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Effect of Rhizobium and Mycorrhizal co-inoculation on some of physiologic characteristics related to grain yield of bean *(VisiaFaba)* under salt stress

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ABSTRACT

In order to study the effect of salinity stress onsome physiologicalcharacteristics of bean, (viciafaba) a factorial experiment was conducted with three factors, including Mycorrhizal in three levels (non-Mycorrhizal, G. intraradices and G.mosseae), rhizobium bacteria in three levels (non- rhizobium, naghadeh's rhizobium and oshnawyeh's rhizobium) and salinity at four levels (control, 60, 120 and 180Milimolar sodium chloride) in a completely randomized design with three replications. The experiment carried out in research greenhouse of Islamic Azad University branch of Mahabad, Faculty of Agriculture. Results indicated significant effects of Mycorrhizal and salinity for all factorsbut effect of rhizobium were non-significant for carotenoids and relative water content. Interaction of Mycorrhizal and rhizobium were significant for chlorophyll a, b, carotenoids and relative water content. However interaction effect of Mycorrhizal and salinity were significant for mentioned factors. Interaction effect of rhizobium and salinity were significant for some factors such as chlorophyll a and carotenoids. But the interaction effect of Mycorrhizal, rhizobium and salinity were significant just for chlorophyll a.

Key words*:* Bean,Mycorrhizae, Pigments Content, Rhizobium, Salinity Stress.

INTRODUTION

Plants during their growth period exposed on stressful environmental factors. One of the most significant of them is salinity stress that restricts plants growth. Soil salinity is a widespread problem that restricts plant growth and biomass production, especially in arid, semi-arid and tropical areas [3].Work on salinity in relation to legume-*Rhizobium*symbiosis using small seeded legumes has shown that *Rhizobium*is more tolerant of salts than the host. Plasmamembrane injury induced by salt stress is related to an increased production of highly toxic oxygen free radicals [18]. Under salt stress, both superoxidedismutase (SOD) and catalaze (CAT) activities decline in plants [12] and malondialdehyde (MDA) accumulates rapidly [13], which results inan increase in permeability of plasma membranes. Salinity stress triggers various interacting events including the increase of ABA concentration, decrease of xylem pH and conductivity [9]. Under stress conditions, reactive oxygen species (ROS) such as superoxide and hydroxyl radicals can be produced in large. Hydrogen peroxide and superoxide radicals are relatively unreactive, but they can form hydroxyl radicals, which can damage proteins, lipids and DNA [15].Salinity affects plants through nonspecific and specific mechanisms. The nonspecific mechanism is related tothe decreasing osmotic potential of the soil solution that impedestranspiration and photosynthesis.Specific mechanisms relate to ion uptake and altered physiologicalprocesses resulting from toxicity, deficiency, or changes in mineral balance. Salt tolerance is the ability of plants to survive and grow under saline conditions and is a variable trait that depends on many factors, including species.

Arbuscular Mycorrhizal (AM; *Glomusfasciculatum*) fungi areubiquitous among a wide array of soil microorganisms inhabiting the rhizosphere [8]. The symbiotic association of aplant with AM fungi allows access to mobile nutrients in nutrient poor soils [13]. AM fungi constitute an integral component of the natural ecosystem, and are known to exist in saline environments where they improve early plantgrowth and tolerance to salinity. Many researchers have reported that AM fungi could enhancethe ability of plants to cope with salt stress [16] by improving plant nutrient uptake and ion balance, protecting enzyme activity [8] and facilitating water uptake[17].In salt-stressed soil, AM fungi are thought to improve the supply of mineral nutrients to the plants, especially the supply ofP, as it tends to be precipitated by ions like Ca2+, Mg2+ and Zn2+ [2]. Giri [8] reported that AM fungicounter-balanced the adverse effects of salinity stress and therebyincreased plant growth. Rabie [16] suggested that AM fungiprotected the host plants against the detrimental effects of salt.

