Effects of mycorrhizal fungi on some physiological characteristics of salt stressed *Ocimum basilicum* L.

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Abstract

The present study investigates the effects of *Glomus mosseae* and *Glomus intraradices* on the resistance of green basil plants to salinity stress. The findings suggested that there was an interaction of effects between mycorrhiza fungi inoculation and salt stress on the physiological characteristics of *Ocimum basilicum* L. On one hand, salinity decreased percentage of root colonization, root length, fresh and dry weight of shoot and root and content of photosynthetic pigments in inoculated basil plants. On the other hand, mycorrhiza fungus – inoculated plants experienced increase in root length, dry and fresh weights of shoot and content of photosynthetic.

Keywords: mycorrhizal fungi; pigment contents; growth parameters

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Introduction

Plants always encounter with different stress conditions. Salt stress is one of the main stresses in the world that decreases agricultural products and deficits of plants in many regions. During development of salt stress in plants, all processes are influenced such as photosynthesis, protein synthesis and lipid and energy metabolism (Evelin et al., 2009). Some biochemical and molecular mechanisms that are increased in plants under salt stress include selective storage or discharging ions, controlling ions uptake by roots and transferring them into leaves, synthesizing compatible solution materials, changing photosynthesis pathway, changing membrane structure, inducing antioxidant enzymes, inducing plant hormones and antioxidative mechanisms (Parida and Das, 2005). It was reported that salinity decreases chlorophyll and photosynthetic activity as well as rubisco activity in maize plants (Khodary, 2004).

Many studies have been reported on the use of growth regulators or mycorrhizal fungi in decreasing harmful effects of environmental stress. Mycorrhizal fungi, vesicular arbuscular, are unique microorganisms residing in rhizosphere. These fungi form symbiotic colonies with most plants and in addition to increasing inorganic nutrients in plant, they can increase the resistance of plants to environmental stresses by stimulating growth regulators, increasing photosynthesis, and improving regulation of

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osmotic adjustment (Rabie and Almadani, 2005). Of course, unfavorable environmental conditions such as salinity can have negative effects on inoculation and survival of mycorrhizas. Al- Karaki (2000) lists some mechanisms against salt stress by VAM fungi as:

- increasing absorption of ingredients having a little movement in soil such as copper, zinc and phosphorus,
- increasing water absorption that dilutes effects of toxic ions,
- balancing ingredients of plants in salinity conditions, and
- increasing soluble glucoses in root that leads to decrease in osmotic potential of root.

Mycorrhiza fungi increase growth, photosynthetic pigments and photosynthesis of host plants by better mineral nutrition. They cause chlorophyll organs of plant to grow by absorbing required carbon, giving nutriments to plant and increasing efficiency of photosynthesis. Al-Karaki (2000) showed that mycorrhiza – inoculated maize plants have more dry matter than non – inoculated plants due to salinity. Also inoculation of salt stressed tomatoes with mycorrhiza meaningfully increased their dry weight of root and shoots compared to non–mycorrhiza–inoculated plants (Al-Karaki 2000). Similarly, lotus glaber plants that were inoculated with *Glomus intraradices* had higher pure growth, root to stem ratio, sodium to potassium ratio and protein and chlorophyll density than non–mycorrhiza–inoculated plants (Sannazzora et al., 2005).

In this study, the effects of *Glomus mosseae* and *Glomus intraradices* on the resistance of green basil plants to salinity stress were investigated.

