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Effect of two species of Mycorrhizal fungi and salinity on proline amount, absorption and transmission of elements on Ocimum basilicum

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ABSTRACT: In order to investigate the effect of coexistence of two species of Mycorrhizal fungus and salinity on chlorophyll and transmission of elements to shoot, a pot experiment in factorial was conducted in a completely randomized design with four replications on basil (Ocimum basilicum L.). Factors examined include three levels of salinity (1, 3 and 5 ds/ m) and applying two Mycorrhizal fungi (Glomus intraradices and Glomus mosseae). Results showed that salinity had a significant effect on investigated traits, such that with increasing salinity, proline content increased. Plants inoculated with AM fungi compared to plants not inoculated, the absorption rate is higher in salt stress and non-stress conditions, respectively. The results showed that the effect of fungus G. mosseae in reducing the salinity was higher than the fungus G. intraradice. Therefore it can be concluded that in the condition of AM fungi to salinity, by increasing nutrient uptake requirements, by adjusting the effects of tensions, by growing proline, improve the growth and function of basil plant.
Keywords: basil, proline, salinity, Mycorrhiza fungi

INTRODUCTION

One of the main problems of agriculture in arid and semi-arid areas is the problem of salinity and concentration of solute in the soil surface, which reduces the yield and acreage (Al-karaki and Hammad 2001). According to UN estimates, approximately 20% of the environment, agricultural land and 50% of arable agricultural lands in the world are exposed to salinity (Flower and Yeo 1995). The soil in the area which offer a total of 32 million hectares are under salt stress that covers nearly 30% of the whole country and 55% of arable land (Anonymous 1994). There are Two views on the effects of salinity on plant growth and metabolism. The Present study investigates the effects of two species of arbuscular mycorrhizal fungi on the rate of root proline and shoots organs that fungus maykvryza symbiotic with root basil can be effective on salt tolerance and uptake and transport plant organs to plant tissues.

MATERIALS AND METHODS

This study investigated the effect of using two species of fungus Glomus intraradices and Glomus mosseae under salinity stress on growth and some physiological factors of basil, into a pot experiment was conducted in a greenhouse of Islamic Azad University. This factorial experiment was conducted in a completely randomized design with four replications. Salinity treatments consisted of three levels including 1, 3 and 5 ds m salinity Mycorrhiza application of the two species G. intraradices and G. mosseae) and the non-application of fungus (control).

The sandy soil used for 1 h with a temperature of 121 ° C for 5 min and then rinsed with tap water sterilized pots, were surface sterilized by alcohol. Physical and chemical properties of soil were determined. The research field had a sandy loam soil. Details of soil properties are shown in Table 1.

Table 1. chemical and physical properties of the studied soil

. depth	Clay (%)	Silt (%)	Sand (%)	texture	EC (dS/m)	CEC (meq/100g)	O.C (%)	Total N (%)	Available P (ppm)	Available K (ppm)
0 - 30	12	23	65	Sandy Loam	1.50	6.4	0.8	0.09	7	120

Preparation of inoculums

According to Dan Kerr for surface disinfection, the seeds were soaked in 5% bleach for 7 min and were washed 8 to 10 times with distilled water. For each hole, a number of seed placements on the inoculum were covered with soil.

Measurement of sodium and potassium

In order to measure the amount of leaves sodium and potassium by the method of Hamada and Elena (1994), first take the shoot and then dry the samples in an oven at 70 ° C for a period of 48 hours. The dried samples using the 0.1 mill powder sample is poured into a 15-mL Falcon and 10 ml of acetic acid is added to the normal 1.0 Glaysyal and maintained in vitro for 24 h. After 24 hours the samples are in a water bath (water bath) at 70 ° C for two hours. After two hours the samples were removed from the water bath and filter them by filter paper (Whatman 41) and funnel. Extract was transferred to another 15-mL Falcon. Flame Photometer device was used for reading.

