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ORIGINAL ARTICLE

Evaluation of Two Biochemical Markers for Salt Stress in Three Pistachio Rootstocks Inoculated with Arbuscular Mycorrhiza (*Glomus mosseae*)

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Key words: Methylglyoxal, Mycorrhiza, Pistachio, Proline, Salt stress

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Key words: Methylglyoxal, Mycorrhiza, Pistachio, Proline, Salt stress

Salinity is an abiotic stress factor that limits plant development (Sengupta and Majumder, 2009). As a result of high salt concentrations, ionic imbalance and hyper-osmotic stress are triggered in plants, which consequently elicit secondary stresses such as oxidative damage (Zhu, 2001).

Salinization of soil is a serious problem and is increasing steadily in many arid and semi-arid parts of Kerman province especially in Rafsanjan region that is thought to be the largest center of pistachio production in Iran and in the world (Bagheri *et al*., 2011a). High levels of soil salinity in Rafsanjan region is mainly due to the soluble salts in irrigation water and fertilizers used by pistachio producers annually, low precipitation and high temperature in this region. According to this problem, recognition of tolerant rootstocks of pistachio to salinity has a specific importance and can play a key role in sustainable production.

Arbuscular mycorrhizal fungi (AMF) widely occur

in salty soils (Aliasgharzadeh *et al*., 2001). Recently, many researchers reported that AMF could enhance the ability of plants to cope with salt stress (Yano-Melo *et al*., 2003; Rabie, 2005; Jahromi *et al*., 2008) by improving plant nutrient uptake (Cantrell and Linderman, 2001; Asghari *et al*., 2005) and ion balance (Zandavalli *et al*., 2004; Giri *et al*., 2007), protecting enzyme activity (Rabie and Almadini, 2005; Giri and Mukerji, 2004), and facilitating water uptake (Ruiz-Lozano *et al*., 1995).

Methylglyoxal (MG), is a potent cytotoxic compound produced spontaneously under physiological conditions from the glycolysis and photosynthesis intermediates, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Richard, 1993). Under stress, the rate of glycolysis increases leading to an imbalance in the pathway. Triose phosphates are very unstable metabolites, and removal of the phosphoryl group of these trioses leads to the formation of MG (Richard, 1993). Therefore, spontaneous production of MG is an unavoidable consequence of the glycolysis pathway during different stresses like salinity, drought and cold stresses (Yadav *et al*., 2005; Singla-Pareek *et al*., 2006). In plants, MG is detoxified mainly via the glyoxalase system. Besides detoxification of MG, the glyoxalase system could also play a role in oxidative stress tolerance by recycling reduced glutathione that would be trapped non enzymatically by MG to form hemithioacetal, thereby maintaining glutathione homeostasis.

It is well known, that one of the most common responses to water deficit and saline environments is the accumulation of proline (Pro) which acts as a compatible solute, an osmoprotectant, and a protective agent for cytosolic enzymes and cellular organelles (Delauney and Verma, 1993; Bohnert *et al*., 1995; Demir, 2004). Additionally, Pro is a nitrogen source available for recovery from stress and for restoration of growth (Trotel *et al*., 1996). Based on previous researches, accumulation of proline in pistachio under both drought and salinity stresses have been proved (Bagheri *et al*., 2011b; Kamiab *et al*., 2012).

Development of salt tolerant plants depend on the basis of physiological, biochemical and molecular markers are recommended and may provide mechanistic understanding the term of tolerance. Hence many metabolic changes are known to occur in plants subjected to salt stress such as proline and methylglyoxal accumulation. The aim of this study was to evaluate proline and methylglyoxal as biochemical markers for salt tolerance of three pistachio rootstocks inoculated with AMF.

MATERIALS AND METHODS

Experimental site

A greenhouse experiment was conducted in 2013 at the Agri-college of Vali-e-Asr university of Rafsanjan (30°23′06″ N, 55°55′30″ E), at1523m a.s.l.

AM inoculum production

Glomus mosseae (Nicolson & Gerdemann) was propagated in a sterile potted soil cropped with *Zea mays* L. between June and October 2012. AM fungal inoculum consisted of a mixture of rhizospheric soil from trap cultures containing spores, hyphae and mycorrhizal root fragments.

