. mosseae*, and against increased malondialdehyde (MDA), other aldehydes, reduced sugars and proline in aerial organs and protein in aerial organs and root. In salinity conditions, sodium content increased in aerial organs and root of basil plant, which is an indicator of damage resulting from salinity. In pretreatment of plants with salicylic acid (0.2 mM), lipid peroxidation was decreased; proline and protein content in aerial organs and root, reduced sugars of root and potassium in aerial organs were increased. Mycorrhizal inoculation decreased lipid peroxidation and increased reduced sugars, proline, protein and potassium contents in aerial organs and roots. In comparison of mycorrhizal inoculation and SA pretreatment, it was observed that effect mycorrhizal inoculation on increase proline, protein and potassium in aerial organs and root and on decrease in sodium of aerial organs was more intensive than salicylic acid. On the other hand, the effect of salicylic on increase in proline of aerial organs and on decrease in potassium of root relative to inoculated plants with mycorrhizae was more intensive. Based these results, it can be concluded that the effect of inoculating plants with mycorrhizae relative to salicylic acid causes a better resistance in basil plant relative to tension of salinity.

Key words: Mycorrhizal fungi, salicylic acid, *Ocimum basilicum*.

INTRODUCTION

Salinity is the most wide spreading phenomena, especially in dry and semi-dry religions in the world. In Iran, there are more than 16 ha agricultural lands of which 30% is influenced by salinity (Ahmadi and Ardekani, 2006). On the other hand, salinity is spreading by 10% rate and concerning the sensitivity of agricultural plants to salinity, the efficiency of plant is reduced in this condition. Moreover, due to increasing population, the need for food and fiber is increased, hence it is anticipated that this problem might influence many agricultural products in Iran and the world in the next few years (Ashraf and Neielly, 2004). The main problem of plant in saline environment is to conserve osmotic potential and to increase the content of toxic ions in plant (Bates et al., 1973, Begum et al.,1992).

At present, many researchers focus on using growth regulators, or mycorrhizal fungi in decreasing the harmful effects resulting from environmental stress. Mycorrhizal, vesicular-arbuscular fungi are unique between micro-organisms occupying rhizosphere. These fungi form symbiotic colonies with most plants and increase inorganic nutrients in plant. They can increase the tolerance of plants due to environmental stress by stimulating growth regulators, increasing photosynthesis, and improving regulation of osmotic pressure (Rabie and Almadani, 2005). Of course, unsuitable conditions of environment such as salinity can have negative effect on inoculation and surviving mycorrhizae. One of the

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effective mechanisms reported for increasing plant resistance to salinity is stimulating synthesis of osmotic materials such as carbohydrates and proline, increasing proteins, increasing the absorption of nutrients and decreasing the content of membranous peroxidation. In mycorrhizal plants, the content of reduced sugars is increased that is mainly due to increase in photo-synthesis. Also, mycorrhizal fungi increase the content of protein by increasing nitrate reductase activity. Increase in content of proline in *Vigna radiate* and *Vicia faba* was reported when inoculated with arbuscular mycorrhizal fungi (sharifi et al., 2007). In other reports, improving absorption of phosphor and potassium of inoculated tomato with mycorrhizae relative to not inoculated plants was observed (Al-karaki and Hammad, 2001).

On the other hand, salicylic or orthohydroxy benzomethylic acid and its compounds are derivatives of plant phenols that are very soluble in water and organic polar solutions. Their effect in removing toxicity from heavy metals, sodium chloride and harmful effects from drought stress were reported (Munne- Bosch and Penuelas, 2003). It was reported that in barley plant, pretreated plants with salicylic acid causes a significant decrease in soluble sugars in aerial organs and an increase in root under salinity. Also, in grape, pretreat-ment with salicylic acid, different content of malondial-dehyde and other aldehyde decrease under cold environ-ment (Wang et al., 2005). In other report, it was showed that salicylic acid caused an increase in antioxidative enzyme activities in tomato and bean and as a result, prevents oxidation of proteins and increases the content of protein (Gapinska et al., 2008).

In this study, the effect of salicylic acid and mycorrhizal on salinity stress resulting from using sodium chloride in basil plant was studied: The purpose of this study was to compare the role of salicylic acid and inoculation with mycorrhizal fungi for increasing of basil plant to salinity.

MATERIALS AND METHODS

Seeds of basil plant (*Ocimum basilicum* L.) were prepared from Pakan bazr Company Isfahan, Iran. The seeds were washed with distilled water three times after disinfecting by sodium hypochloride (10%). Applied treatments included: Sodium chloride (0, 75 and 150 mM), *Glomus mosseae* and *Glomus intraradices* mycorrhizae and salicylic acid (0, 0.2 mM). For inoculating plants with mycorrhizae in Petri dishes, 5 g soil containing spore of mycorrhizal species was separately poured in Petri dishes and the seeds were placed on it .The soil is moistened with distilled water and the seeds were placed in germinator in order to sprout under light (16:8) and temperature (16 ± 2 and 23 ± 2°C) condition.

Sowing of plants was performed in plastic pot with 12 cm diameter containing perlite. For mycorrhizae treatment, 50 g soil containing spore of mycorrhizae was poured on surface of perlite in every pot and budded embryos in mycorrhizal soil were placed on them. The pots were placed in greenhouse under light (14:10) and temperature (22 ± 2 and 17 ± 2°C) condition and intensity of light 11000 KLUX and 60% humidity. For irrigation of pots, distilled water and nutrient solution (long Aston) were used. For pretreatment with salicylic acid after spreading forth leaf, leaves of plants were sprayed with salicylic acid solution (0.2 mM) for five days at light phase. Five days after final treatment with salicylic acid, saline treatments with sodium chloride were performed three times for 72 h.

Measurement of colonization (infection) mycorrhizal percentage of root

For this measurement, Rajapakes and Miler (1992) method was used. The roots were painted with fuchsine acid color and the percentage of inoculated root was measured by using anatomical microscope, unit of arbuscular- vesicular mycorrhizae.

Estimation of lipid peroxidation

For the estimation of lipid peroxidation, the density of malondialdehyde and other aldehydes was measured by the methods of Meir et al. (1992) and Heath and Packer 1968. Based on these methods, fresh leaves were ground in a china mortar containing 5 cc trichloroacetic acid (TCA) 0.1%. The resulting essence was centrifuged in 10000 g for 5 min. 4.5 cc of TCA solution (20%) containing 0/5% thiobarbituric acid (TBA) was added to 1 cc of mixture and was then heated in 95°C for 30 min. Subsequently, it was cooled in ice and again the solution was centrifuged in 10000 g for 10 min. The absorption degree of solutions was read by spectrophotometer in wavelength 532 nm for malondialdehyde and 455 nm for other aldehydes absorbing. Other non special dyes was determined in 600 nm and subtracted. To calculate the concentration malondialdehyde, silence coefficient equal to 155 $cm^{-1}MM^{-1}$ was used and the results were calculated and introduced according to microgram on gram of fresh weight.

Determination of protein content

The content of proteins in root and leaf was measured according to Lowry et al. (1951) method. 0.02 g tissue of fresh leaf and root was weighed and every sample was ground separately by 4 cc of salt phosphate buffer in china mortar within ice. Absorption intensity of solutions was determined in wave length 660 nm. Albumin of cow was used for preparing standard curve and the results were reported according to milligram on gram of fresh weight.