Proline measurement

To determine the amount of proline in root and shoot, the method of Bates, et al (1973) was used. Thus, for the preparation of dimenhydrinate Nin, the amount of 1.25 g of this material is poured into the flask and then 30 mL of acetic acid of analytical grade and 20 mL of 6 M phosphoric acid was added. Then slowly heated to Nin dimenhydrinate completely resolved. To measure proline, first the amount of 2.0 g of fresh plant samples were weighed, then they were well worn in porcelain mortar in 10 ml of sulfosalicylic acid 3%. The resulting homogenous material centrifuged in centrifuges around 13,000 rpm at 4 ° C and for 10 min. Then, 2 ml of the filtered extract was transferred to a capped tube and 2 ml of the reagent and 2 ml of acetic acid of analytical grade Nin dimenhydrinate was added to all tubes, respectively. After closing the pipes lid, they were put at 100 ° C water for 1 hour. After cooling, the amount of 4 ml of toluene was added to each of the tubes. For mixing the two solutions, the tubes were shaken using a vortex for 15-20 seconds. At last the supernatant phase which is red and contains proline dissolved in toluene was taken and in conjunction with standard samples, was placed in a spectrophotometer. Facts were read at a wavelength of 520 nm and the proline concentration in milligrams per gram of fresh tissue was determined using a standard curve.
Finally, all data were analyzed using SPSS software.

RESULTS

In Figure 1 the data obtained from the comparison of the mean difference shows a significant difference in terms of the mean of proline shoot between the control plants and plants treated with two fungi in 3 Salinity levels. Results of Duncan's test indicated that the salinity level 5, the mean of aerial proline is more than 1 and 3 levels and with increasing salinity levels the amount of plant shoots increases. However, no significant difference was observed between levels 1 and 3 ds/m salinity.

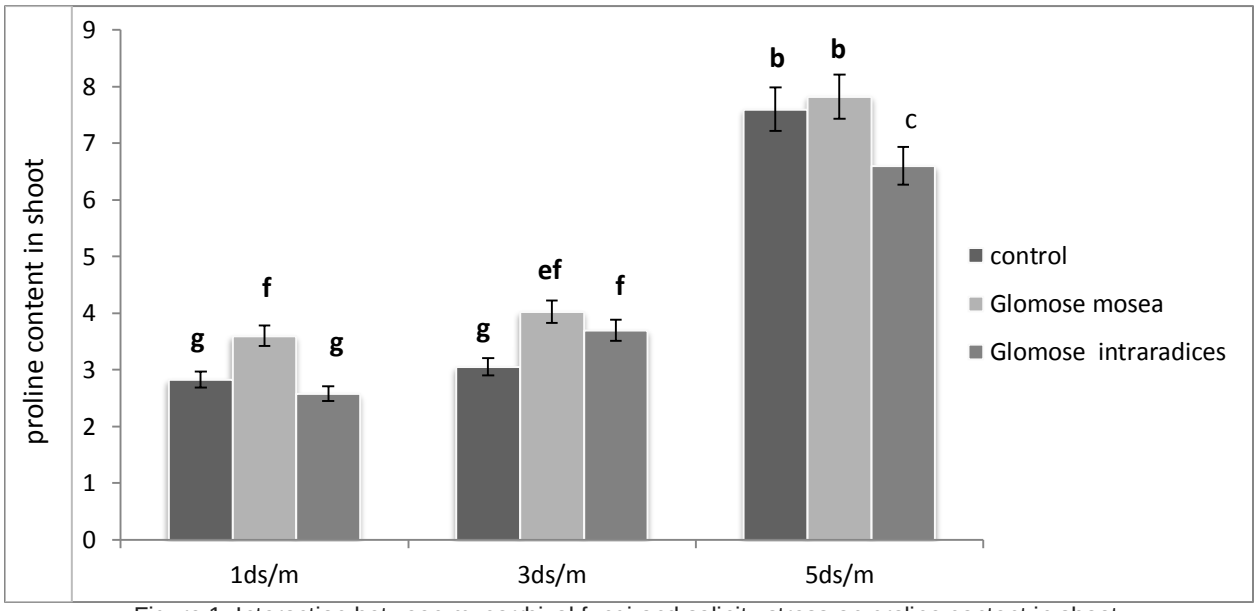

Figure 1. Interaction between mycorrhizal fungi and salinity stress on proline content in shoot.

Information obtained from the mean comparison shows a significant difference of root proline mean between the treated plants and control plants with the fungus at three levels of salinity. Results of Duncan's test indicated that with increasing salinity level, a significant decrease was observed at 5% level in the amount of proline in roots. But the decrease between salinity levels 1 and 3 ds/ m was not significant.