Soil preparation and seed sowing

The soil used was an autoclaved (121°C for 2 h) sandy loam with the following characteristics: sand 70.2%, silt 14.6%, clay 15.2%, pH 7.2, P 7.4 mg kg^{-1} soil, K 23.4 mg kg^{-1} soil, Fe 1.3 $\mu g.g^{-1}$, Zn 0.03 $\mu g.g^{-1}$, Mn 0.13 $\mu g.g^{-1}$, Cu 0.03 $\mu g.g^{-1}$ and cation exchange

capacity 1.7 $dS.m^{-1}$. Adequate amount of fertilizers (NH_4NO_3, K_2SO_4, $MnSO_4$ and Fe EDDHA) were added to soil, based on soil analysis results.

Seeds of three pistachio rootstocks, *P. vera* cv. Sarakhs, *P. vera* cv. Abareqi and Bane baghi (*P. eurycarpa* × *P. mutica*) were surface sterilized in 10% sodium hypochlorite for 10 min and then incubated at 25°C on sterile moist cloth for one week. Six germinated seeds were sown in each pot containing 5 kg of autoclaved soil. The number of seedlings per pot was reduced to 4 within 21 days of germination.

Mycorrhizal inoculation

One hundred gram (fresh mass) of inoculum having on average of 90% of infected roots was placed on the soil surface immediately before planting and after placing the germinated seeds on it, seeds were covered with sterilized sand. Control plants received the same amount of an autoclaved inocula. The growth of seedlings continued for 180 days in greenhouse (T: 27±5°C; RH: 30±2%; 12–14 h day light without additional artificial lightening) before the start of salt treatments and during this period, the seedlings were watered every two days up to FC level with distilled water. At the end of this stage, sampled roots showed an average of 85% colonization for Sarakhs, 80% for Abareqi and 90% for Bane baghi.

Salt treatments

Four salt levels including EC of 0.5 (tap water as control), 5, 10 and 15 dSm^{-1}, achieved by adding NaCl in irrigation water. To avoid osmotic shock, salt solution in two higher levels (EC of 10 and 15 dSm^{-1}) was introduced gradually by 2 and 3 steps respectively. Plants were harvested 70 days after the commencement of salt treatments and during this period, they were irrigated every two days 30% more than predetermined FC level to avoid salt accumulation in the soil.

Root sampling and assessment of arbuscular mycorrhizal colonization

The experiment was terminated by separating shoots from roots days after treatments commencement. Roots were carefully rinsed with running tap water and then the roots of 4 plants in each pot was mixed and cut into 1cm in length segments. Samples for mycorrhizal assessment were prepared according to method of Phillips and Haymann (1970). Roots were boiled for 1 h in 15 % KOH and then washed with tap water. Staining was performed in 0.05 % trypan blue by autoclaving the samples for 15 min. Thereafter, Samples were stored in lacto glycerol [mixture of lactic acid, glycerol, water 1:1:1 (v/v/v)]. Root segments were mounted on glass slides and examined under a compound microscope (*CHS, Olympus optical Co., LTD, Japan*). Mycorrhizal colonization (abundance of hyphae, vesicles and arbuscules) was estimated using 50 root segments of each sample (Giovannetti and Mosse, 1980).

Sample preparation for MG estimation

Methylglyoxal was estimated basically according to the method of Yadav *et al*. (2005a) with some modification. About 0.5 g of leaf and root tissue was homogenized in 3 mL of 0.5 M perchloric acid. After incubating for 15 min on ice, the mixture was centrifuged at 4ºC for 10 min at 11,000 g. The supernatant was decolorized by adding charcoal (10mgmL-1), kept for 15 min at room temperature, and centrifuged at 11,000 g for 10 min. Before using this supernatant for MG assay, it was neutralized by keeping for 15 min with saturated solution of potassium carbonate at room temperature and centrifuged again at 11,000 g for 10 min. The neutralized supernatant was used for MG

estimation.

Methylglyoxal assay

In a total volume of 1ml, 250 µL of 7.2 mM 1, 2-diaminobenzene, 100 µL of 5 M perchloric acid, and 650 µL of the neutralized supernatant were added in that order. The absorbance at 335 nm of the derivatized MG was read after 25 min in a spectrophotometer (PG-instrument, T80, China). The final concentration of MG was calculated from the standard curve and expressed in terms of $\mu mol.g^{-1}$FW.

Proline determination

Proline colorimetric determination proceeded based on reaction with ninhydrin (Bates *et al.*, 1973). A 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hour. The reaction was arrested in an iced bath and the cromophore was extracted with 4 ml toluene and its absorbance at 520 nm was determined. Proline concentration was calculated with a standard curve and expressed as $\mu g.g^{-1}$ fresh mass.