Determination of proline content

Proline was measured by the method of Bates et al. (1973). 0.04 g fresh tissue of leaf and 0.08 g fresh tissue of root were ground in sulfosalicylic acid (3%), and the resulting educe was centrifuged in 10000 g for 5 min. Then, 2 cc surface solution was mixed by 2cc pure salicylic acid and heated in 100°C for 1 h, the tubes were cooled in ice bath and 4 cc toluene was added to each other and shaken well. By fixing tubes, two layers were formed separately, and that upper color layer was used for measuring the content of proline in wavelength 56 nm, while data from pure proline was used for standard curve. The results were calculated and introduced according to milligram on gram of fresh weight.

Measuring the content of reduced sugar

0.04 g fresh tissue of leaf and 0.08 g fresh tissue of root were weighed and every sample was ground separately by 10 cc distilled water in china mortar. The content of reduced sugars in leaf and root was measured by Nelson–Somogyi (1952). The absorption intensity of solutions was read in wavelength 600 nm, and then the

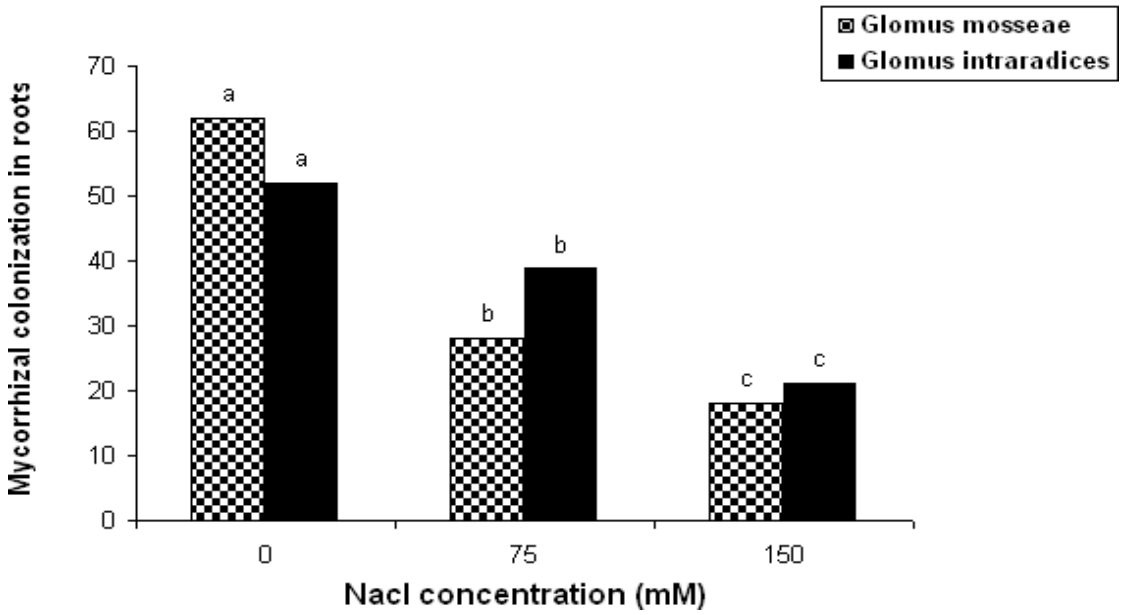

Figure 1. Root colonization in root green basil plant under salinity (a)

content of reduced sugars was calculated and reported according to milligram on gram of wet weigh by using standard curve.

Data analysis and statistical studies

This experiment was performed with four replicate in completely random design Framework. Data analysis was performed by MSTATS and SAS software. The figures were drawn by Excel software.

RESULTS

The amount of mycorrhizal colonization root

According to Figure 1, root colonization percentage in inoculated plants with *G. mosseae* and *G. intraradices* was reduced by increasing the content of sodium chloride that is significant at 5% level and is index of negative effect of salinity on root colonization degree by these fungi. The results were not significance between *G. mosseae* and *G. intraradices* and salinity (Figure 1).

Membranous lipid peroxidation

The obtained results from experiments related to membranous lipid peroxidation showed that the content of malondialdehyde and other aldehydes was increased significantly relative to control in plants that treated with NaCl. Pretreated plants with salicylic acid or inoculating them with mycorrhizae decreased significantly these parameters in plants under salinity stress than to control plant. Also, the effect of mycorrhizal fungi *G. mosseae* on reducing the content of malondialdehyde relative to salicylic acid was higher (Figure 2).

Proline content

The results of this study showed that proline content in aerial organs significantly increased in 150 mM sodium chloride. Salicylic acid increased the content of this parameter relative to control plants, so that increasing effects of salicylic acid was more prominent than mycorrhizal fungi. On inoculating plants with *G. mosseae*, proline content of aerial organs relative to control significantly increased in plants treated with 75 and 150 mM NaCl. However, in plants inoculated with *G. intraradices*, the content of this parameter increased significantly only in 75 mM NaCl salinity, while in 150 mM NaCl it was decreased relative to control plant. In root, sodium chloride treatment reduced proline content in root significantly. Pre-treating plants with salicylic acid and or inoculating them with mycorrhizae caused a significant increase of this parameter in plants under tension of sodium chloride relative to control plant. In inoculating plants with mycorrhizal fungi, the highest content of root proline in 150 mM salinity was observed for *G. mosseae*, while in 75 mM salinity, it was observed for *G. intraradices* (Figure 3).

Reduced sugar content

Reduced sugar content of aerial organs in plants under salinity increased significantly. In plants inoculated with