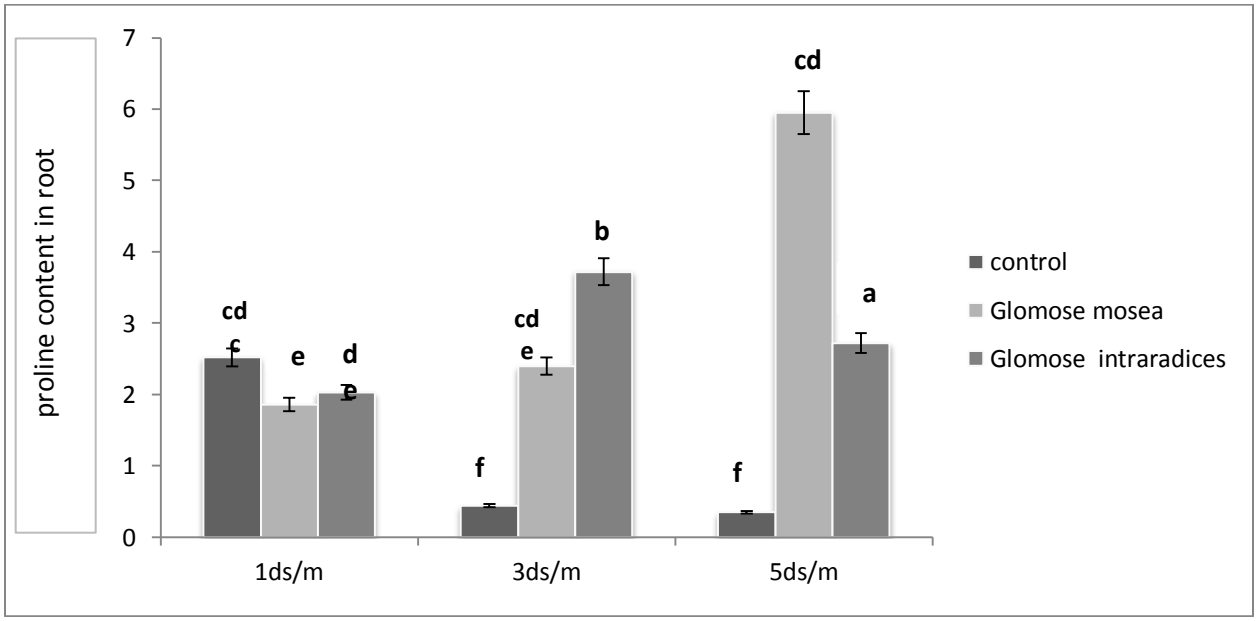

Figure 2. Interaction of salinity stress on mycorrhizal fungi and root proline

Information obtained from the mean comparison shows a significant difference for intake of sodium and potassium ions between control plants and plants treated with the fungus at three levels of salinity. Duncan test results indicate that increased salinity caused a significant increase in the amount of sodium in shoots. The effects of sodium reduction glomos mossa shoot were more intense than Glomose intraradices.