Experimental Design

The experiment was a completely randomized design with three replicates and a factorial combination of two mycorrhiza treatments (with or without mycorrhizal), four salinity levels (S_1:0.5, S_2:5, S_3:10 and S_4:15 dSm^{-1}) and three rootstocks (Abareqi, Sarakhs and Bane baghi). Data were statistically analyzed by analysis of variance with the MSTATC PROGRAM (Michigan State University, East Lansing, Mich., USA). Probabilities of significance were used to test for significance among treatments and interactions and Duncan's multiple range test at 5% significant level was used to compare means.

RESULTS

All salt levels, AMF treatments and pistachio rootstocks had significant effect on almost all of measured parameters except root proline by mycorrhiza and leaf MG by pistachio rootstocks. The most significant interactions were recorded for M×S and S×PR (Table 1).

Methylglyoxal levels in leaf

It was found that leaf MG levels increased drastically due to different salt stress treatments in both -M and +M plants but in any salt level, -M plants had higher amount of MG (Fig. 1). In -M plants, Abareqi had the least and Sarakhs had the most leaf MG while the opposite results were obtained with +M plants (Fig. 2).

Methylglyoxal levels in root

In comparison with leaf, accumulation of MG was much lower in root. Regardless of salt treatments and pistachio rootstocks, MG content of -M plants was 16% more against +M plants (Fig. 3). Similar to leaf, MG was increased with increasing in salt stress levels but the results were significant just with S_3 and S_4. At stress levels of S_1 and S_2, there were no significant differences between rootstocks while in higher levels, Sarakhs and Abareqi showed the highest and lowest MG respectively (Fig. 4).

Proline accumulation in leaf

Leaf accumulation of proline in response to salt stress and mycorrhizal symbiosis in different pistachio rootstocks presented in Fig. 5. Despite of Mycorrhizal treatment, increasing concentration of NaCl from EC of 0.5 to 15 $dS.m^{-1}$, progressively increased proline concentration in leaf tissue of Sarakhs, Bane baghi and Abareqi by about 4.7, 6.3 and 4.8 fold respectively over than control treatment. It is interested to note that cv. Sarahks had significantly lower proline concentration at salt level of S_4 in -M and +M treatments compared to the other rootstocks. +M plants of all rootstocks

showed lower proline at salt levels of S_3 and S_4 in comparison with respected control -M (Fig. 6).

Proline accumulation in root

Root proline content in the salt stressed seedlings reached to 88.8 (4.93 folds of control) and 75.8 µg g^{-1} FW (3 folds of control) in -M and +M respectively under stress level of S_4 (Fig. 7). At each salinity level, no significant difference was observed between +M and -M plants except at S_4 where -M plants had higher amount of proline. No significant difference in root proline was observed between pistachio rootstocks at salinity levels of S_1 and S_2 but at higher salinity levels, cv. Abareqi had higher proline content (Fig. 8).

Root colonization

None of the pistachio plants in the non-inoculated treatments were colonized by *G. mosseae*. The extent of AM colonization decreased significantly with increase in soil salinity (10, 15 dS m^{-1}) in Abareqi and Bane baghi by 58 and 29% respectively over respected control while root colonization of cv. Sarakhs was not affected by salinity except at S_3 level (Fig. 9).

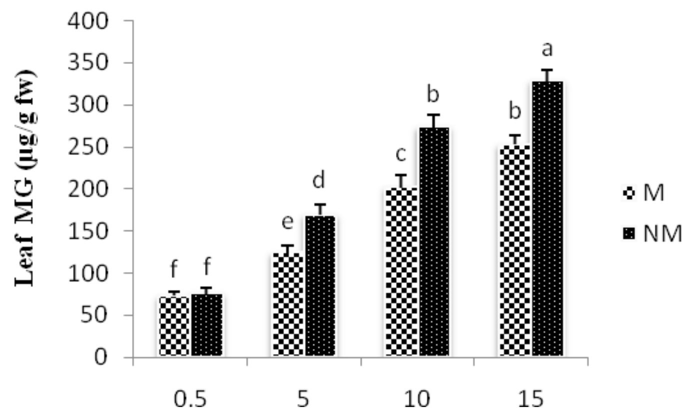

Figure 1. Interaction effects of mycorrhizal symbiosis and salt intensities (EC of 0.5, 5, 10 and 15 dS m^{-1}) on leaf MG levels of pistachio rootstocks. Bars indicate standard error. Colomns with different letters aresignificantly different at P≤ 0.05.