Materials and Methods

Seeds of basil plant (*Ocimum basilicum* L.) were obtained from Pakan Bazr Company Isfahan, Iran. The seeds were washed with distilled water three times, immediately after disinfecting by sodium hypochlorite (10%). Applied treatments included sodium chloride (0, 75, 150mM), and *Glomus mosseae* and *Glomus intraradices* mycorrhiza. For inoculating the seeds with mycorrhiza, they were placed in Petri dishes containing 5 g soil and spore of mycorrhizal species. The soil was moistened with distilled water and the dishes were placed in germinator in order to germinate under light 16h and alternate temperature (16±2 °C and 23±2 °C) condition. The seedlings were then transferred to plastic pots with 12 cm diameter. Ten seedlings were placed in each pot containing perlite. To ensure the inoculation of mycorrhiza, 50 g soil containing spores of mycorrhiza was put on the surface of perlite in every pot and budded embryos in mycorrhizal soil were placed on them. The pots were placed in greenhouse conditions with light period 14h, temperature 22±2 °C and 17±2 °C, light intensity of 1100 KLUX and humidity 60%. For irrigation of the pots, distilled water and nutrient solution (long Aston) were used. Saline treatments with sodium chloride were performed three times during 72 hours.

Measuring percentage of mycorrhizal colonization in root

Rajapakes and Miler (1992) method was used for measuring percentage of colonization of fungi. The roots of inoculated plants were randomly selected and painted with fuchsine acid and the percentage of inoculated root was measured using an anatomical microscope (unit of arbuscular- vesicular mycorrhiza).

Measuring length of plant root

The root lengths were measured by a ruler and recorded in centimeter. For each treatment group, four repetitions were recorded and the mean was reported in cm.

Measuring dry weight of root and of shoot

After separating roots from shoots, each of them was separately placed in aluminum sheet and then put in oven at 80 ^{o}C for 10 days until their weight was fixed and their dry weight was measured in terms of grams.

Measuring fresh weight of root and of shoot

After separating roots from shoots, their fresh weights were measured in terms of gram. For each treatment, four replications were measured and mean was recorded in terms of gram.

Measuring chlorophyll and carotenoid content

Chlorophyll and carotenoid contents of the plants were measured according to the method suggested by Lichtenthaler (1987). In this method, 0.2 g fresh texture of leaf was weighed and then ground in Chinese mortar containing 80% acetone. Then 5 ml Acetone was added to it and solution volume was reached to 15 ml. Three ml of this solution was poured in a cuvette and its absorption intensity was read in 470, 663, 647 nm by Spectrophotometer. For regulating Fi spectrophotometer, 80% Acetone was used as witness. Pigment density was determined in terms of mg/g fresh weight of the plant essence.

Data analysis and statistical studies

This experiment was performed with four replications based on a completely randomized design. Data analysis was performed by SAS software. The Figures were drawn by Excel soft ware.

Results

The content of root mycorrhizal colonization

Root colonization percentage in inoculated plants with *Glomus mosseae* and *Glomus intraradices* was reduced by increasing the salinity (Fig. I). The lowest colonization level was observed in 150 mM salinity. This indicates that sodium chloride can significantly reduce root colonization. Fig II shows the penetration of fungi into root cells.

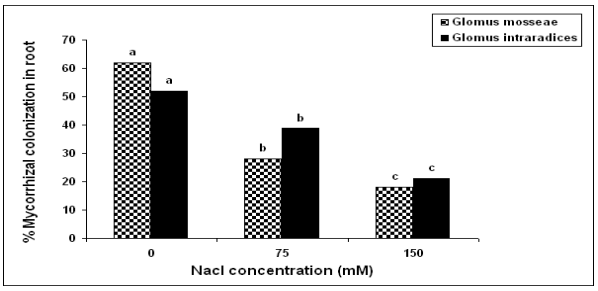


Fig. I. Mycorrhizal colonization in root of green basil plant under salinity stress

