



RESEARCH PAPER

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Physiological and mineral nutrient changes in safflower cultivars in response to different salinity level and mycorrhizal inoculation

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Abstract

Two Safflower (*Carthamus tinctorius* .L) cultivars seeds consist were used to study the influence of inoculation with mycorrhiza arbuscular fungi under salinity stress condition. Factorial experiment based on completely randomized design (three-way ANOVA) with 3 replications was used. Salinity treatment with 3 level (0.5, 6, and 12 dS/m) and mycorrhizal arbuscular inoculation with two species (Three level consist of on inoculation, and inoculation with *Glomus intraradices* and *Glomus moseae*) were applied on two cultivar of safflower (Goldasht and Padide) in this experiment. The content of macro and micro nutrient such as total Nitrogen (N), potassium (K), sodium (Na), chlorine (Cl), phosphorus (P), magnesium (Mg), calcium (Ca), copper (Cu) and zinc (Zn) were measure in this study. The results Showed that salinity had a great impact on nutrient deficiency of safflower plants with increasing the content of some elements such as Na, Cl, and Cu in plant's leaf, but decreasing other important nutrients such as N, P, K and Mg. Inoculation of mycorrhizal fungi could reduce the negative effect of salinity by increasing content of important elements in this condition such as P, N, K. Also, these nutrients were higher in inoculated plant than non-inoculated under non-saline condition, indicating positive effects of mycorrhizal inoculation in all stressful or non-stressful conditions. Mg, Ca, and Zn are among the micro nutrient which are important for plant to produce higher yield. It is seems that Goldasht cultivar has a better contribution to inoculation with mycorrhizal inoculation and it showed relatively higher amount of macro nutrient and lower amount of Na and Cl.

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Introduction

In the recent years, using biological methods and considering the potential of beneficial bacteria and fungi to elevate defense mechanisms in plants dealing with stress conditions has received increased attention (Aroca *et al.*, 2007; Giri *et al.*, 2003; Mirzakhani *et al.*, 2009; Moucheshi *et al.*, 2012; Ruiz-Lozano and Aroca, 2010; Saed-Moucheshi *et al.*, 2013). Arbuscular mycorrhizal (AM) fungi have been shown to enhance plant growth and stresses tolerance by altering the physiological properties of the host plant, increasing water absorption capacity, increasing root hydraulic conductivity, adjusting osmotic balance, and also the absorption and changing content of mineral element in plants (Al-Karaki, 1998; Augé, 2001; Evelin *et al.*, 2009). It is reported that AM fungi symbiotic system can colonize plant roots in some of the crops and improve their performance (Aliasgharzadeh *et al.*, 2001) by increasing nutrient supplies to the plants and reducing water stress effects (Augé, 2001; Toro *et al.*, 1997). It is reported that AM fungi symbiotic system can colonize plant roots in some of the crops and improve their performance (Aliasgharzadeh *et al.*, 2001) by increasing nutrient supplies to the plants and reducing water stress effects (Augé, 2001; Toro *et al.*, 1997). Safflower has been grown worldwide for centuries, primarily for its colorful petals to use as a food coloring and flavoring agent, for vegetable oils and also for preparing textile dye in the Far East, central and northern Asia and European Caucasian (Hojati *et al.*, 2011). It has also received considerable interest recently as forage plant (Mirzakhani *et al.*, 2009). Furthermore, since consumers have demanded healthier oil for cooking, safflower oil is more demanded in these days (Rahamatalla *et al.*, 2001).

Regards to importance of the safflower plant as its different consume in human activities and industry, and also significant effect of salinity stress as one of the most important environmental stresses, this study was carried out to survey the influence of mycorrhizal colonization of AM fungi with different cultivars of safflower under salinity stress condition on its

different mineral contents and also its physiological properties.

