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Research Article

The effect of Mycorrhizal fungi on the amount of glycine betaine, soluble sugar, proline, leaf water content and leaf chlorophyll of the white seedless grape under drought stress conditions

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ABSTRACT
Mycorrhizal fungi have a positive role in moderating the devastating effects of drought in most plants; on this basis to determine the effects of inoculation ofroots ofgrape var*Sefidbidaneh*with three species of Mycorrhizal fungi namelyGlomusfasiculatum, Glomusintraradices and Glomusmosseae on the amount of chlorophyll, soluble sugars, betaine glycine and proline under water stress, the present study was conducted as a factorial experiment based onrandomizedcomplete block design in four replications.Compared to control treatment,inoculating with Mycorrhizal fungi could have significant effectson aforementioned traits so that the amount of betaine glycine, proline and soluble sugars increasedbut chlorophyll decreasedin control. The highestamounts of soluble sugars,betaine glycine and proline were observedatthe irrigation levelof 25% of stress. Furthermore, the highest amount of chlorophyll was related to the irrigation levels of 50% and 75%. At all levels of interaction between fungus and moisture stress, the highest amount of proline synthesis was observed in control,indicatingthe positive role of Mycorrhizal fungi in moderating the negative effects of water stress and in increasingof water absorption in drought stress conditions.

Keywords: Water stress, Physiological traits, Yield moderation, Nutrient absorption, Interaction between fungus and irrigation

INTRODUCTION
White seedless, red currants, Asgari, Yaghuti, Shahroudi, RishBaba,Peikani, Fakhri, SiahSardasht, Sahebi and several othercultivars are important vinevarieties cultivated in Iran. All edible grapesof Iranbelongto Vinifera species (Dowlati et al., 2005). Grapes is one of the main horticultural crops of Iranso that it has the first position among fruit trees with respect to the area of cultivation and economically isplaced after pistachio and date (Nazemiyeh1999). Causing a huge damage annually, drought is one of the most important factors limiting the crops production in the world.Environmental conditionswhich preventthe access of plant toadequate waterto performits vital function andrepetition of this process which leads

tothedehydrationof plant tissues iscalleddrought(Levitt, 1980).When plant cells lose their swelling state and speed of transpiration is greater than the absorption, plant is dehydrated (Kozlowski and Palardy, 1997).If water stress is high, it causes a sharp decline in photosynthesis, disruption tophysiological processes, reduction of yield, and finally drying of plant. Transpiration is controlled by factors such as structure and area of leaves, size of stomata pores, number of stomata and othereffective factorson water vapor gradient between plant and atmosphere (Ghaderi et al., 2006). The response plants show to water shortage depends on the plant species, length and duration of drought stress, age and stage of plant development, type of cell and plant, cellular components and its structure. Drought stress triggers different impacts on morphological characteristicsof plant such as leaf area, shoots growth and root development, plant pigments, fresh and dry weight of leaf and root and on the physiological characteristics such as leaf water potential, stomata resistance, relative water content of leaf, photosynthetic activity, absorptionof photosynthetic carbon dioxide, transpiration and proline accumulation (Sadrzadeh et al., 2007; Zarrabi et al., 2009). Moreover,it results inlosing of water and plant cells and shortage of food as well asreducingof carbon dioxide absorption due to stomata closure, and finally causes thestarvation of plant (Kramer and Boyer, 1995).

Mycorrhizalword is formed fromtwo words of (Myco) means fungus and (Rhiza) means root, which represents theparticipation in symbiosis between fungus and the root of the host plant. In this system,fungus forms an extensive coverage of thread-like hyphae called mycelium around the root of host plant. Most plants are able to form Mycorrhizalsystem (Mostajeranand Zoee, 2006). In plants withgood symbiotic relationship, Mycorrhizal fungusis an important factor to increase the resistanceand growth of plant especially in adverse environmental conditions such as drought stress and food shortage.Mycorrhizalfungi help plants to absorb fluid and non-fluid resourcesby forming individual and developed network. Networkhyphae generally increases the receiving of fluid food resources insome plantsandaffects the growth of plants in different ways, and, by contrast, receives its required materials bysendingitssuckersinto thehost cell, particularly inthe area under theepidermis(the cortex),(Ameriyan, 1992).

One of the most important physiological roles of prolineaccumulation in response to water shortage isthe role it plays as a regulator of osmotic pressure and protective agent of cytoplasmic enzymes and membrane structure. In a study about the effect ofdrought stress on proline level, soluble and insolublesugars of some grape varieties, drought stress ledto the increase inthe concentration of prolineand soluble sugars, and reduction of insoluble sugars in leaf (Rabie et al., 2009; Azizi et al., 2009). Increase of proline in stress periodmay be the result of proteins breakdown as well as reduction of their use due to the reductionin plant growth. Under stress conditions,proline accumulationoccurs in all organs of the plant, but the rate of accumulation in leaves is quicker than other organs. As thestored amino acid in the cytoplasm,proline probably has a vital role in protecting the structure of macromolecules to play (Heuer, 1994). The accumulation of soluble sugars (sucrose, glucose and fructose) is associated with the resistanceof plants to drought (Hokstira and Booting, 2001). Sugars retain the cells during drought by two mechanisms: (1) water is replaced withhydrocele groups of sugars in other tomaintain the hydrophilic interactions in proteins and membranes during dehydration. So sugars are linked with proteins and membranes by hydrogen; that is why degradation of proteins is prevented(Leopold et al., 1994).