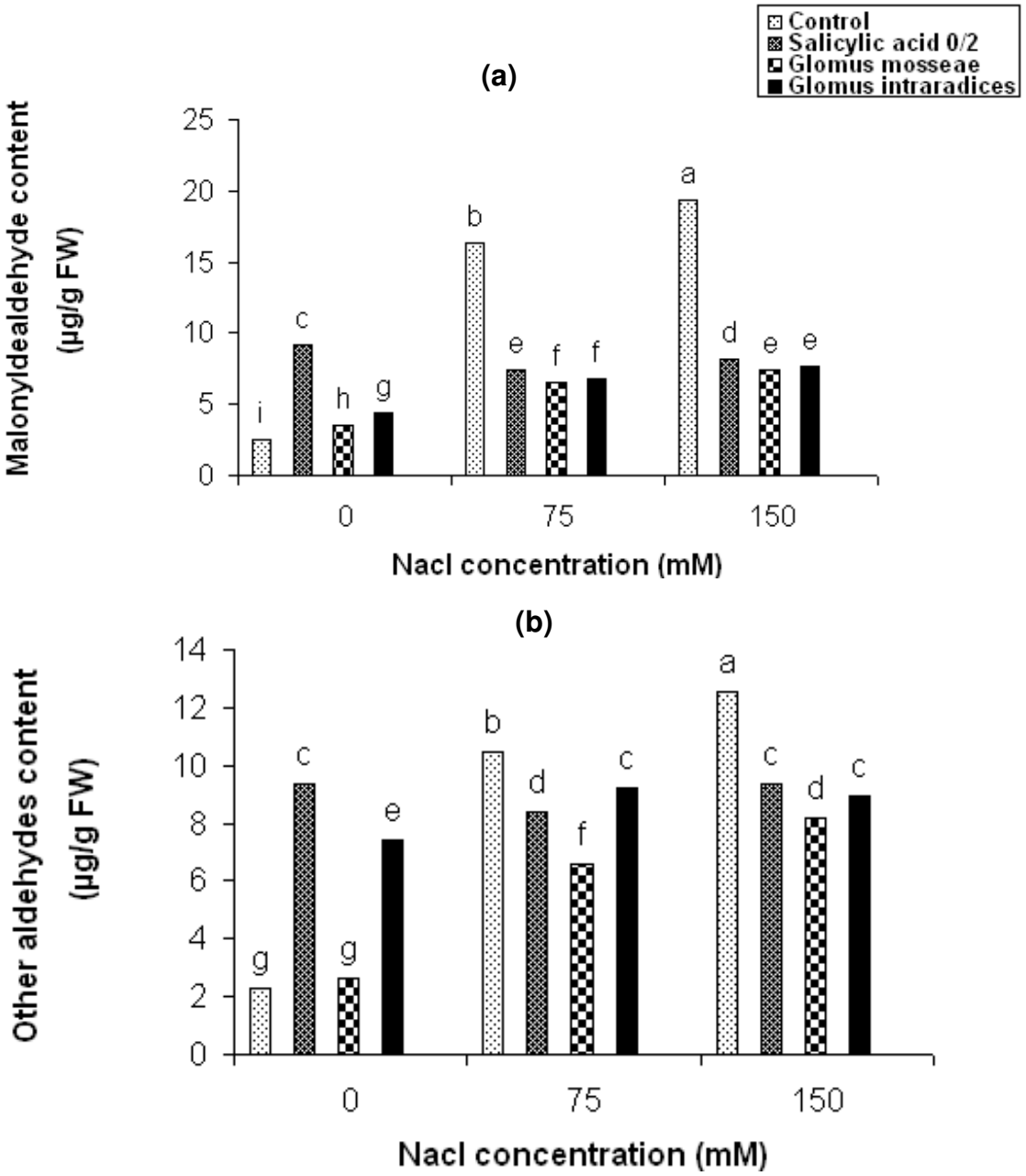

Figure 2. Interactive effect of mycorrhizal fungus, salicylic acid and salinity on the content of malondialdehyde (a) and other aldehydes (b).

mycorrhizae and treated with sodium chloride, the content of this parameter was significantly increased so that the effect of *G. mosseae* relative to *G. intraradices* was more prominent. While in plant sprayed with salicylic acid and treated with salinity, reduced sugar content of aerial organs was decreased significantly. In considering the results from reduced sugar content of root, for plants under salinity, the most content of this parameter was observed in 75 mM sodium chloride. Pretreated plants with salicylic acid or inoculating them with *G. mosseae* ceased to increase significantly this parameter in plants under sodium chloride relative to control plant. Inoculating plants with *G. intraradices* increased reduced sugar content of root in 150 mM sodium chloride relative to control plant and the results was not significant in two other levels (Figure 4).

Protein content

The results from this study showed that protein content of aerial organs was increased significantly in 150 mM

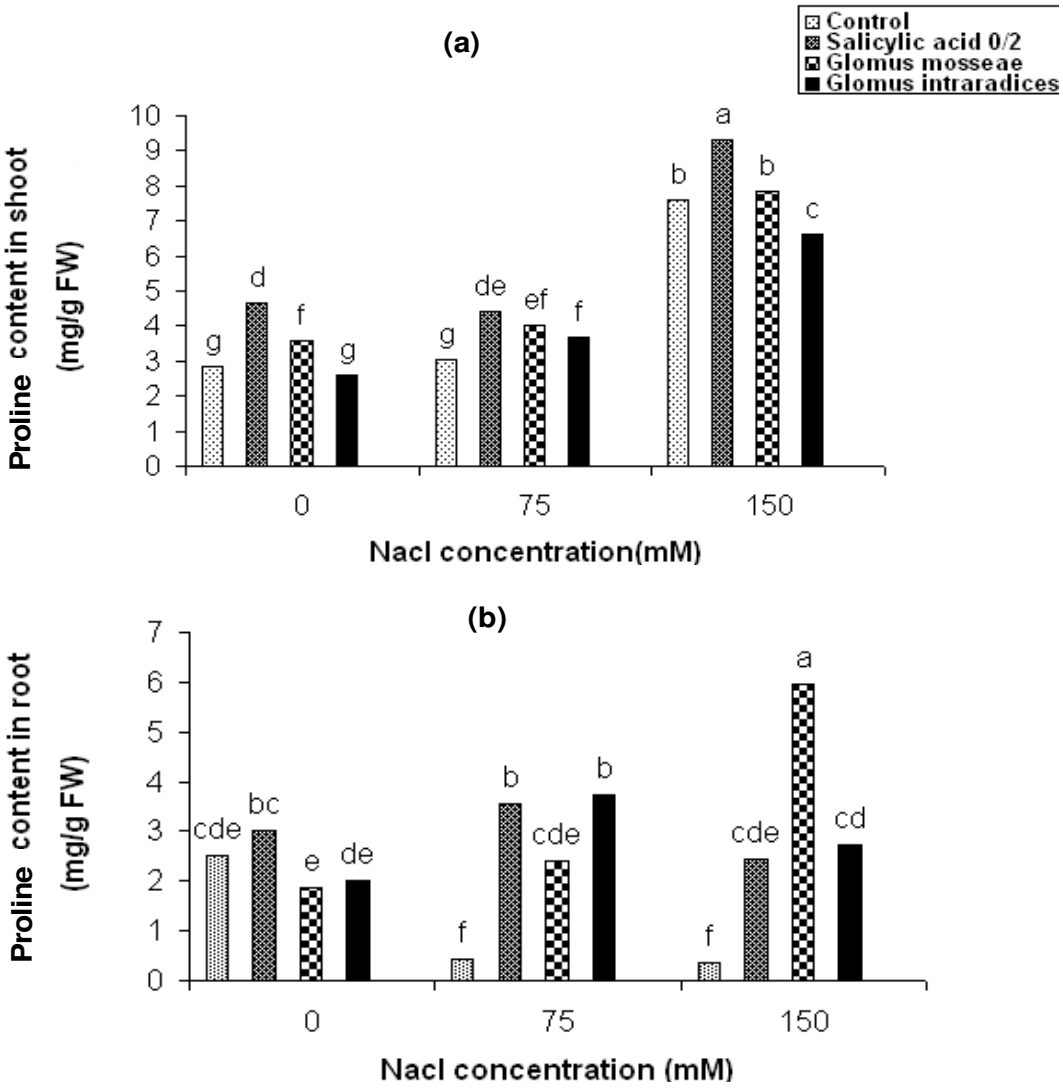

Figure 3. Interactive effect of mycorrhizal fungus, salicylic acid and chloride sodium concentration on proline content of aerial organs (a) and root (b).

sodium chloride. In 75 mM salinity, pre-treating with salicylic acid or inoculating plants with mycorrhizal fungi caused an increase of this parameter, while in 150 mM, inoculating with *G. mosseae* or pretreatment with salicylic acid caused an increase in the content of this parameter. But pretreatment with salicylic acid increase protein content in 75 mM sodium chloride and reduction in 150 mM sodium chloride significantly. In considering the results from protein content of aerial organs and root, increasing effects of *G. mosseae* were more prominent than *G. intraradices* (Figure 5).