Fig. II. Mycorrhizal fungi penetration into root cells

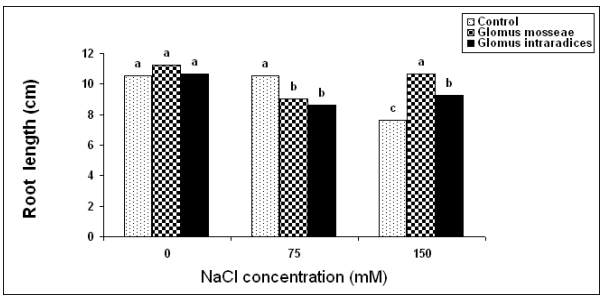


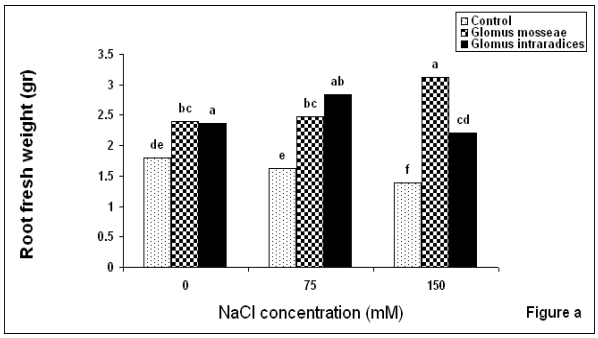
Fig. III. Interactive effect of mycorrhizal fungus and salinity on the root length

Root length

The results of this study showed that root length was decreased by increasing salinity relative to control group and the reduction was meaningful at p≤ 0.05, but no meaningful difference was seen between 0 and 75mM. In salinity–mycorrhiza fungus treatment there was a decrease in root length growth at 75 mM chloride sodium while this parameter increased was at 150 mM chloride sodium relative to the control plants (Fig. III).

Root dry and fresh weights

Root fresh weight was decreased relative to control by increasing salinity, but meaningful difference was not observed between 0 and 75-mM salinity. Effect of *Glomus mosseae* and *Glomus intraradices* fungus and of salt stress on basil plant showed increased root fresh weight relative to the control plants. Root dry weight was also increased by increasing salinity and this decrease was meaningful at p≤ 0.05, but a meaningful difference was not seen at 75 and 150 mM. The amount of this parameter was meaningfully increased in treating plants with *Glomus mosseae* while in treatments with *Glomus intraradices* increase in root dry weight was only observed at 75 mM salinity relative to control plant (Fig. IV).



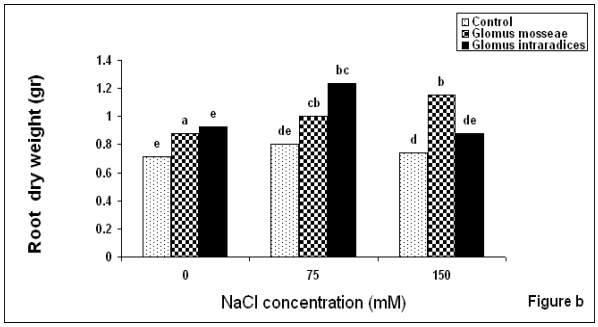
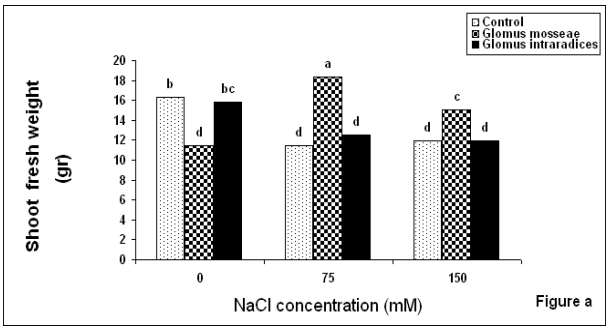


Fig.IV. Interaction of mycorrhizal fungus and salinity on the root fresh (a) and dry (b) weight

Shoot dry and fresh weights

Shoot fresh weight in the study was decreased relative to control by increasing salinity; however, between 75 and 150 mM salinity the decrease was not meaningful. The effect of *Glomus mosseae* fungus and of salt stress on basil plant showed an increase in shoot fresh weight relative to control plant. In treating with *Glomus intraradices* fungus, meaningful results were not observed in any sodium chloride densities.

The change in shoot dry weight was not meaningful under the applied salinity levels. The effects of *Glomus mosseae* and *Glomus intraradices* fungus and of salt stress on basil plant show that there was an increase in shoot dry weight relative to control plant (Fig. V).