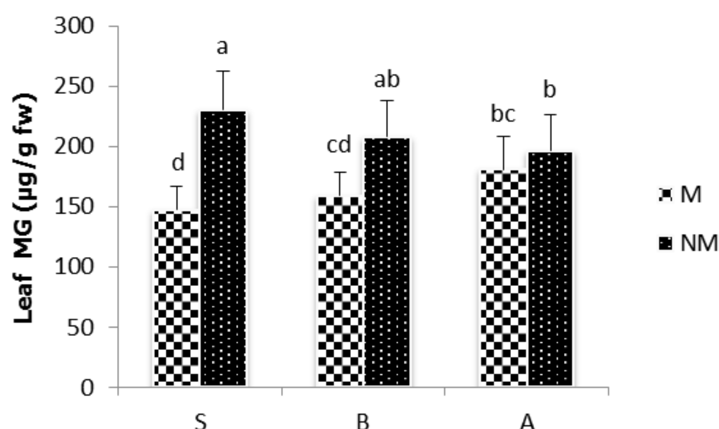

Figure 2. Effects of mycorrhizal symbiosis on leaf MG levels in three pistachio rootstocks (S: Sarakhs, B: Bane baghi, A: Abareqi) grown 70 days under different salt intensities (EC of 0.5, 5, 10 and 15 dS m^{-1}). Bars indicate standard error. Colomns with different letters aresignificantly different at P≤ 0.05.

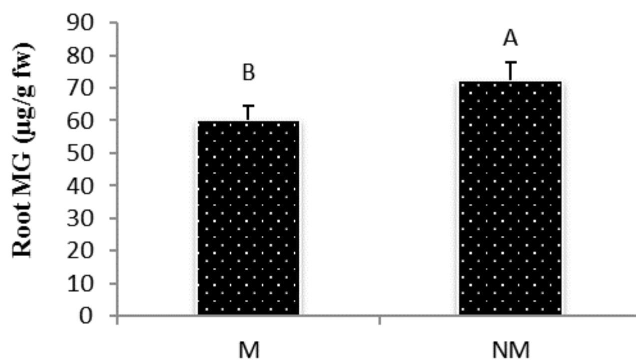

Figure 3. Effect of mycorrhizal symbiosis on root MG level of pistachio rootstocks. Bars indicate standard error. Colomns with different letters aresignificantly different at P≤ 0.05.

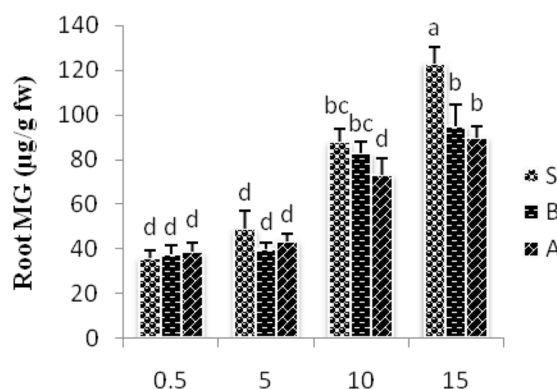

Figure 4. Effects of different salt intensities (EC of 0.5, 5, 10 and 15 dS m^{-1}) on root MG levels in three pistachio rootstocks (S: Sarakhs, B: Bane baghi, A: Abareqi). Bars indicate standard error. Colomns with different letters aresignificantly different at P≤ 0.05.