Sodium content

The results from this study showed that sodium content of aerial organs and root was increased in treatment resulting from sodium chloride using salicylic acid, and mycorrhizal fungus significantly decreased sodium content of aerial organs relative to control plants. Decreasing effects of *G. mosseae* were more sensible than other treatments. In considering sodium content of root, pretreating with salicylic acid caused this parameter to decrease significantly relative to control plant. In inoculating plants with mycorrhizal fungi, only significant effects of *G. mosseae* caused this parameter to increase significantly in 150 mM sodium chloride (Figure 6).

Potassium content

Potassium content of aerial organs decreased in treatments resulting from sodium chloride. Using salicylic acid, mycorrhizal fungus increased significantly the

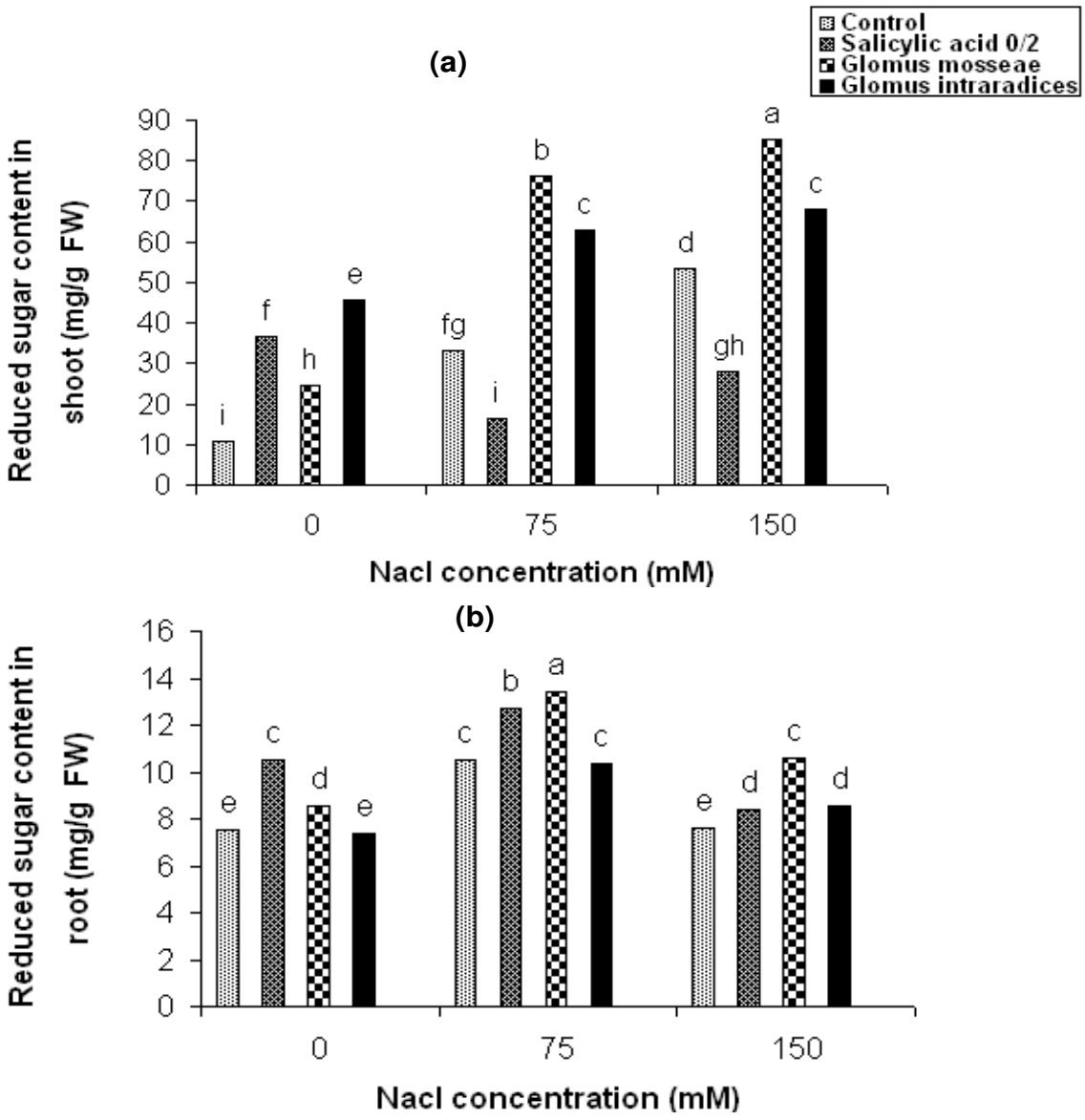

Figure 4. Interactive effect of mycorrhizal fungus, salicylic acid and salinity on reduced sugar content of aerial organs (a) and root (b).

content of this parameter relative to control plants. The increasing effects of *G. mosseas* were more sensible than *G. intraradices.* Potassium content of root increased in 75 mM under the treatments of salinity and decreased in 150 mM. Using salicylic acid and mycorrhizal fungus treatments decreased the content of this parameter significantly relative to control groups, and in comparison of two fungi, decreasing effects of *G. intraradices* was more prominent that *G. mosseae* (Figure 7).

DISCUSSION

In this study, mycorrhizal colonization content of root in basil plant decreased. It was reported that mycorrhizal colonization content of root decreased significantly in tomato plants (Abdel and Chaoxing, 2011) and *Jatropha curcas* L. (Ashwani et al., 2010) under salt stress inoculating by mycorrhizal fungus, which is similar to the results of this study that specified that increasing salinity can have negative effects on leaf growth and viability *Glomus* species.

Producing radical oxygen in cell causes peroxidation in membranous lipids and compounds such as malondialdehyde, propanal, butanal and so on. Increasing the content of lipid peroxidation is an index of increase in oxidative stress (Sairam et al., 1998). In this study, treatment of salinity caused the increased content of MDA and other aldehydes in aerial part of plant, which is sign of stress intensity applied to plant. The same result