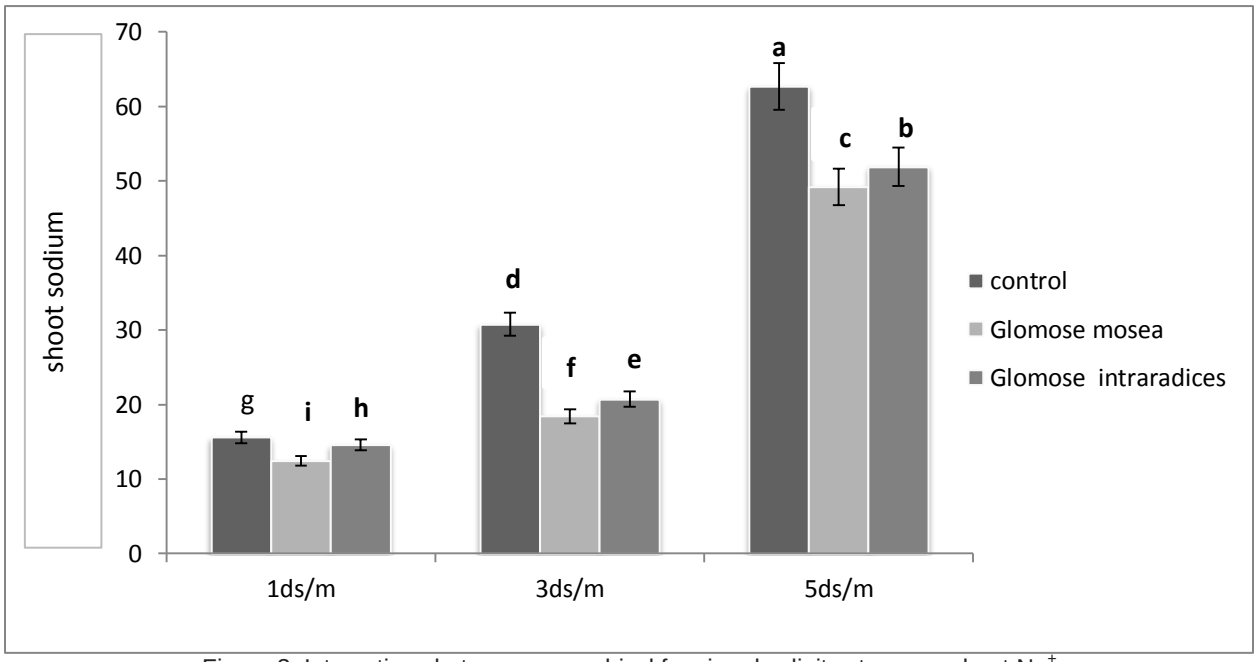

Figure 3. Interactions between mycorrhizal fungi and salinity stress on shoot Na^+

Information obtained from the comparison of the mean shows a significance difference in terms of root mean sodium ion uptake between control plants and plants treated with the fungus in 3 Salinity levels. The results of this study indicated that increased salinity caused a significant increase in the amount of sodium in the root. In plants treated with the fungus glomos mossa alone at level 3 and 5 ds/m NaCl salinity, sodium roots grew more than control plants at 5% level of significance, but at the level of 3 ds/m salinity, significant differences were not observed. The effects of salinity on plant basil, mushrooms and green Glomose intraradices was not significant.