Vesicular Arbuscular Mycorrhizal (VAM) are important components of the rhizosphere and they have the ability to create amutually beneficial root fungi association. Generally, legumes are quiteresponsive to VAM especially in soils with low available phosphorus. Mycorrhizal plants are very efficient in P absorption and accumulation,and have a greater tolerance to toxic heavy metals, root pathogens,drought, high temperature, saline conditions and adverse soil pH thannon-Mycorrhizal plants. Mycorrhizal research in theSudan revealed that nodulation and growth of legumes can be significantly enhanced by both rhizobium and Mycorrhizal inoculation.

MATERIALS AND METHODS

This experiment is carried out in the greenhouse of Islamic Azad University branch of Mahabad, Faculty of Agriculture, in 1390. The locus of the experiment, from geographical perspective, is located between 35 degrees and 58 minutes to 39 degrees and 47 minutes of north latitude (from equator) and 44 degrees and 3 minutes to 47 degrees and 23 minutes longitude from Greenwich meridian. The altitude is 1358 meters.This experiment is carried out as a factorial based of complete randomized block in triplicated performance. The firs factor: Mycorrhizalfungi that includes 3 levels of control (non-inoculated Mycorrhizal), G. intraradices, and G. mosseae; The second factor: Rhizobium bacteria which includes three levels of control (non-inoculated Rhizobium) , Naqadeh's Rhizobium, and Oshnavieh's Rhizobium, and the third factor: salinity stress that includes 4 levels of control (zero), 60, 120, 180 (Milimolar sodium chloride).In this experiment, bean was used (as a *sayad* figure).For each treatment 3 pots were considered and each block contained 36 pots, with the sum of 108 pots in 80*30*30 cm within the whole experiment. At first, the pots were filled by perlite and after that, in each pot, 15 seeds were cultivated after disinfection with Coptain fungicide in concentration of two in thousand. Every four days irrigation with saline water was carried out. In order to avoid abundance of salt in the root's area, suitable holes were prepared at the end of the pots. To provide necessary nutrition for plant's growth, they were fed by Hogland nutrient solution at the four leaves stage the action of pruning was done and the number of plantlets was decreased to eight per pot, that three of them were utilized for physiological experiments and five for estimating yield of the grain. In order to measure the leave relative water content, Wetherley method [20] was used and for extraction of chlorophyll and carotenoids, Asetone was used and it's measurement was carried out with a modified method of Strain and Svec [19]. Finally, the data were analyzed statistically via SPSS, and comparison of means was done through Ducan's multiple range test in 5% level. For drawing graphs, Excel was used.

RESULTS

Photosynthesis pigments (chlorophyll a, b, and carotenoids)

In order to the obtained results of the study of the content of chlorophyll a, b, and carotenoids in inoculated with a significant difference (P<%1) was observed between Mycorrhizal plants and non-Mycorrhizal ones in the salt stress-free environment. Based on analysis of the table of data variance (table 1), the effect of Rhizobium bacteria on chlorophyll a and b is meaningful in the possibility level of 1%, while it didn't show any meaningful impact on Carotenoids. With the salinity levels of zero, 60, 120, and 180 mM a significant difference was seen between Mycorrhizal and non-Mycorrhizal plants, which indicates the positive effect of Mycorrhizal over beans under salt-stress conditions. According to the results of variance of analysis (table 1), the interaction of Mycorrhizal and Rhizobium on photosynthesis pigments and also the interaction of Mycorrhizal and salinity on the mentioned characteristics (chlorophyll a, b and Carotenoids) became meaningful at the possibility of 1% (P<%1). It is while, the tripartite interaction of Mycorrhizal, Rhizobium and salinity was significant over chlorophyll a but not on chlorophyll b and Carotenoids.