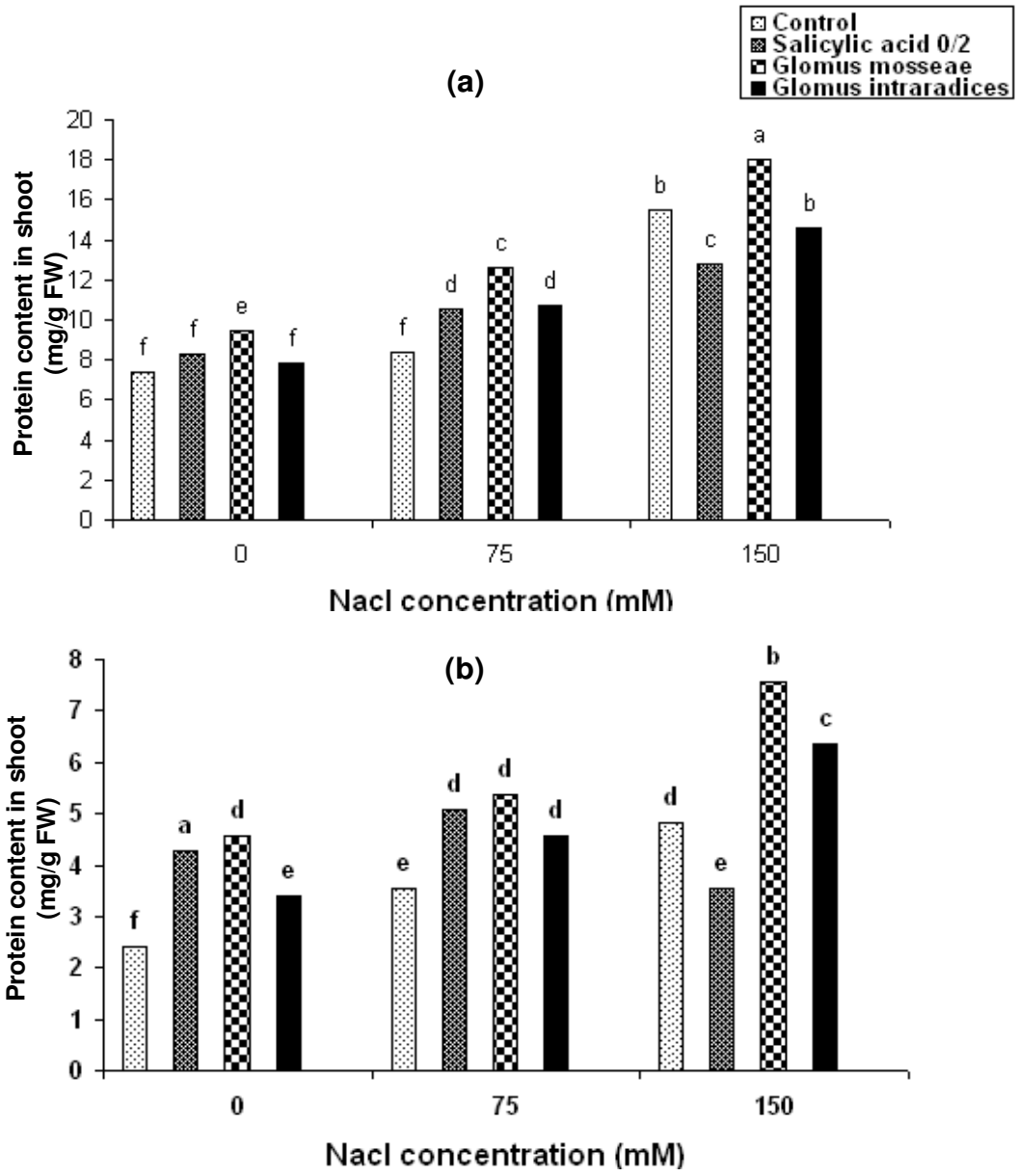

Figure 5. Interactive effect of mycorrhizal fungus, salicylic acid and salinity on protein content of aerial organs (a) and root (b).

was observed in barley and two species of sugar beet under saline stress (Gao et al., 2004) and in maize under drought stress (Maksymiec and Krupa, 2002). Using pretreatment with salicylic acid or mycorrhizal inoculation in plants under sodium chloride, however, decreased the content of these parameters. Thus, it can be concluded that using these pretreatments caused a decrease of the destructive effects from sodium chloride in green basil plant. It was reported that in wheat treated with salicylic acid compared to wheat under salinity, the content of MDA was reduced (Agarawal et al., 2005). Furthermore, inoculating tomato plants with *G. mosseae* decreased MDA content under saline stress compared to not inoculated plants (Abdel and Chaoxing, 2011). In other reports, symbiotic mycorrhizae decreased relative membranous penetration (Figure 8) and malondialdehyde in leaves and roots of maize under heat stress (Zhu et al., 2009) in *Cajanus cajan* L. under saline stress (Garg and Manchanda, 2009) and bean under drought stress (Porcel and Ruiz-Lozano, 2004). Also mycorrhizal damage resulting from stress conditions is consistent with this study and is a sign of less oxidative damage in inoculated plants.

Also, in plants under saline stress reduced sugar content and protein in aerial organs and root and proline in aerial organs increased. This implies that the resistant

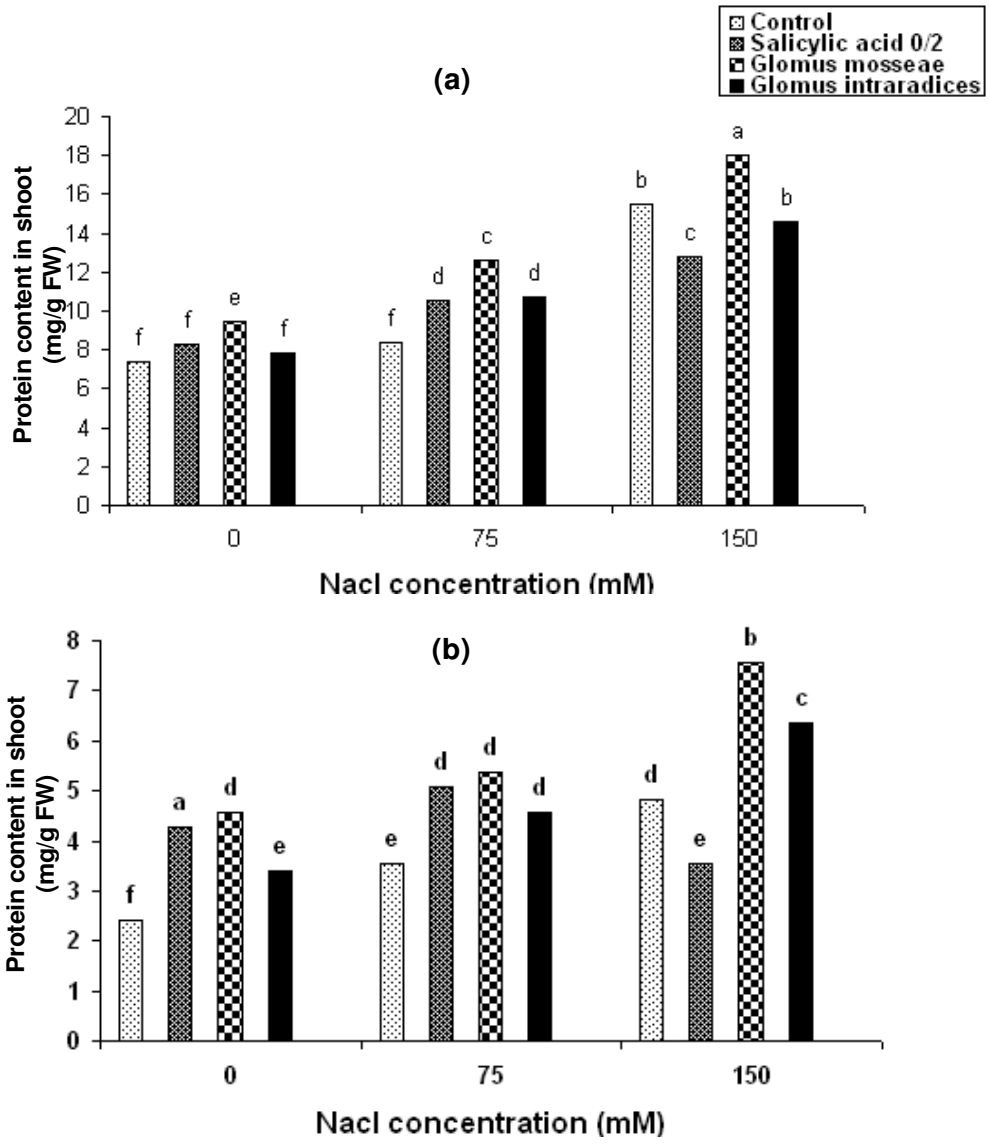

Figure 5. Interactive effect of mycorrhizal fungus, salicylic acid and salinity on protein content of aerial organs (a) and root (b).