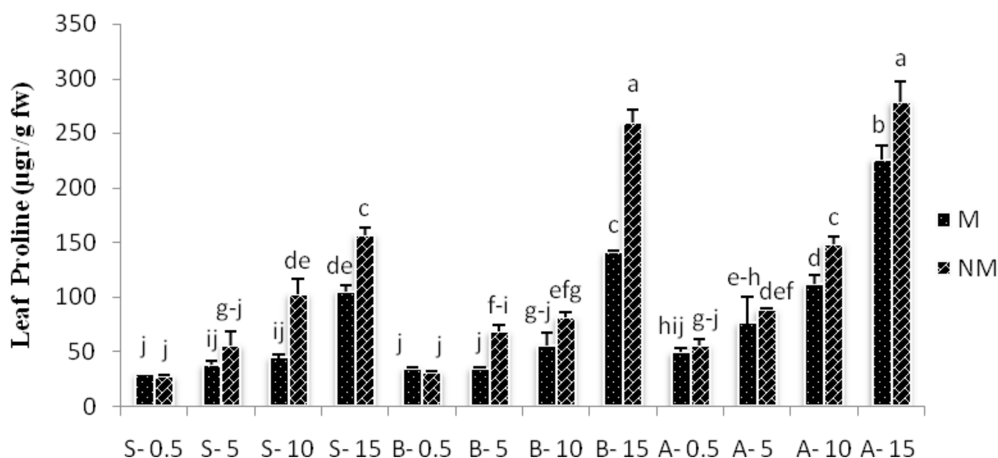

Figure 5. Effects of mycorrhizal symbiosis (M: with mycorrhizae, NM: without mycorrhizae) on leaf proline levels in three pistachio rootstocks (S: Sarakhs, B: Bane baghi, A: Abareqi) grown 70 days under different salt intensities (EC of 0.5, 5, 10 and 15 dS m^{-1}). Bars indicate standard error. Colomns with different letters aresignificantly different at P≤ 0.05.

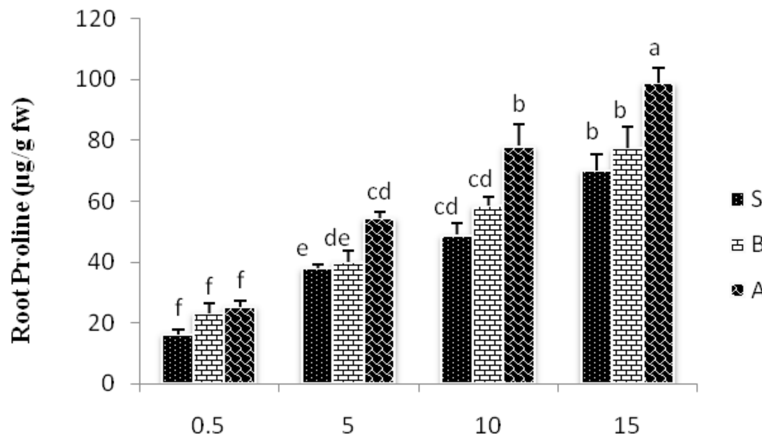

Figure 6. Effect of different salt levels (EC of 0.5, 5, 10 and 15 dS m^{-1}) on root proline content of three pistachio rootstocks (S: Sarakhs, B: Bane baghi, A: Abareqi) 70 days after salt stress commencement. Bars indicate standard error. Colomns with different letters aresignificantly different at P≤ 0.05.

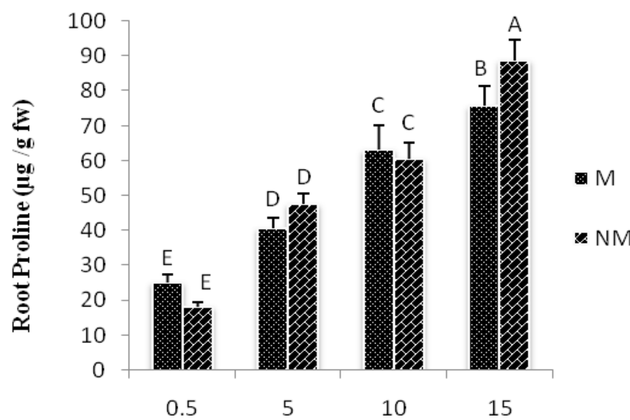

Figure 7. Interaction effects of mycorrhizal symbiosis and salt intensities (EC of 0.5, 5, 10 and 15 dS m^{-1}) on root proline contentof pistachio rootstocks. Bars indicate standard error. Colomns with different letters aresignificantly different at P≤ 0.05.