Materials and methods

Two safflower (*Carthamus tinctorius .L*) cultivars consist of Goldasht and Padide were prepared from Darab Research Station of Agriculture and Natural Resources. In order to sterilization of the seeds, they were treated with sodium hypochlorite 10% for about 1 min and then washed three times with distilled water. After sterilization, the seeds were placed in petri dishes containing 5 gr of biological soil having mycorrhizal spore of *Glomus intraradices* and *Glomus mosseae* species. The mycorrhizal spores were prepared by Zist Fanavaran Turan Co. located in Semnan Province, Iran. The biological soil was contained 50 mycorrhizal spores in every 1 gr soil. The petri dishes containing biological soil and placed seeds were moisturized by distilled water and then were set in a germinator having 16:8 light conditions for which temperature of 23 ± 2 and 16 ± 2 °C. After about 4 days when all seeds were germinated, they transmitted to the pots. The pots with 240 g weight, 30 cm diameter, and 20 cm height were filled with 3 kg perlite and cocoperlite (3:1). Fifty gr of biological soil containing mycorrhizal spores were put on the perlite and cocoperlite mixture surface and then the seedlings were sown in the pots. The pots were kept in greenhouse at light condition of 14:10 with temperature of regularly 22 ± 2 and 17 ± 2 °C, light density of 11000 klx and 60% air moisture. Salinity treatment was applied using sodium chlorite in mixed with distilled irrigation water and Hogland nutrient solution. Salinity treatment was consisting of four levels contain 0.5 (control), 6, and 12 dS/m.

Mineral measurements in leaves

For determination of total Nitrogen (N), potassium (K), sodium (Na), chlorine (Cl), phosphorus (P), magnesium (Mg), calcium (Ca), copper (Cu) and zinc (Zn) (Chapman and Pratt, 1961), the samples were dry ash and the at 550 °C. then 2 mol HCl solution were used to extraction, subsequently Total Na and K content were determined through atomic absorption spectrophotometer (Varjan model Spectera aa 220

made by Australia).

Statistical analysis

Factorial experiment based on completely randomized design (three-way ANOVA) with 3 replications was used. Salinity treatment with 3 level (0.5, 6, and 12 dS/m) and mycorrhizal arbuscular inoculation with different species (Three level consist of control, and inoculation with *Glomus intraradices* and *Glomus mosseae*) were applied on two cultivar of safflower (Goldasht and Padide). The data were subjected to three-way ANOVA using SAS 9.2 with GLM procedure (proc GLM), and LSD method of mean comparisons. The univariate normality test was carried out on residuals of the ANOVA model for all measured traits for testing hypothesis of normal distribution of the data. Corr procedure of SAS software was used for carrying out correlation between measured traits.

Results and discussions

Using SAS software and proc glm, the result of ANOVA and mean comparison were prepared. Total nitrogen, phosphorus, and potassium contents as macro nutrients in plants were affected by salinity, but mycorrhizal inoculation and cultivar were significant just P and K (Table 1). The interaction for mycorrhizal inoculation with cultivar was significant for abovementioned traits which indicate different response of cultivars to inoculation of mycorrhiza

fungi. Salinity decreased the content of total nitrogen but the rates of decrease were lower in mycorrhizal inoculated plants (Figure 1). Under the salinity conditions (6 and 12 ds/m), the content of G. intraradices were higher than G. moseae, but under the control condition G. moseae was higher. Goldasht cultivar showed higher amount of N under control condition but lower under the salinity and mycorrhizal inoculation. The control of salinity showed highest amount of phosphorus content in compare to both salinity levels (Figure 2). Inoculation of mycorrhizal fungi increased the content of P in safflower but this rate was lower under salinity's levels. Goldasht cultivar had higher amount of P but it shows greater content under control condition. Salinity also clearly reduced the amount of potassium in safflower plants. Inoculated plants showed more amount of K under all treated condition in compare to not inoculate ones. Padide cultivar had higher amount of K than Goldasht cultivar, but their differences under salinity levels were lower than control condition. These results is in line with the result of Caravaca *et al* and Habibi *et al* which reported that salinity significantly decrease the amount of all three above-mentioned minerals (Caravaca *et al.*, 2003; Habibi *et al.*, 2008). In the study of Sefik *et al* (2006) AMF applications significantly affected the K content. Also, the interaction of N application with AMF on the content of N, P and K I the shoot of soybea was significant.

Table 1. Analysis of variance related to measured mineral nutrient in response to inoculation and salinity stress in two cultivar of safflower.

