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The Role of Arbuscular Mycorrhizal Fungi and Salicylic Acid in Success of Culturing *Ocimum Basilicum* L. in Aluminum – Contaminated Lands

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Abstract: Aluminum stress is considered as limiting factor of plant performance. Many studies showed that inoculating plants by mycorrhizal fungi and using salicylic acid increased the resistance of many plants against heavy metals toxicity. The purpose of this study was to consider the role of *Glomus mosseae* and *Glomus intraradices* fungi and of salicylic acid in increasing resistance of green basil against aluminum toxicity. This experiment was performed in factorial plan based on random blocks in greenhouse conditions. The results of this study showed that aluminum caused increase in reduced sugar in shoot and root and increase in proline in shoot, while destructive effects of aluminum chloride were adjusted in pretreated – salicylic acid or inoculated – mycorrhizal fungi plants.
Keywords: Aluminum; salicylic acid; arbuscular mycorrhizal fungi; *Ocimum basilicum*

INTRODUCTION

Plants are exposed wide types of environmental stresses during their life cycles that we can point to heavy metals stress. The main problem of plant in medium containing heavy metals is that toxic ion is accumulated and concentration of essential cations is decreased such as iron, potassium and manganese (van–Assache and Clijsters 1990). The aluminum is one of heavy metals that produce wide range of disorders in cells and whole of plant such as shortening roots, decreasing photosynthesis activity, leaves chlorosis and necrosis, paraoxidation of membranous lipids and also this mental produces proline. Plants produce key molecules such as salicylic acid that plays role in creating resistance of plants against environment stresses. Salicylic acid belongs to group from phenyl compounds and it is considered as a like–hormone material. Preventing toxicity in high amounts of heavy metals is benefit of peaceful relation from symbiosis type that is created between mycorrhizal fungi and many plants. There is a few data about role of Arbuscular mycorrhizal in absorbing heavy metals.

The studies showed that using species of *Glomus* separated from heavy mental environment and inoculating them of plant cause increase in plant tolerance against high amounts of metals and plant absorbs less metal. The purpose of this study was to compare the role of salicylic acid and inoculation with mycorrhizal fungus in increasing resistance of basil plant against aluminum toxicity.

METHODS AND MATERIALS

Providing seed and producing plant material and performing treatments

Seeds of basil plant (*Ocimum basilicum* L.) prepared from Pakan Bazr Company Isfahan-Iran. The seeds were washed with distilled water three times after disinfecting by sodium hypo chloride (10%). Applied treatments included: Aluminum chloride (0, 75, 150mM), *Glomus mosseae* and *Glomus intraradices* mycorrhize, and salicylic acid (0, 0.2mM).

For inoculating of plants with mycorrhize in Petri dishes, 5gr soil containing spore of mycorrhizal species was separately poured in Petri dishes and the seeds were placed on it .The soil was 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) condition .

Plants' planting was performed in plastic pot with 12cm diameter containing perlite. For mycorrhize treatment, 50gr soil containing spore of mycorrhize 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±2C) 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 (0.2mM) for five days at light phase. Five days after final treatment with salicylic acid, saline treatments with aluminum chloride were performed three times during 72 hours.

BIOCHEMICAL STUDIES

Measuring the content of proline

Proline was measured by Bates *et al* (1973) method 0.04gr fresh tissue of leaf and 0.08gr fresh tissue of root were grounded in sulfosalicylic acid (3%) and the resulting educe was centrifuged in 10000g for 5 minutes. Then 2cc surface solution was mixed by 2cc and 2cc pure salicylic acid and heated in 100^0c for one hour, the tubes were cooled in ice bath and 4cc toluene was added to each other and shacked well. By fixing tubes, two layers were formed separately that upper color layer was used for measuring the content of proline in wavelength 56 nanometer from pure proline was used for standard curve and the results were calculated and introduced according to milligram on gram of fresh weight.

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Measuring the content of reduced sugar

0.04gr fresh tissue of leaf and 0/08gr fresh tissue of root were weighted and every sample was grounded separately by 10cc 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 nanometer. Then 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 replications in completely random design framework. Data analysis was performed by MSTATS and SAS software. The Figures were drawn by Excel software.