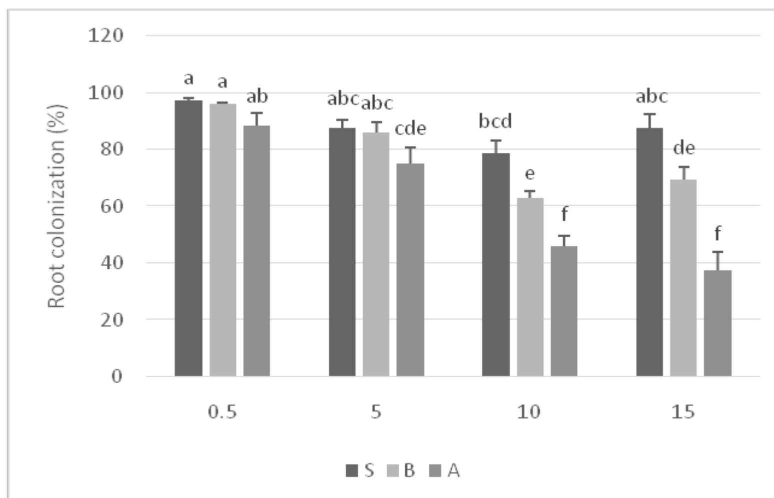

Figure 8. Effect of salinity levels (EC of 0.5, 5, 10 and 15 dS m^{-1}) on mycorrhizal root colonization percentage of three pistachio rootstocks (S: Sarakhs, B: Bane baghi, A: Abareqi) 70 days after salt stress commencement. Bars indicate standard error. Colomns with different letters aresignificantly different at P≤ 0.05.

Table 1. *ANOVA* results for mycorrhizal infection percentage(MI), leaf methylglyoxal (LMG), root methylglyoxal (RMG), leaf proline (LPRO) and root proline (RPRO)cotentof Abareqi, Sarakhs and Bane baghi rootstocks (PR) exposed to varying intensities of salt stress (S) andAMF (M) treatments.

Parameters	M	S	PR	M×S	M×PR	S×PR	M×S×PR
MI%	-	***	***	-	-	***	-
LMG	***	***	ns	**	**	ns	ns
RMG	***	***	***	ns	ns	*	ns
LPRO	***	***	***	***	ns	***	**
RPRO	ns	***	***	**	ns	*	ns

*** –significant (*P*<0.001); ** – significant (*P*<0.01), * – significant(*P*<0.05); ns – not significant.

Table 2. Relationship between the biomarker (Y) and salt stress (x) levels and their respective correlation coefficients for mycorrhizal and non-mycorrhizal pistachio plants.

	Mycorrhizal treatments	
Biomarkers	-M	+M
LMG	Y= 17.7X + 76.12 R^2= 0.98***	Y=12.7X + 65.53 R^2= 0.99***
RMG	Y= 5.4X + 31.2 R^2= 0.96***	Y= 4.25X + 27.55 R^2= 0.94***
LPRO	Y= 12.89X + 14.44 R^2= 0.91***	Y= 7.9X + 18.48 R^2= 0.83**
RPRO	Y= 4.62X + 18.59 R^2= 0.98***	Y= 3.59X + 23.78 R^2= 0.99***

DISCUSSION

In an attempt to determine whether the accumulation of MG and proline in leaf and root tissues of three different pistachio rootstocks, pre-treated with mycorrhiza, in response to various salt stress levels is a common phenomenon, MG and proline levels were measured in this study. Significant increase of MG levels were observed due to different concentration of NaCl that is in agree with previous studies (Yadav *et al.*, 2005). The sharper increase were observed in leaf tissues where cells become metabolically active under stress condition which is mirrored by upregulation of enzymes involved in glycolysis and TCA cycles (Sommer *et al.*, 2001) and as a result, flux of triose phosphates increases which, instead of giving only pyruvate could be converted to MG. Previously, It has been reported that MG accumulation in rice roots is lower than shoots under salt stress (Yadav *et al.*, 2005) which is confirmed by our results. It seems that increased levels of MG could act as a signal for plants to respond to the stress (Yadav *et al.*, 2005; Singla-Pareek *et al.*, 2006). However, very little works have been done in plant systems regarding the endogenous production of MG. Results presented in Fig.2 showed that leaf MG level in non-mycorrhized cv.Sarakhs seedlings was highest while the lowest values were recorded for the mycorrhized seedlings of the same rootstock. The opposite results were observed in cv. Abareqi. It can be attributed to the mycorrhization extent of these rootstocks under different salinity levels since root colonization of Abareqi seedlings was reduced severely as the effect of salinity whereas no obvious reduction occurred in cv. Sarakhs under the same conditions.

Leaf and root proline content was increased with increase in the level of salinity. The increase of proline level in roots was less and gradual in compare with leaves which is in agree with previous

results (Sofo *et al.*, 2004). Abareqi possessed maximum values for leaf proline content at salinity levels of S_3 and S_4 compared to the others. Proline acts as a cytosolic osmoticum, scavenger of $OH\cdot^-$ radical and can interact with cellular macromolecules, such as DNA, protein, membranes, and can stabilize their structure and function (Kishor *et al.*, 2005). Therefore, it was expected that the exposure of pistachio plants to NaCl could higher the level of proline in order to overcome the oxidative stress generated by the salinity. A possible reason for this increased level of proline during the salt stress could be an alteration in the activities of the enzymes involved in the biosynthesis and degradation of proline.