system of plant was initiated and osmolyte was produced against damages from salt stress in plant. Proline protects cell membrane in biotic and abiotic stresses. Sairam et al. (1998) showed that increase in proline and reduced sugar causes an increase plant resistance against salinity. In *in vitro* environment, proline has a role of sweeper of ROS and causes to increase cellular synthesis in plants during stress (El-Tayeb, 2005). Increase in proline content in this experiment can be due to increase proline synthesis or reduced decomposition in order to confront saline stress. In general, proline accumulation in saline and drought stresses was reported by many researchers. For example, Meloni et al. (2001) on studying cotton, reported that proline can regulate osmosis in osmotic conditions (Meloni et al., 2001). Also, it was reported that sodium chloride causes an increase in soluble sugars in barely and tomato (Gao et al., 2004). This increase in glucose, fructose and sucrose creates osmotic balance in tomato because salinity causes an increase in enzymatic activity of sucrose phosphate and fruit sugar and leaf of tomato (Rawa- Miszczak et al., 2003).

In addition, increase of proteins was observed in saline stress in many species of plant. For example Amini and Ehsanpour (2005) reported an increase in soluble proteins in root and leaf of tomato while increasing salinity from 0 to 80 mM. They assumed that increase in soluble proteins resulted from protein synthesis or structural proteins; especially they contribute to synthesize proteins changing properties of cellular wall

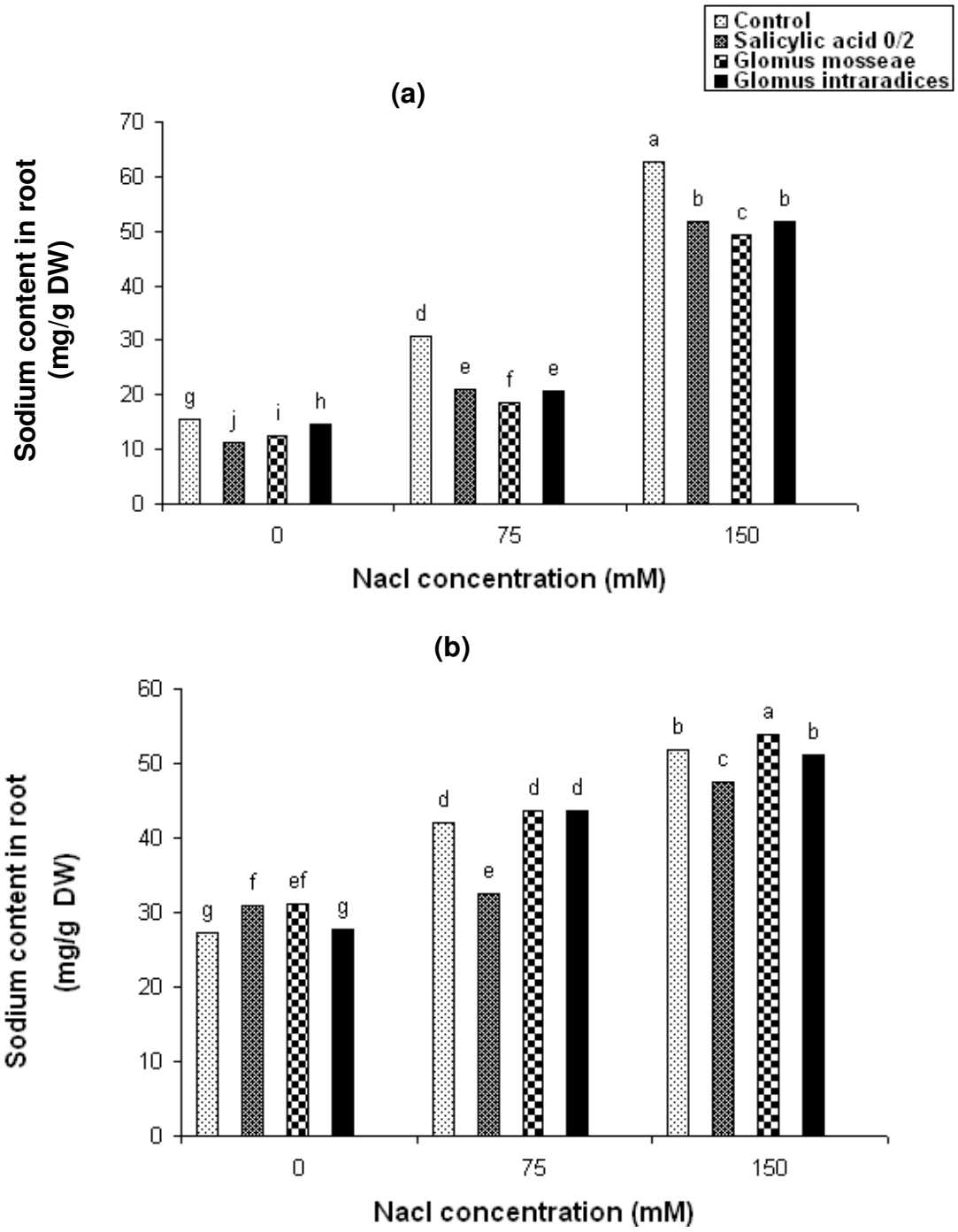

Figure 6. Interactive effect of mycorrhizal fungus, salicylic acid and salinity on sodium content of aerial organs (a) and root (b).

(Amini and Ehsanpour, 2005). While pretreating plants with salicylic acid decreased content of reduced sugar of aerial organs in sodium chloride stress and increased reduced sugar of root content, protein content of aerial organs and root only in 75 mM sodium chloride and proline of aerial organs and root. In the same experiment in barley plant under saline stress, pretreating with salicylic acid caused to significantly decrease soluble sugar content in aerial organs and to increase it in roots (El-Tayeb, 2005). In other report, salicylic acid induces synthesis of HSP proteins in *Tobacco* leaves (Burkhanova et al., 1999). Pretreatment with salicylic acid may stimulate hydrolysis of insoluble sugars or proteins and created an osmotic source that is important to