Source	Degree of freedom	Mean squares								
		N	P	K	Na	Mg	Cl	Ca	Cu	Zn
Stress (A)	2	13.32**	0.0364**	44.48**	350.17**	0.3374**	264.4**	12.99**	611.96**	1410.44**
cultivar (B)	2	0.13ns	0.0049*	13.39**	1.62ns	0.0244**	7.02**	0.16**	42.63**	443.43**
Inoculation (C)	2	0.48ns	0.0279**	2.61**	5**	0.0419**	11.48**	0.26**	2.01ns	387.45**
A*B	2	0.05ns	0.0037ns	3.75**	0.96ns	0.0039ns	8.97**	0.09*	6.19ns	1178.83**
A*C	4	0.34ns	0.00095ns	0.02ns	2.23**	0.0026ns	9.92**	0.16**	40.69**	465.89**
B*C	2	2.98**	0.0082**	0.54*	1.72*	0.00229ns	0.52ns	0.14**	13.32*	1102.65**
A*B*C	4	0.04ns	0.0009ns	0.68*	1.58*	0.0012ns	0.53ns	0.1**	34.29*	591.62**
Error	35	0.25	0.00151	0.14	0.51	0.0021	0.33	0.02	3.7	19.86
coefficient of variation		15.02	14.44	10.9	15.2	8.58	9.37	9.02	22.54	5.1

Main cause of salinity is by means of sodium, and in this study the salinity stress were iduced by the 6 and 12 ds/m concentration of Na. results showed that the

effect of salinity stress ad mycorrhizal inoculation were significant but the effect of cultivar was not significant (Table 1). Furthermore, except of

interaction of salinity by cultivar, all other interactions were significant. Salinity levels showed highly differences for Na content in compare to control condition (Figure 3). Content of Na for inoculated plant with mycorrhiza were lower than non-inoculated ones. *G. intraradices* showed lower content of Na than *G. moseae*. The amount of sodium in Padide cultivar was higher than Goldasht cultivar. This results is showing that mycorrhizal inoculation has a great impact on plants under saline conditions and it can reduced the negative effect caused by salinity and higher Na concentration.

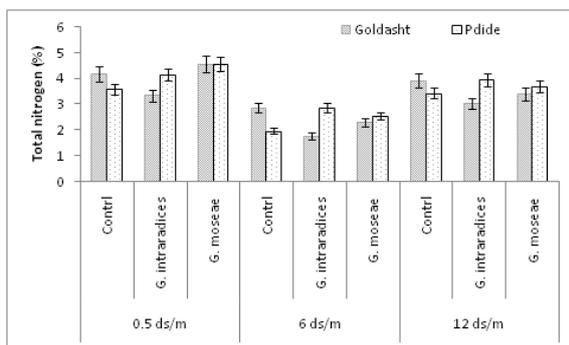


Fig. 1. Nitrogen changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

The amount micro nutrient such as chlorine, magnesium, calcium, copper, and zinc were also measured in this study. The effect of salinity and cultivar were significant on all micro nutrients. Effect of inoculation was significant on Mg, Cl, Ca, and Zn, but not significant for Cu (Table 1). Most of the interaction effects were significant for all micro nutrients showing different interacting of factors with each other. Increasing levels of salinity induced higher Cl content. Under control and salinity level of 6 ds/m, no significant different were observed for comparing cultivars and also inoculation levels, but under salinity of 12 ds/m, *G. intraradices* and *G. Moseae* decreased content of Cl (Figure 5). Padide cultivar showed higher content of Cl than Goldasht cultivar under 12 ds/m salinity level. This results is showing that mycorrhizal inoculation is more affective under sever salinity related to chlorine deficiency. Magnesium is an important elements contributing to chlorophyll and it has a central role in photosynthesis. The content of Mg was decreased by salinity, but the rate of decreasing under mycorrhizal

inoculation was lower (Figure 6). The effect of *G. intraradices* was higher than *G. moseae*, and Goldasht cultivar showed higher Mg than Padide. Salinity reduced the rate of Ca content in safflower leaves. Under control condition, the content of Ca was higher in no-inoculated plants, while under salinity, this content were lower (Figure 7). Therefore, inoculation under salinity condition is more effective for higher absorption of Ca. Goldasht cultivar showed higher amount of Ca under the most of the considered conditions. Sefik *et al* (2006) reported that mycorrhizal inoculation significantly affected the content of some micro nutrient such as Ca and Mg contents of soybean shoots.