We found a very strong relationship between salinity and measured biochemical markers (Table 2). Significant positive correlation between leaf MG level and salt stress intensity in -M (R^2 = 0.98***) and +M (R^2 = 0.99***) pistachio rootstocks and between root proline and salinity levels in -M ((R^2 = 0.98***) and +M (R^2 = 0.99***) plants revealed that both of them could be used as a biochemical marker of salt stress level in pistachio plants.

The fact that under S_4, cv. Sarakhs had the highest and lowest accumulation of leaf MG and proline (Fig. 2, 5) respectively and also cv. Abareqi had the highest and lowest of root proline and MG respectively under S_3 and S_4 could be related to the role of proline in enhancing MG detoxification systems (Hossain and Fujita, 2010).

In our experiment, root colonization extent of all rootstocks were not changed up to EC of 5 dS m^{-1} and thereafter, it was reduced in Abareqi and Bane baghi while did not change in Sarakhs (Fig. 8). Salinity, not only affects the host plant but also the AMF. It can hamper colonization capacity, spore germination and growth of hyphae of the fungus. Several researchers have reported the negative effects of salinity on the fungus (Jahromi *et al.*, 2008). A few studies reported that AMF colonization is not reduced in the presence of NaCl (Levy *et al.*, 1983). Increased AMF sporulation and colonization under salt-stress conditions has also been reported (Aliasgharzadeh *et al.*, 2001). However, unchanged colonization percentage in cv. Sarakhs under salt stress remains ambiguous, although it can be attributed to the quantity and quality of root exudates (Giovannetti *et al.*, 1996), more research and understanding are needed to clarify its accurate mechanism.

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REFERENCES

Aliasgharzadeh, N., Rastin, S.N., Towfighi, H. and Alizadeh, A. (2001) Occurrence of arbuscular mycorrhizal fungi in saline soils of the tabriz plain of iran in relation to some physical and chemical properties of soil. *Mycorrhiza*., **11**, 119-122.

Asghari, H.R., Marschner, P., Smith, S.E. and Smith, F.A. (2005) Growth response of atriplex nummularia to inoculation with arbuscular mycorrhizal fungi at different salinity levels. *Plant Soil*., **273**, 245-256.

Bagheri, V., Shamshiri, M., Shirani, H. and Roosta, H. (2011a) Effect of mycorrhizal inoculation on ecophysiological responses of pistachio plants grown under different water regimes. *Photosynthetica*., **49**, 531-538.

Bagheri, V., Shamshiri, M. H., Shirani, H. and Roosta, H. R. (2011b) Effect of arbuscular mycorrhizae and drought stress on growth indexes, water

relations and proline as well as soluble carbohydrate content in pistachio (pistacia vera l.) rootstock seedlings. *Iranian Journal of Horticultural Sciences,* **42**, 365-377.

Bates, L., Waldren, R. and Teare, I. (1973) Rapid determination of free proline for water-stress studies. *Plant soil*., **39**, 205-207.

Bohnert, H. J., Nelson, D. E. and Jensen, R. G. (1995) Adaptations to environmental stresses. *Plant cell*., **7**, 1099-1111.

Cantrell, I. C. and Linderman, R. G. (2001) Preinoculation of lettuce and onion with va mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant Soil*.**, 233**, 269-281.

Delauney, A. J. and Verma, D. P. S. (1993) Proline biosynthesis and osmoregulation in plants. *The Plant Journal*, **4**, 215-223.

Demir, S. (2004) Influence of arbuscular mycorrhiza on some physiological growth parameters of pepper. *Turk J Biol*., **28**, 85-90.

Giovannetti, M. and Mosse, B. (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol*., **84**, 489-500.

Giovannetti, M., Sbrana, C., Citernesi, A. S. and Avio, L. (1996) Analysis of factors involved in fungal recognition responses to host-derived signals by arbuscular mycorrhizal fungi. *New Phytol*., 65-71.

Giri, B., Kapoor, R. and Mukerji, K. (2007) Improved tolerance of acacia nilotica to salt stress by arbuscular mycorrhiza, glomus fasciculatum may be partly related to elevated k/na ratios in root and shoot tissues. *Microb Ecol*., **54**, 753-760.