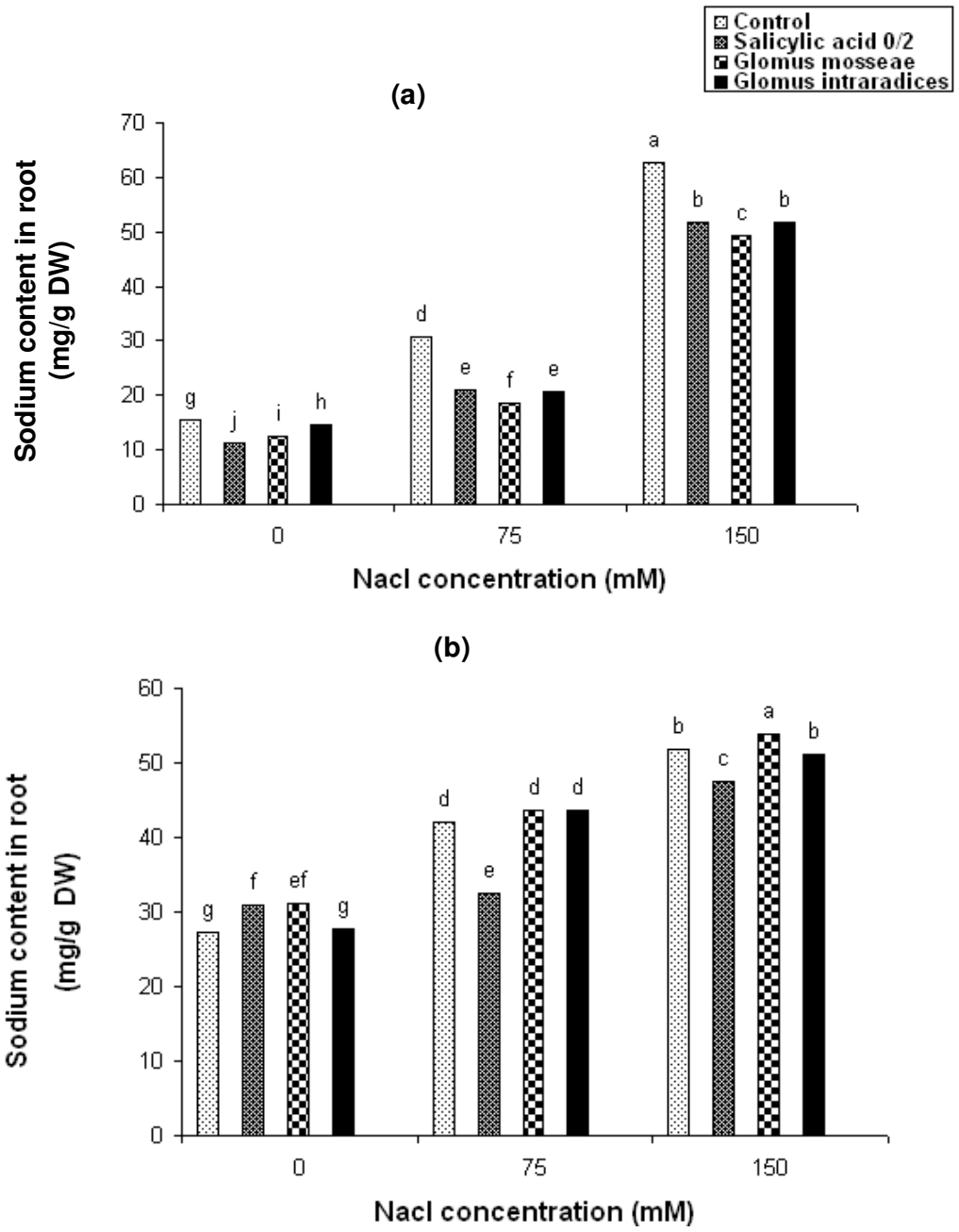

Figure 6. Interactive effect of mycorrhizal fungus, salicylic acid and salinity on sodium content of aerial organs (a) and root (b).

osmotic regulation (Shakirova and Sahabutdinova, 2003). The results of this study are consistent with other reports. Inoculating plants with mycorrhizal fungi also reduced sugar content and protein increased in aerial organs and root and so did proline of root too, and proline content of aerial organs increased only in 75 mM sodium chloride that is the sign of stimulating osmotic material synthesis in stress conditions by these fungi. The researchers reported mechanisms different for justifying increase in sugar by VAM fungi. For example, it was stated of VAM fungi significantly increasing photosynthetic and stomata conductance of host plants and thereby causing an increase in sugar content. Also, increase of sugars in salinity can be as a result of starch (Marschner and Dell, 1994).

In inoculating the plant, *Prosopis juliflora*, with *Glomus fascisulatum*, soluble sugar of leaf and roots increased (Selvaraj and Chelapan, 2006). More also, the plant, *Lotus glaber*, inoculated with *G. intraradices* have more root to stem ratio, sodium to potassium ratio and protein