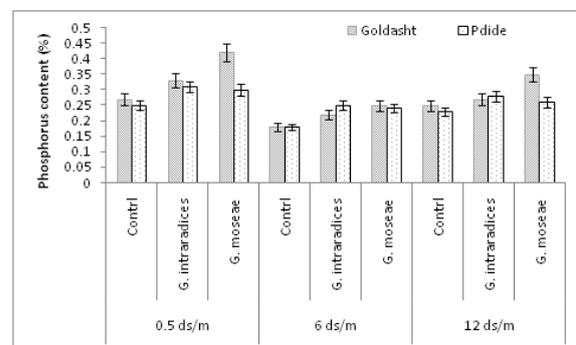


Fig. 2. Phosphorus changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

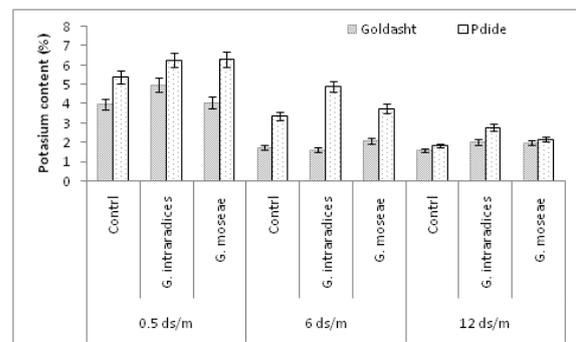


Fig. 3. Potassium changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

Figure 8 shows content of copper in inoculated and non-inoculated safflower plants under salinity condition. There was no significant difference related to control and 6 ds/m salinity level for copper, but its content in 12 ds/m salinity level was significantly higher than two other levels. Also, no significant differences was observed for inoculated plants in compare to non-inoculation ones. Padide cultivar

showed significant higher content of Cu in the most of the conditions comparing with Goldasht cultivar. Zinc content showed no significant differences for both control and salinity level of 6 ds/m, but its content was relatively higher in 12 ds/m (Figure 9). Inoculation with mycorrhizal fungi had different impact on Zn content, where *G. intraradices* showed relatively higher Zn content I compare with *G. moseae*. Furthermore, content of zinc in Goldasht cultivar was higher than that in Padide cultivar.

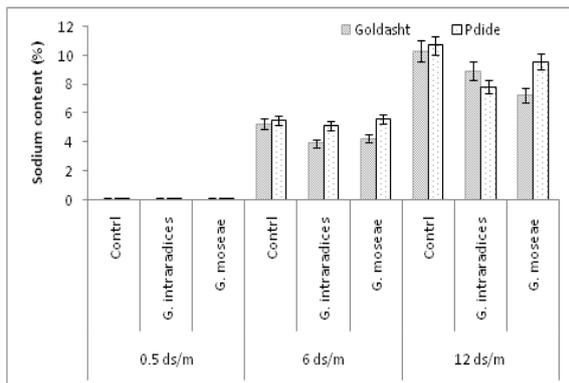


Fig. 4. Sodium changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

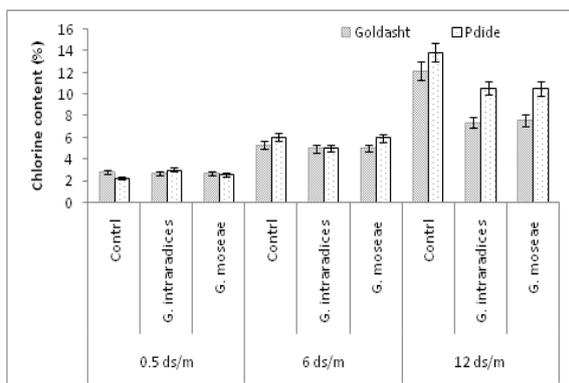


Fig. 5. Chlorine changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

Salinity which is caused by the effect of Na, disorders the ionic equilibrium in the soil and finally decreases the absorption of the other elements such p and mg. Mycorrhizal plants showed higher content of the all measured minerals in compare to control. Abdel Latif study on tomato (Abdel Latif and Chaoxing, 2011) and belterano on pepper (Beltrano *et al.*, 2013) obtained similar results. Higher area of the root of the colonized plant can absorb more mineral such as p, N and mg by assisting of mycorrhizal hyphae, so that

mycorrhizal colonization can play an important role in ionic equilibrium of the plant in the salinity condition (Miransari, 2011). It is stated that mycorrhiza fungi has three important mechanism for absorbing more nitrogen consist of activation of nitrate reductase (NADH) enzyme resulted in higher concentration of nitrogen and transforming to arginine and finally higher production of ammonium compound, increasing the amount of nitrate fixation and decreasing the toxicity effect of Na in the host plants (Evelin *et al.*, 2012). Higher absorption of p in mycorrhizal colonized plant suppresses the negative effect of Na in metabolic pathways results in higher growth of the plants (Garg and Manchanda, 2008). On the other hands, more absorption of the mg has an important role in increasing amount of pigments content in compare to non-mycorrhizal plants (Evelin *et al.*, 2009). Also, since mg is a key element in photosynthetic pathway and pigments content, which mycorrhizal inoculation plant showed higher content for this element; inoculated plants can manage more plant production in this condition.