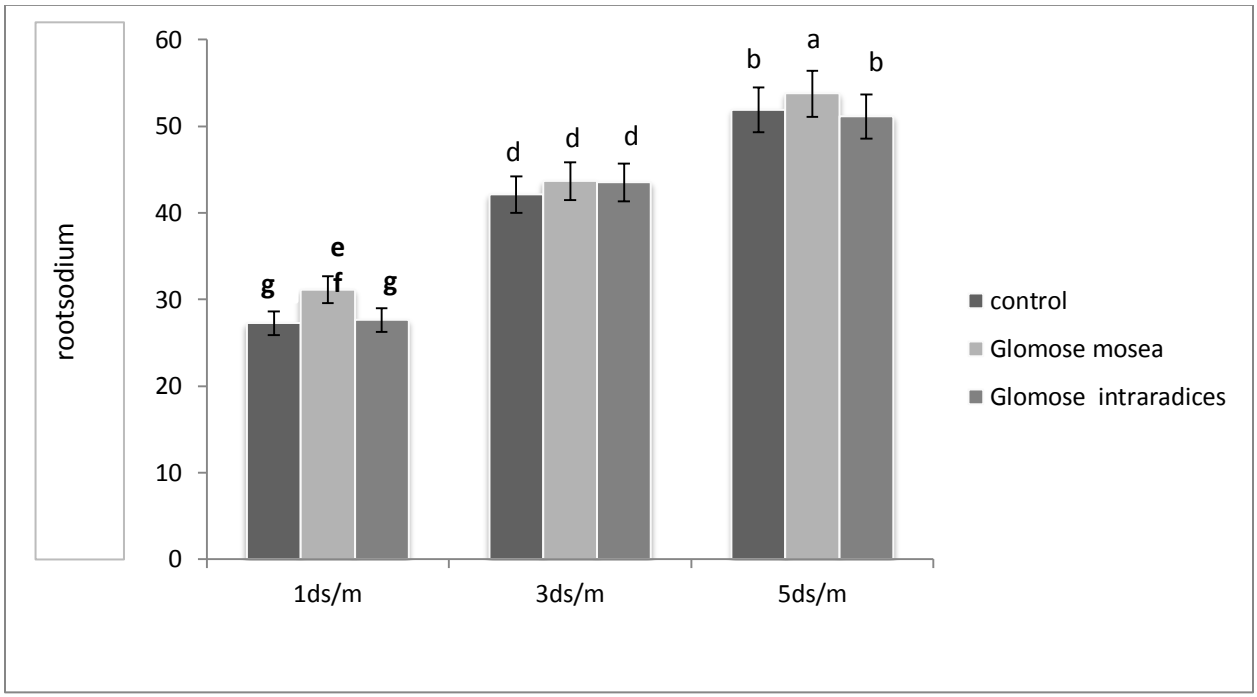

Figure 4. Interaction between mycorrhizal fungi and salinity stress on Na root

Information obtained from the mean comparison shows a significant difference in terms of root Potassium mean levels between control plants and plants treated with 3 salinity levels with the fungus. The results of this

study indicate that increased salinity caused a significant reduction in the amount of potassium in the shoot that is significant in 5%. Effects of treatment of glomos mossa in increasing potassium shoots are more apparent than Glomose intraradices.

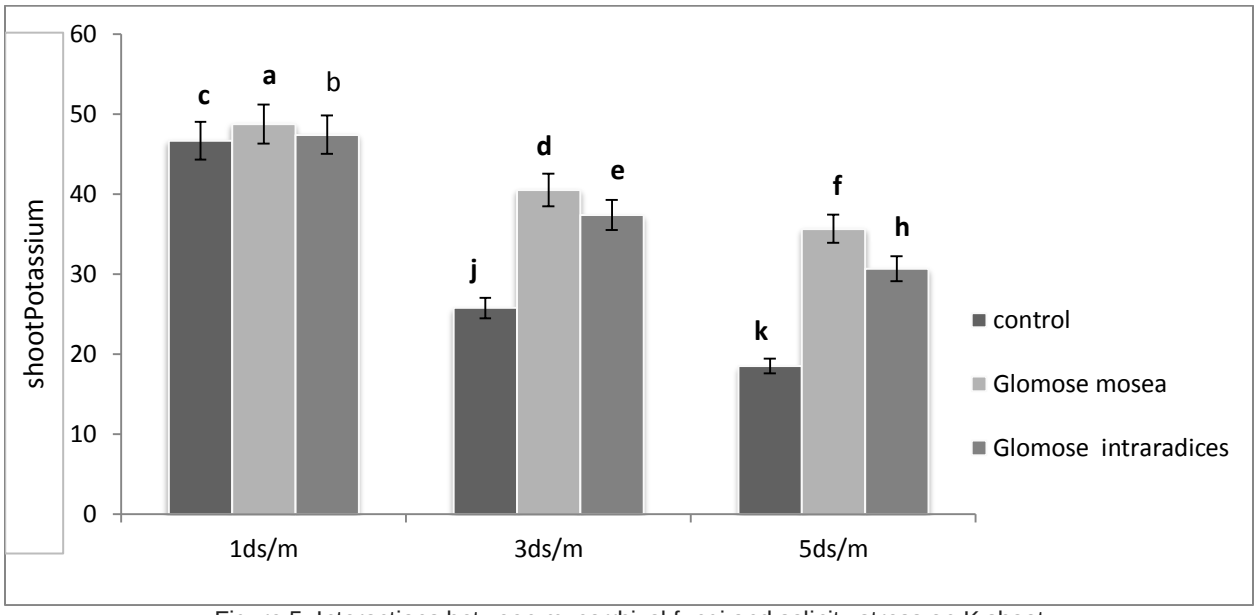

Figure 5. Interactions between mycorrhizal fungi and salinity stress on K shoot

Information obtained from the comparison of the mean shows a significant difference in mean intake of potassium ions in the roots between the control plants and plants treated with fungus at three levels of salinity. The results of this study indicated that the highest Salinity levels in the root potassium salt of 3 ds/m and the lowest rate was observed at 5 ds/m, which is significant in the level of 5%. Treatment plants and fungi glomos mossa Glomose intraradices was also significantly decreased with increasing salinity in the root potassium levels compared with controls. Which is significant at 5% level, but the mycorrhizal treatments alone increased value of this parameter rather than control.