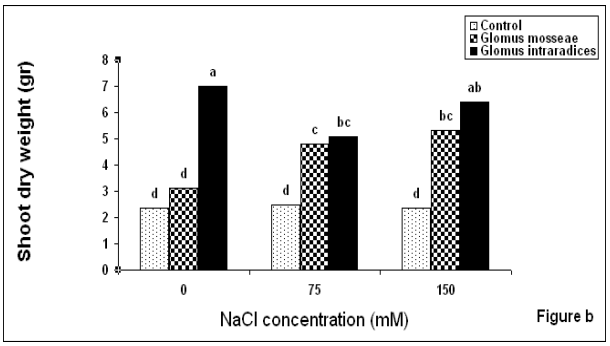


Fig.V. Interaction of mycorrhizal fungus and salinity on the shoot fresh (a) and dry (b) weight

Chlorophyll a

Chlorophyll a content in salinity treatments meaningfully decreased at p≤ 0.05. However, treating plants with mycorrhiza fungi, meaningfully increased chlorophyll a content (Fig. VI).

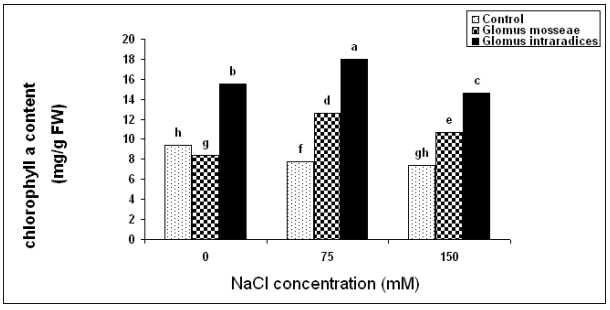


Fig. VI. Interaction of mycorrhizal fungus and salinity on the chlorophyll a content

Chlorophyll b

Increasing salinity, reduced chlorophyll b content. In plants inoculated with mycorrhiza fungi, chlorophyll b meaningfully increased ($p \leq 0.05$) relative to control plants (Fig.VII).

Total chlorophyll

Increasing of salinity causes a decrease in total chlorophyll content which is meaningful at $p \leq 0.05$. In inoculated plants with mycorrhiza fungi, chlorophyll b content had meaningfully increased at $p \leq 0.05$ relative to control plant (Fig. VIII). In these experiments, increasing effects of *Glomus intraradices* were more prominent on total chlorophyll content and on mitigating salinity stress effects compared to *Glomus mosseae.*

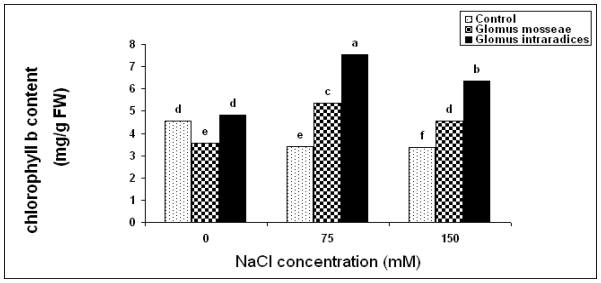


Fig.VII. Interaction of mycorrhizal fungus and salinity on the chlorophyll b content

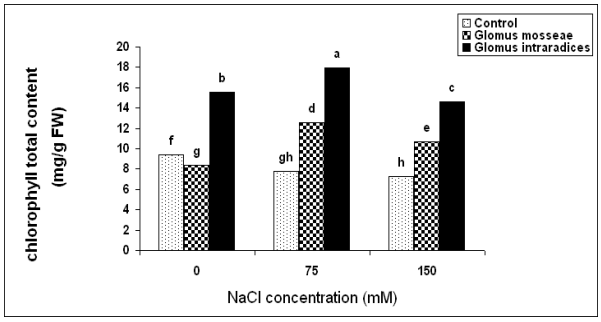


Fig. VIII. Interaction of mycorrhizal fungus and salinity on the total chlorophyll content

Carotenoids

The results of this study showed that salinity generally decreased carotenoids content. Furthermore, the decrease in carotenoids content was meaningful only at 75mM salinity relative to control plants treated with *Glomus mosseae* while it was not meaningful at the other 2 levels. In inoculated plants with *Glomus intraradices*, carotenoid content had meaningfully increased at $p \leq 0.05$ relative to the control plants (Fig. IX).