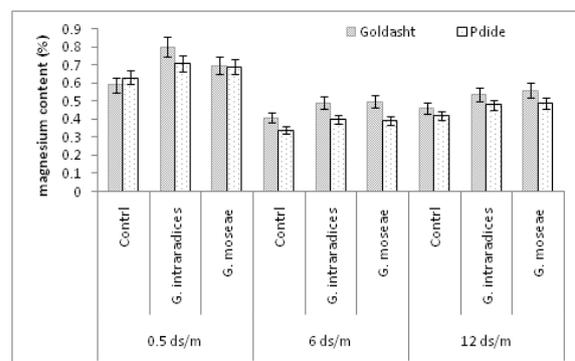


Fig. 6. Magnesium changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

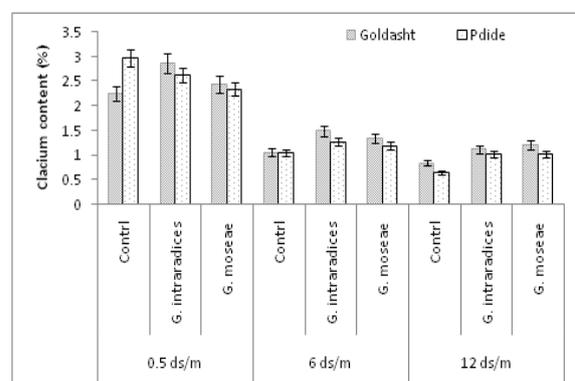


Fig. 7. Calcium changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

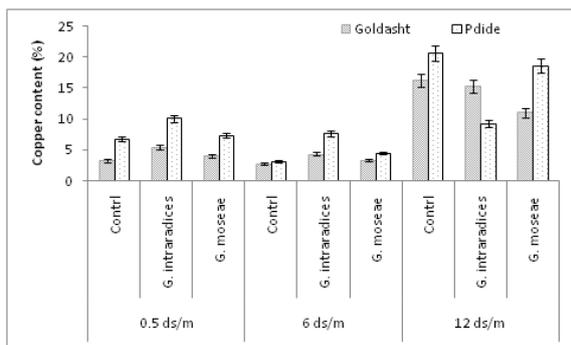


Fig. 8. copper changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

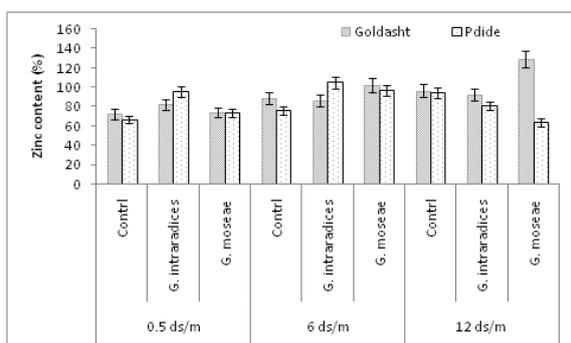


Fig. 9. Zinc changes in response to salinity and mycorrhizal inoculation in safflower cultivars.

Overall results indicated that salinity had a great impact on nutrient deficiency of safflower plants with increasing the content of some elements such as Na, Cl, and Cu in plant's leaf, but decreasing other important nutrients such as N, P, K and Mg. Inoculation of mycorrhizal fungi could reduce the negative effect of salinity by increasing content of important elements in this condition such as P, N, K. Also, these nutrients were higher in inoculated plant than non-inoculated under non-saline condition, indicating positive effects of mycorrhizal inoculation in all stressful or non-stressful conditions. Mg, Ca, and Zn are among the micro nutrient which are important for plant to produce higher yield. It is seems that Goldasht cultivar has a better contribution to inoculation with mycorrhizal inoculation and it showed relatively higher amount of macro nutrient and lower amount of Na and Cl.

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