Giri, B. and Mukerji, K. (2004) Mycorrhizal inoculant alleviates salt stress in sesbania aegyptiaca and sesbania grandiflora under field conditions: Evidence for reduced sodium and improved magnesium uptake. *Mycorrhiza*., **14**, 307-312.

Hossain, M. A. and Fujita, M. (2010) Evidence for a role of exogenous glycinebetaine and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under salt stress. *Physiol Mol Biol Plants*., **16**, 19-29.

Jahromi, F.,Aroca, R.,Porcel, R. and Ruiz-Lozano, J. M. (2008) Influence of salinity on the in vitro development of glomus intraradices and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. *Microb Ecol*., **55**, 45-53.

Kamiab, F., Talaie, A., Javanshah, A., Khezri, M. and Khalighi, A. (2012) Effect of long-term salinity on growth, chemical composition and mineral elements of pistachio (*pistacia vera* cv. Badami-zarand) rootstock seedlings. *Annals of Biological Research*., 3, 5545-5551.

Kishor, P. K., Sangam, S., Amrutha, R., Laxmi, P. S., Naidu, K., Rao, K., Rao, S., Reddy, K., Theriappan, P. and Sreenivasulu, N. (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr Sci*., **88**, 424-438.

Levy, Y., Dodd, J. and Krikun, J. (1983) Effect of irrigation, water salinity and rootstock on the vertical distribution of vesicular-arbuscular mycorrhiza in citrus roots. *New phytol*., **95**, 397-403.

Rabie, G. (2005) Influence of arbuscular mycorrhizal fungi and kinetin on the response of mungbean plants to irrigation with seawater. *Mycorrhiza*., **15**, 225-230.

Rabie, G. and Almadini, A. (2005) Role of bioinoculants in development of salt-tolerance of vicia faba plants under salinity stress. *African Biotechnology Journal*., **4**, 210-222.

Richard, J. (1993) Mechanism for the formation of methylglyoxal from triosephosphates. *Biochem Soc Trans*., **21**, 549-553.

Ruiz-Lozano, J., Azcón, R. and Gomez, M. (1995) Effects of arbuscular-mycorrhizal glomus species on drought tolerance: Physiological and nutritional plant responses. *Appl Environ Microbiol*., **61**, 456-460.

Sengupta, S. and Majumder, A. L. (2009) Insight into the salt tolerance factors of a wild halophytic rice, porteresia coarctata: A physiological and proteomic approach. *Planta*., **229**, 911-929.

Singla-Pareek, S. L., Yadav, S. K., Pareek, A., Reddy, M. and Sopory, S. (2006) Transgenic tobacco overexpressing glyoxalase pathway enzymes grow and set viable seeds in zinc-spiked soils. *Plant Physiol*., **140**, 613-623.

Sofo, A., Dichio, B., Xiloyannis, C. and Masia, A. (2004) Lipoxygenase activity and proline accumulation in leaves and roots of olive trees in response to drought stress. *Physiol Plantarum*., **121**, 58-65.

Sommer, A., Fischer, P., Krause, K., Boettcher, K., Brophy, P., Walter, R. and Liebau, E. (2001) A stress-responsive glyoxalase i from the parasitic nematode onchocerca volvulus. *Biochem J*., **353**, 445-452.

Trotel, P., Bouchereau, A., Niogret, M. and Larher, F. (1996) The fate of osmo-accumulated proline in leaf discs of rape (*Brassica napus* L.) incubated in a medium of low osmolarity. *Plant Sci*., **118**, 31-45.

Yadav, S. K., Singla-Pareek, S. L., Ray, M., Reddy, M. and Sopory, S. (2005) Methylglyoxal levels in plants under salinity stress are dependent on glyoxalase i and glutathione. *Biochem Biophys Res Commun*., **337**, 61-67.

Yano-Melo, A. M., Saggin, O. J. and Costa Maia, L. (2003) Tolerance of mycorrhized banana (*Musa sp.* cv. Pacovan) plantlets to saline stress. *Agr Ecosyst Environ.*, **95**, 343-348.

Zandavalli, R. B., Dillenburg, L. R. and De Souza, P. V. D. (2004) Growth responses of *araucaria angustifolia* (araucariaceae) to inoculation with the mycorrhizal fungus *Glomus clarum*. *Appl Soil Ecol*., **25**, 245-255.

Zhu, J.-K. (2001) Plant salt tolerance. *Trends Plant Sci*., **6**, 66-71.