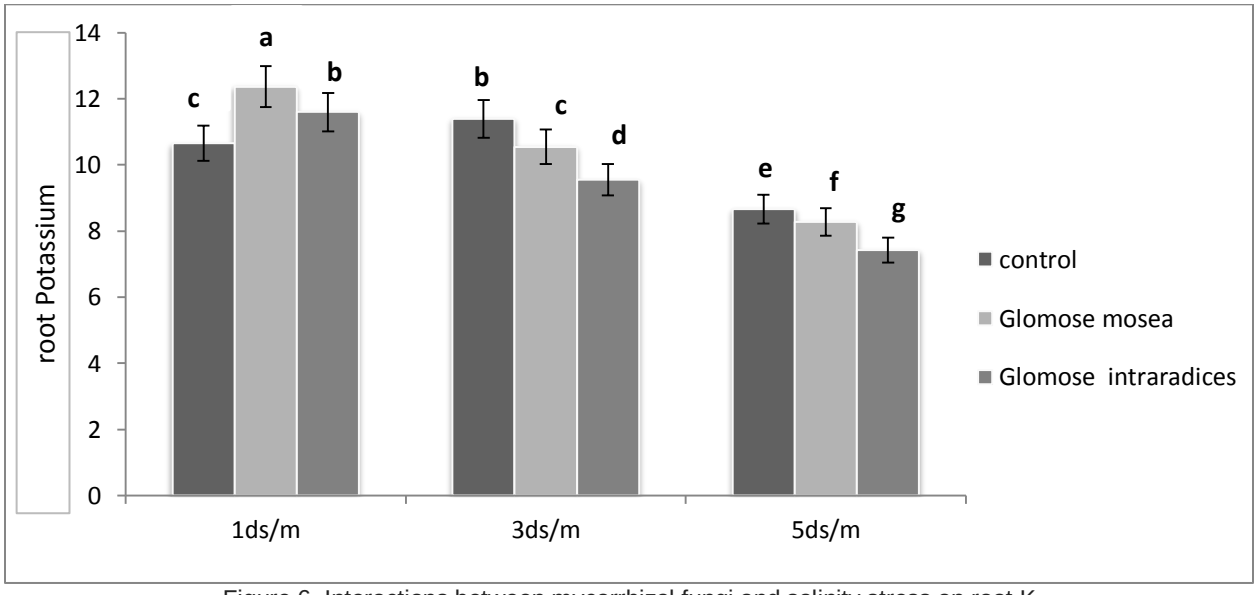

Figure 6. Interactions between mycorrhizal fungi and salinity stress on root K

Table 2.

Shoot proline	Root Proline	Shoot Potassium	Root Potassium	Shoot sodium	Root sodium	df	S.O.V
5/86**	3/880**	385/322**	68/971**	66/058**	3/419**	2	Glomos
110/129**	2/840**	7172/369**	1537/713*	811/738**	23/674**	2	NaCl
5/279**	12/613**	295/968**	94/257**	312/285**	3/213**	4	Glomos×NaCl
0/143	0/247	0/305	1/018	0/160	0/067	18	Error
0.85	0.97	0.95	0.85	0.80	0.70		C.V

* and ** showed significant differences at 5 and 1 %, respectively.

DISCUSSION AND INTERPRETATION

Adaptation of plants to environmental stresses such as salinity and accumulation of metabolites such as nitrogen compounds (proline and other amino acids and spermidine) and carbohydrates (sucrose and other oligosaccharides and polysaccharides) are done. Proline has multiple roles, such as regulation of cell pH, protein stability, and increased protection from the cold and adjusts the redox potential, the enzyme increase leads to greater cell compromise with stress and protect the cytosol and the structures of the cell. Proline accumulated predominantly in the cytoplasm to balance the osmotic potential vacuole (Arnon 1949). Many plants synthesize proline as non-toxic protective at salinity conditions. During stress, proline synthesise is induced and its amount becomes large, because proline is a key amino acid in adjusting osmotic.