Leaf relative water content

Based on the variance of analysis table (table 1), leaf relative water content of bean was significantly reduced under conditions of salinity and inoculation with Mycorrhizal fungi. The results of the study of Mycorrhizal inoculation and the amount of leaf relative water contentindicated that there is a significant difference (P<%1) among

Mycorrhizal and non-Mycorrhizal plants. In difference levels of salinity a significant difference was also seen for the amount of the mentioned characteristic. It is while, with increasing salinity level, the amount of relative water content had a significant reduction. Interaction of Mycorrhizal inoculation and exerting salinity also became significant (P<1%).

DISCUSSION

The results showed that the application of Mycorrhizal fungi, alone or in combination with Rhizobium bacteria, caused an increase in chlorophyll a,band Carotenoids. Increasing the amount of chlorophyll in the leaves with the effect of Mycorrhizal symbiosis could be due to increase absorption of phosphorus from the soil by the fungi. Phosphorus has an important role in the metabolism of plant materials.

Auge [4] showed that Mycorrhizalfungi can indeed cause an increase in chlorophyll concentration in the levels of the beans by increasing phosphorus concentration to 40% in the plant and absorbing water twice more than the usual. Rhizobium bacteria, depending on their breed, increased chlorophyll a and b. In this regard, Zahir [21] noted a 40% increase in corn nitrogen uptake in treatments containing Azotobacter. Mohsen [14] observed the highest leaf nitrogen content aerial organs with the presence Mycorrhizalfungi is also affected with salinity stress and in this condition Mycorrhizal colonization of plants is reduced and the fungi has more tendency to sporulation. With the excess salinity and it's bad effect on the structure of chlorophyll and therefore degradation of chloroplasts, chlorophyll content decreases [6]. One possible reason for the decrease in chlorophyll within salinity stress in non-Mycorrhizal plants is salt interference with chlorophyll synthesis [8]. Another possible reason for decrease is in chlorophyll concentration can be antagonistic effects of sodium ions on the absorption of Magnesium [1].

According to the results obtained from this research, combined inoculation of Mycorrhizaland Rhizobium caused an increase in chlorophyll a, which can be due to the synergistic effects of Rhizobium bacteria and Mycorrhizal fungi on root growth. These results have been confirmed by Kumutha [11] and also Dudde and Raut [7]. Synergistic effect could be due to the role of phosphorus on the formation of nodules and Nitrogen fixation in legume plant species [5].Reduction in leaf relative water content shows the reduction in the amount of water within the plant. Reduction in leaf relative water content under salinity stress may be due to decreased osmotic potential of water by the excessive dissolving of minerals in the soil solution and reduced water uptake by the plant roots.

Krishnan and Kumari [10] reported that under salinity stress conditions, leaf relative water content decreases. This researchers claimed that under salinity stress conditions, thus reducing the amount of water absorbed by the root, the amount of cell swelling reduces and this will decrease the under salinity stress conditions. According to observations obtained from this research, the application of Mycorrhizal fungi decreases damage to plant cells in low levels of salinity stress (60mM) which with severe stress due to damage to the cell membrane, resistance of Mycorrhizal plants against stress is reduced.

Table 1 variance analysis of chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoids (Car) and relative water content (RWC)

SOV	DOF	Chl a	Chl b	Car	RWC
M	2	3.293**	1.984**	0.005**	412.825**
R	2	0.405**	0.284**	7.954E-6	6.722
S	3	6.286**	5.916**	0.012**	1462.195
M*R	4	2.252**	0.225**	6.759E-5**	3.908
M*S	6	0.149**	0.134**	0**	74.778
R*S	6	0.145**	0.03	7.120E-5**	3.822
M*R*S	12	0.112**	0.044	0.090E-5	5.007
ERROR	72	0.038	0.037	1.749E-5	3.747
CV%		5.9	7.94	0.19	2.21

***:significant in the possibility level of 1%; M(Mycorrhiza), R(Rhizobium), S(Salinity)*

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