Proline content

The results of this study showed that proline amount of shoot had a significantly increase in 50mM aluminum chloride and this parameter amount had no significantly change by using salicylic acid. In inoculating plants with *Glomus mosseae*, proline amount of shoot was increased in 25mM aluminum chloride in interaction between *Glomus mosseae* and salicylic acid and the results were not significant in other cases.

In considering proline amount of root, this parameter was decreased in plants under aluminum stress. Pretreating plants by salicylic acid caused to significant increase of this parameter in plants under aluminum chloride stress relative to control plant. In inoculating plants by *Glomus intraradices*, in interaction between *Glomus mosseae* and salicylic acid and between *Glomus intraradices* and salicylic acid proline amount of root was increased relatively to control plant (Figure 1)

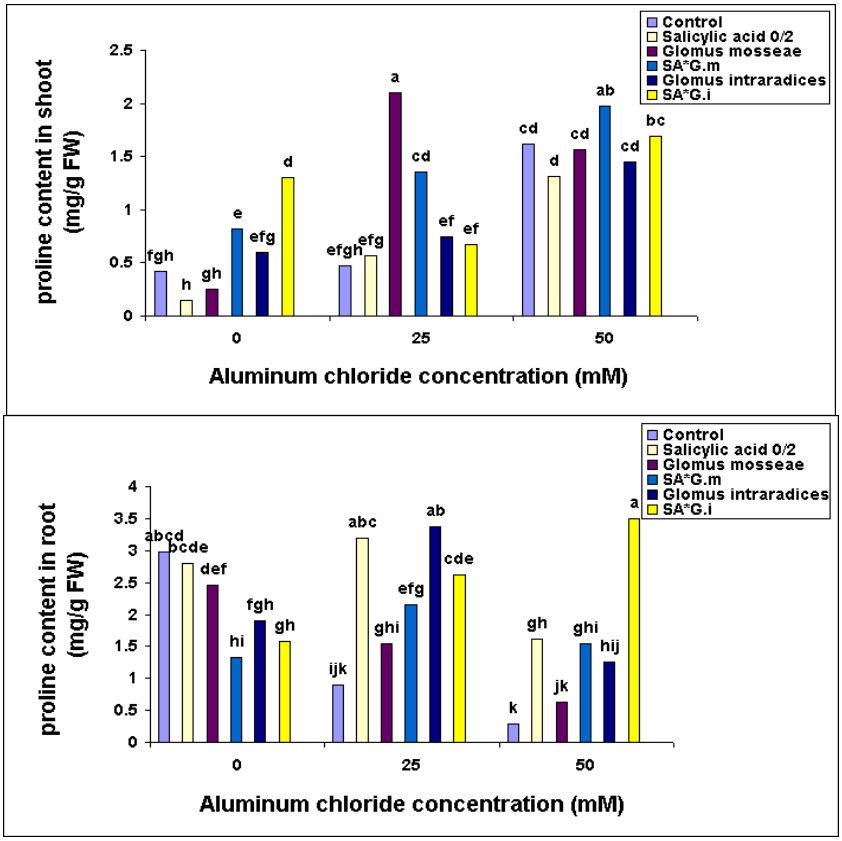

Figure 1: Interactive effect of mycorrhizal fungus, salicylic acid and aluminum chloride on proline amount of shoot and root

Reducing sugar amount

Reducing sugar amount of shoot and root was increased significantly in plants under aluminum stress. The amount of these two parameters was increased significantly in interactions and in plants inoculated by mycorrhizae. While reduced sugar amount of shoot was decreased significantly in sprayed – salicylic acid plants under aluminum stress. This parameter was increased relatively to control in inoculating plants with mycorrhizal fungi and in interaction with salicylic acid.

In considering reducing sugar amount of root, this parameter amount was increased in plants under aluminum stress. Pretreating plants by salicylic acid caused to increase reducing sugar amount of root relative to control plant in 25mM aluminum chloride and in inoculating plants by mycorrhizal fungi and in interaction with salicylic acid (Figure 2).