In this study, plants that were subjected to salinity stress, the amount of proline shoot in high salinity increased, which indicates the failure resistance of plants and production systems damage Asmvlyt to salt stress in plants, While it declined in roots. Increased amount of proline in this experiment could be due to increase of proline synthesis or decreased breakdown to combat salinity. In general Proline accumulation in drought and salinity stresses has been reported by many researchers. For example, Maloney, et al (2001) studied the role of proline in osmotic adjustment in cotton as their osmotic conditions (Meloni et al. 2001).

However, with increasing NaCl, accumulation of proline induces and proline dehydrogenase enzyme activity is reduced (Parida and Das 2005). A decrease in enzyme activity of proline oxidase with increasing alpha-glutamyl kinase may be the reason for the increase in proline accumulation. Proline accumulation in peanut due to activation of proline synthesis via the glutamate pathway and enzyme activity of alpha-glutamyl kinase, glutamyl phosphate reductase and Δ- Pyrvlyn5-carboxylate reductase has been reported (Bates et al. 1973). Jybvn et al (2000) measured proline accumulation in canola leaf discs by osmotic stress and showed that after 24 hours; its value increases (Gibon et al. 2000). Inoculating plants with mycorrhizal fungi, the amount of shoot proline at a concentration of 75 mm NaCl and root proline increased which indicate that stimulating osmotic in stress situation is by the fungus. In vigna radiata and vicia faba, proline concentration increases in contamination with arbuscular mycorrhizal fungi (Sharifi et al. 2007). By examining the content of sodium and potassium ions in the plant, it was found that in both shoot and root, the amount of Na ions increased by the treatment of sodium chloride. And potassium ion concentrations in the shoots were reduced significantly, which could be due to competition in the uptake and transport of sodium and potassium ions. While with an increase in sodium ions, the presence of potassium and its uptake through the leaf and root can reduce the effects of this ion in compete with sodium. Maintaining the proper level of potassium is essential for the survival of plants under saline conditions. Potassium has a huge impact on the bottom to keep the osmotic potential of the root cells and to maintain the pressure, inflammation, and regulating the water balance in plants is critical (Munns and Schachtman 1993). In a similar study, it has been reported that the concentration of sodium ions in Triglochin bulbosa and Triglochin straiata increased stress and decreased concentration of potassium ions (Hagemeyer 1996). Potassium deficiency is a common complication of competing sodium and potassium occurs for uptake at the root. Because of transporting of sodium and potassium cations with a common protein, sodium competes with potassium to flow into the cell (Hasegawa et al. 2000). Sodium ions with features like potassium ions through the potassium channels with a less choice are included than nonspecific cation channels that are known to adversely affect calcium ion. Plants resistant to salt with accumulating sodium in roots and preventing air from reaching the sensitive tissues, cope with salinity. But Sensitive species by increasing the amount of sodium in shoot increase the amount of this ions rather than potassium ions and lead to lipid peroxidation and degradation of proteins (Parida and Das 2005). Reports suggest that damage caused by salt in salt-sensitive tomato is due to the imbalance of ions (Hagemeyer 1996). Sodium concentration in the roots and stems of rice plants grown under salinity and sodium levels in salt tolerant rice samples is more sensitive (Kang et al. 2005). Mycorrhizal inoculation in plants that were stressed with sodium

chloride, potassium shoot levels were increased and significantly reduced shoots sodium and root potassium. It can be concluded that the application of pretreatment in particular mycorrhizal fungi prevents the transmission of sodium to the plants, especially, shoot, followed by reducing adverse effects caused by sodium chloride stress in green basil. Absorbed potassium by the mycorrhizal fungi in the roots may have been transported to the aerial parts. In non-salinity condition in the mycorrhizal plants, the root sodium level and shoot potassium in roots increased. In an olive tree with increasing salinity, sodium and chloride levels increased in roots and leaves of mycorrhizal and non-mycorrhizal plants and root potassium decreased (Rinadelli and Mancuso 1996). In Cajanus cajan L. plants, under salt stress, a higher ratio of potassium to sodium and calcium to sodium was observed in mycorrhizal plants than non-mycorrhizal plants (Garg and Manchanda 2009).

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