Discussion

Mycorrhizal colonization content of root in basil plants decreased in this study. Decrease in mycorrhizal inoculation is due to a decrease in spore stress, hypha growth and effect on formation of Arbuscule (Sannazzaro et al., 2005; Rabia and Almadini, 2005). On the other hand, in mycorrhiza populations, fungus is dependent on plant growth and production of nutriments in the host plant. So every factor influencing carbohydrate production and its transfer into roots can be effective on inoculation amount. Since salinity decreases plant growth and carbohydrate density, it can decrease mycorrhizal inoculation (Nouriana et al., 2007; Asghari, 2008). Decrease in mycorrhiza – inoculation amount of root was reported by other researchers (Al-Karaki and Hammad, 2001, Sannazzaro et al., 2005). Significant decrease was reported in mycorrhizal colonization content of tomato (Abdel latef and Chaoxing, 2011) and *Jatropha curcas* L. roots (Ashwani et al., 2010) under salt stress inoculated by mycorrhizal fungus. This is similar to the results of the present study.

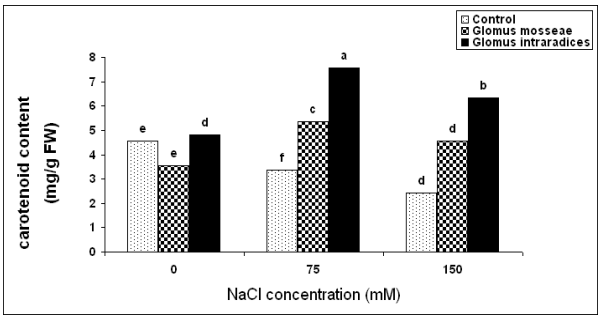


Fig. IX. Interaction of mycorrhizal fungus and salinity on the carotenoid content

Moreover, high – density salinity in this study decreased the root length. There are similar reports about cotton and bean (Dash and Panda, 2001). Salinity decreases plant growth by creating water stress in root region. Elements such as sodium decrease growth through decreasing cell division and elongation. Furthermore, salt stress decreased root growth

and total plant growth by increasing ethylene (Ho et al., 2001). While in treating with mycorrhiza fungi and salinity, the amount of this parameter was increased in high salinity relative to control. One strategy that plants use for avoiding undesirable conditions of soil in terms of water and nutriment deficit is increase in root contact surface by mycorrhiza (Turk et al., 2006). Since there is a negative relation between mycorrhizal inoculation and phosphorus absorption level, it seems that root growth is increased by increasing mycorrhizal inoculation of plant root and providing a desirable limitation of phosphorus. Correlation of root length with mycorrhizal inoculation amount of root is probably related to suitable ventilation of soil, that is the result of hypha network of mycorrhizal fungi that connects particles of soil and as result the root spreads into deep soil (Turk et al., 2006).