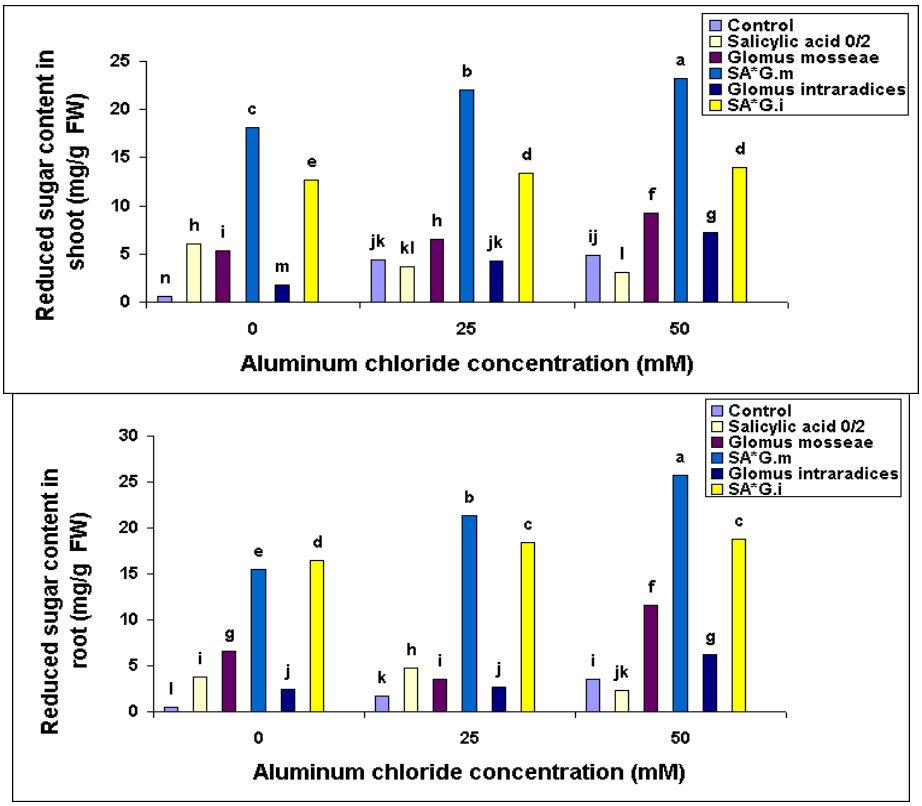

Figure 2: Interactive effect of mycorrhizal fungus, salicylic acid and aluminum chloride on reduced sugar content of shoot and root

DISCUSSION AND CONCLUSION

Reducing sugar amount of shoot and of root and also proline of shoot were increased in plants under aluminum stress that indicates the resistive system of plant and osmolite produced against damages resulting from aluminum stress in plant. Proline protects cell membrane in biotic and abiotic stresses. Sairam and his colleagues (1998) experiments showed that increase in proline and reduced sugar causes to 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).

Reducing sugar amount of shoot was decreased in aluminum chloride stress in pretreating plants with salicylic acid and this factor amount of root was increased in 75mM aluminum chloride and proline of shoot and of root also was increased. Pretreating by salicylic acid may stimulate hydrolysis of insoluble sugars and cause to create osmosis source that is important in osmosis regulation. The results of this study are consistent with other reports. In inoculating plants with mycorrhizal fungi and in interaction between fungi and salicylic acid, the amount of this parameter was also increased. In plants colonization with mycorrhiza, fungous hyphae can cause heavy metal toxicity decrease by maintaining metal and non – transferring it into plant system (Horst 2004). In inoculating *Prosopis juliflora* by *Glomus fasciculatum*, soluble sugar of leaves and roots is increased (Selvaraj and Chelapan 2006). In other report, in wheat plant under cadmium stress inoculated with *Glomus intraradices*, by increasing metal, sugar and proteins content increased compared to not inoculated plants (Jamalabad and Khara 2008).

Therefore, it can be concluded that tolerance of plant can be enhanced against aluminum toxicity by using mycorrhizal fertilizers and because materials special for filtrating water are made of aluminum, and so some aluminum is in water and soil, tolerance of this plant to culture can be increased in these conditions.

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