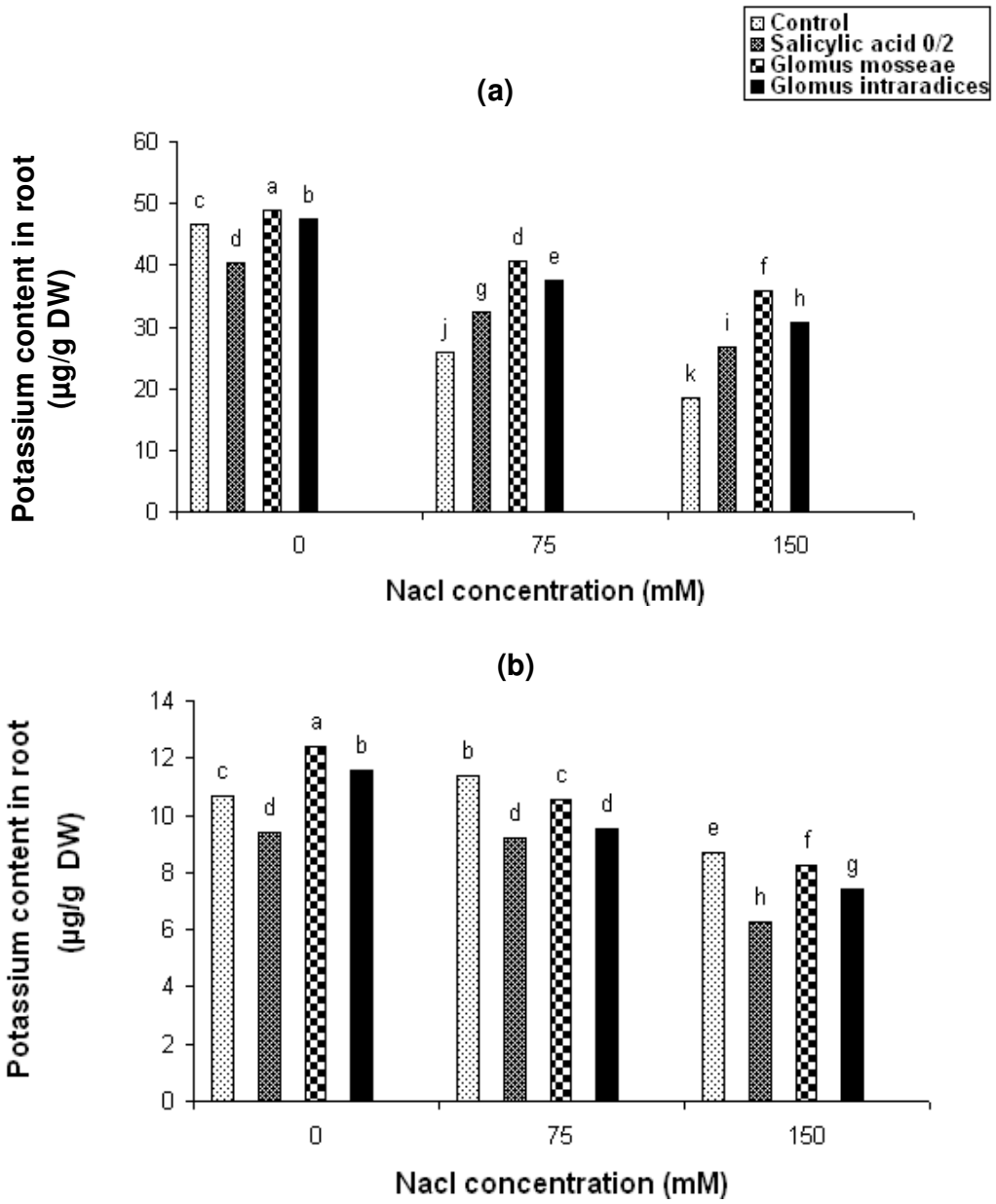

Figure 7. Interactive effect of mycorrhizal fungus, salicylic acid and salinity on potassium content of aerial organs (a) and root (b).

and chlorophyll content than non-mycorrhizal plants (Sannazzaro et al., 2005). Mycorrhizal symbiotic increased soluble sugar and proline content in roots of maize under heat stress, while proline content of leaf was decreased (Zhu et al., 2009). In other reports, in wheat plant under cadmium stress inoculated with *G. intraradices*, by increasing metal, sugar and proteins content increased compared to uninoculated plants (Jamalabad and Khara, 2008). In this study, under treatments resulting from sodium chloride, sodium content of aerial organs and root increased and potassium content of aerial organs and root was decreased. In the same study it was reported that in salt stress, sodium ion content increased in *Triglochin bulbosa* and *Triglochin striata* and potassium ion density decreased (Hagemeyer, 1996). Potassium scarcity is a symptom that occurs due to competition between sodium and potassium cat ions with a common protein, sodium competes with potassium for charging into cell (Hasegawa et al., 2000). Using pretreatment with salicylic acid caused a decrease in sodium content of aerial organs and root, as well as potassium content of root, while potassium ion in wheat (El-Tayeb, 2005). Also, it was reported that salicylic acid increased potassium

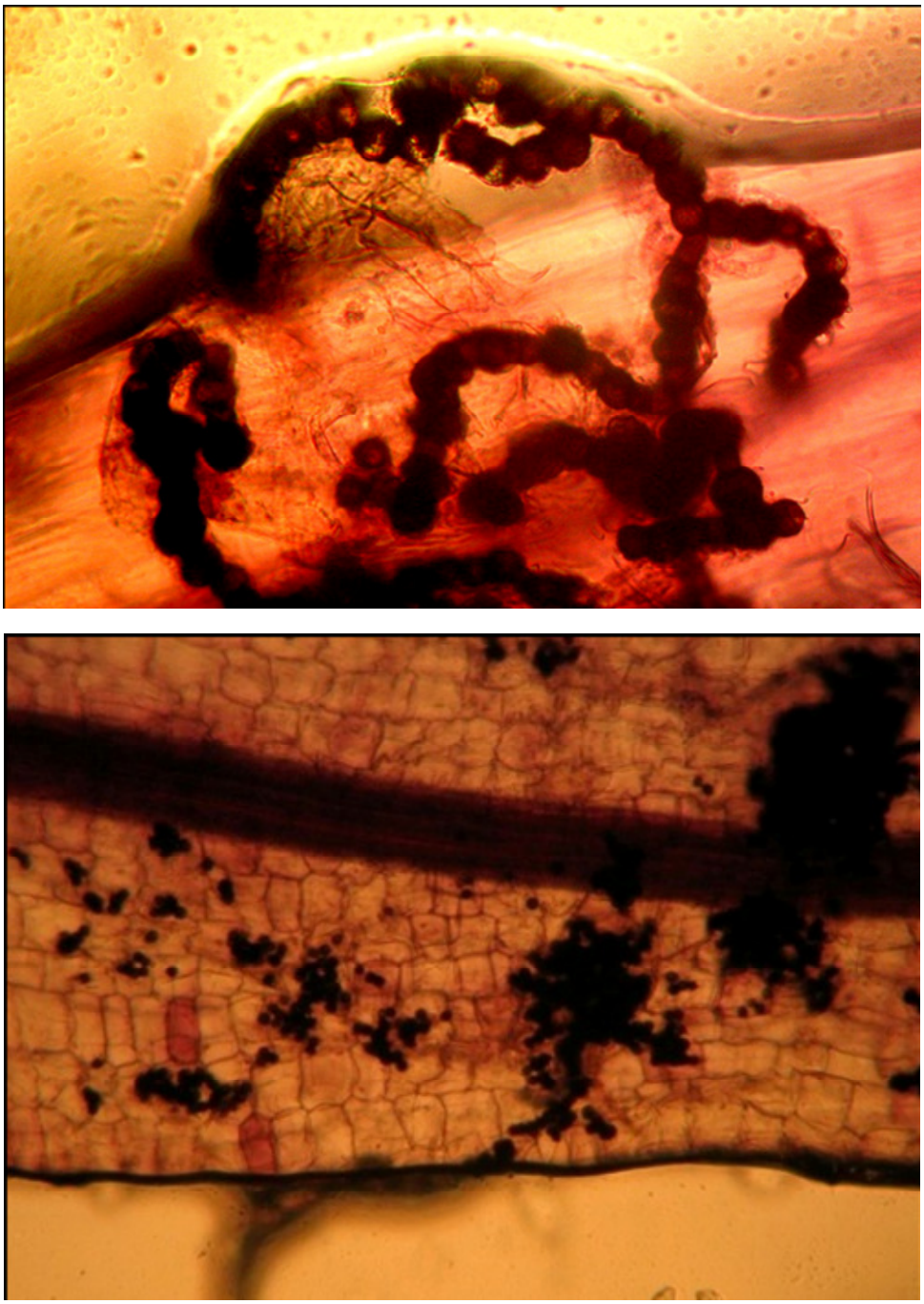

Figure 8. Mycorrhizal fungi penetration into root cells.

density in maize under saline condition, and sodium accumulation and chloride is controlled in maize (Gunes et al., 2005).

Conclusion

Mycorrhizal inoculation in plants under sodium chloride stress increases potassium content of aerial organs and decreases sodium content of aerial organs and potassium of root. Thus, it can be concluded that using this pretreatments, especially VAM fungi, prevented the transfer of sodium to plant, especially aerial organs and decreased destructive effects from sodium chloride stress in green basil plant. Also, inoculating with mycorrhizal fungus could increase transferring potassium to aerial part of plant. In a similar report, sodium and chloride content in roots and leaves of mycorrhizal and non-

mycorrhizal plants increased in olive tree by increase in salinity, and potassium of root was decreased (Rinadelli and Mancuso, 1996). In the plant, *Cajanus cajan* L., a more sodium to potassium ratio and calcium to sodium ratio in mycorrhizal plants than non-mycorrhizal plants was observed (Garg and Manchanda, 2009).

It can be concluded that pretreating leaves of basil plant with salicylic acid or inoculating with mycorrhizal fungus caused to increase resistance of this plant relative to salt stress.

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