In this study, root dry weight was increased by increasing salinity and root fresh weight was decreased by decreasing osmotic potential of soil and due to disturbance in water absorption by plant. In a similar study on barley plant, root fresh weight and aerial part decreased under salt stress by increasing its density. Ghoulam et al. (2002) reported similar results for beetroot. In mycorrhiza fungus – inoculated plants, root fresh and dry weight was increased. Existence of fungus hypha network increases nutrient and water absorption. Fibers of mycorrhizal fungus are divided into two groups; some of them enter the plant system and decrease density of abscisic acid and increase cytokinin content. This action increases water absorption and develops root system of the plant. Second group of fibers are out of root system and secrete organic acids solving phosphorus such as malic acid that increases phosphorus absorption by plant and its dry matter. Phosphorus as one of the elements required for plant increases dry matter because it has an important role in cellular division by regulating plant hormones. Moreover, it has an important role in producing photosynthetic matters and produces energy in plant (Khalvati et al., 2005). Inoculation of *Pistacia vera* by *Glomus etunicatum* arbuscular mycorrhiza fungus increases higher dry weight of root and stem and higher leaf surface relative to non–mycorrhizal plants in low and medium salinity levels. Also similar results were observed in non–salinity conditions (Abbaspour et al., 2005). Similar results were also reported about *Jatropha curcas* plants under salt stress (Ashwani et al., 2010) and *Glomus etunicatum*–inoculated maize plants under cold stress (Zhu et al., 2010) compared to non–mycorrhizal plants.

The results of shoot dry and fresh weights showed no meaningful difference under the applied salinity levels in this study, but shoot fresh weight was decreased in high salinity. This may be because of decrease in water absorption in stress conditions (Jamil et al., 2005). Water content of leaf in cucumber is also decreased under salt stress because the most sensitive factor in salt stress is water content as it is reduced under high salt stress (Stepein and Klobus, 2006). Also it was reported that, in red raspberry, damage of leaf is a result of accumulation of toxic sodium and chlorine ions, ionic unbalance, decrease in nutriments and water stress (Wahome, 2003).

In the plants inoculated with mycorrhiza fungus, increased fresh and dry weight of shoot was observed. This increase in weight can be resulted from the effects of mycorrhiza fungus on absorbing various nutriments such as nitrogen, calcium, potassium, copper, zinc and sulphur. Using mycorrhiza fungus increases plant growth and affects devoting and transferring nutriments between stem and root so that dry weight of shoot is increased by increasing absorption of nutriment and their transfer. Fresh weight of shoot was also increased in Glomus mosseae–treated plants in low salinity. Similar results were also obtained about mycorrhizal barley plant in salt stress conditions (Nourinia et al., 2007).

Decrease in carotenoids and chlorophyll content due to salinity increase was another observation of this study. Salt stress opens porphyrin rings and harmful matters resulting from this dissolution are transferred to vacuole. Existence of these compositions demolishes green color of leaf (Parida and Das, 2005). The results of this study are consistent with the results obtained from maize plant and barley (El-Tayeb, 2005). Carotenoids are also tetratropenoid compositions that maintain chlorophyll against light oxidation, light

absorption and transfer of light energy to chlorophyll a (Devlin and Withman, 2002). One reason for decrease in carotenoids in salt stress is Beta-carotene destruction and zea xanthin formation (Sultana et al., 1999). It was reported that total chlorophyll and carotenoids are decreased in tomato under salt stress (Parida and Das, 2005). Chlorophyll and carotene were decreased by 63% and 75% respectively in Arbutus plant under intense drought stress and carotene destruction is related to oxygen produced in thylakoid (Munne –Bosch and Penuelas, 2003).

In this study, photosynthetic pigments were increased under the effect of mycorrhizal inoculation. One reason of chlorophyll decrease in salt stress is antagonistic effects of sodium on Mg absorption. Since mycorrhiza helps absorb Mg in plant in some cases, it can increase chlorophyll synthesis in plant. Also chlorophyll decrease can be resulted from sodium decrease in shoot of mycorrhizal plants relative to non–mycorrhizal plants. In fact, mycorrhizing decreases role of salt in chlorophyll synthesis (Giri and Mukerji, 2004). In *Glomus etunicatum* inoculated maize plant, increase in photosynthesis speed, pure sweating and chlorophyll a, b density was reported under cold stress (Zhu et al., 2010). Also in *Jatropha curcas* L. mycorrhizal plants higher chlorophyll were reported than non–mycorrhizal plants under salt stress (Ashwani et al., 2010).

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