Sugarsare a major factor in Vitrification (being glassy), a process that forms biological glassesin the cytoplasm of dehydrated cells (Botink et al., 1998). In study about the effects of drought on five grape varieties including *white seedless*, *Shahani, Muskat, Gazanyand Film Seedless*, it was found that increase in the stress intensity resulted inthe enhancement ofprolineand soluble sugars levels, while chlorophyll and soluble proteins levels decreased. The highest amounts of proline and soluble sugars accumulation were due to*Muskat Gordo* and *Gazni* varieties respectively,and,on the contrary, the lowest accumulation rate of both compoundswas observedin *Film Seedless* (Rezai, 2007).Adjustmentof osmotic potential throughArbuscularMycorrhizais probably one of the most important reasons for improving the ability ofhost plant to grow under water stress. Plants reduce the cell osmotic potential by collecting higher amounts of organic products, such as prolineand glycine betaine, and carbohydrates such as sucrose, mannitol and inorganic ions;leading to the retention ofhigher amount of water during drought (Medina et al. 2010; Miransari 2010). Of all these metabolites,proline probably accumulated more than others under the conditions of water shortage (Ruiz-Lozano et al. 2006).Under water stress conditions, plant stomata are closed and subsequently CO_2 concentration is reduced in mesophyll tissue.Following the reduction in CO_2 concentration, darkreactions of photosynthesis aredisrupted and obtained products of light reactions, which include ATP andNADPH, are not consumed. Of the importantfactors reducingyield and quality of grapes are water stress in the critical period of growth and nutrient deficiencies.Furthermore, fixation of a number of elements such as iron and their deficiency is evidentdue to calcareous soilof the vineyards.*White seedless* grape is one of thecommercialvarieties spread in many parts of Iran due to its great growth in clay soil. On thisbasis, study of good methods to control or reduce the adverse effects of drought and other effective methods to increase the efficiency of water use should be taken into consideration. Inoculation ofgrape with Mycorrhizal fungus is one of the important methods for improving the absorption of water and nutrients especially in arid regions and poor soils. The present research was aimed at studying the effects of three species of Mycorrhizal funguson the improvement ofgrowth, and absorption of some nutrients and water under drought stress conditions in grape var.*white seedless*.

MATERIALS AND METHODS

This study was conducted at the Horticultural Research Station ofNakhjavaniUrmiain years 2013 and 2014as factorial experiment based on randomized complete block design with four replications. The first factor included inoculation with three Mycorrhizal fungi *(Glomusmosseae, G. fasciculatum, G. intraradices)* and without inoculation (control), and the second factor was irrigation at three levels (35, 55 and 75% of field capacity water). Cuttings were first prepared innecessary number andthen placed in sand mediumfor rooting. In the same medium, inoculation of half seedlings with suspension ofArbuscularMycorrhizal (AM) fungus was carried out, and the rest of seedlings were considered as control. Mycorrhizal fungi Inoculum (spore, mycelium, Mycorrhizal roots and soil) provided from Turan biotech Company,Sharood, and were reproduced on sorghum roots. For producingMycorrhizal seedlingsofgrape, its hardwoodcuttings were put in rooting medium that was mixed with Mycorrhizal fungi Inoculum with the ratio of15 ppm. To ensure root colonization, sampling from root was performed weekly. After staining the root with Trypan 05% and being ensure aboutcolonization in the last stage,the percentage ofroots colonization was determined and seedlings were transferred to pots for further study. Inoculated rooted seedlings were planted in pots containing sterilized soil. A soil sample of eachpot was prepared for determining the moisture content and physicochemical characteristics.During the growing season,complete cares were takenfrom grapes plants. 20-liter plastic pots were used for cultivation of seedling. Two-bud pruning was done. Two months afterfull establishmentof seedlings in pot and development of the green corona,irrigationtreatments were appliedwith daily weighing of pots and adding consumed water due to evaporation and transpiration. In fact, decrease inweight of each of the pots showed the amount ofconsumed water through evaporation and transpiration. Irrigation regimes were applied on seedlings for three months. At the end of the experiment, physiological traits including leaf chlorophyll index, proline, soluble sugars, and glycinebetaine were measured in all water treatments.

Measuring Chlorophyll level Index

At the end of the experiment in all treatments, pots were randomly selected and the amount of chlorophyll of four leaves (the upper and lower parts of the plant) from each pot was measured with chlorophyll content meter (Model CCM-200-Opti-science).

Measuringthe amount ofleaf proline

Preparation of extract and measurement of leaf proline was performed based on Bates et al (1973) method. For extraction, first 0.3 g of fresh leaf tissue was pulverized with 10 ml of 3%Solfosalicilic acid (w/v) in a Chinese mortar. Then pulverized sample was poured into the test tube and was shaken for 2 minutes.Then both solid and liquid phasesof samples were carefully separated. Liquid phase was centrifuged at the speed of 3500 rpm for 10 minutes and the upper part of it was removed.

To measure the proline level, the amount of 2 ml was separated from the extract and was poured into the test tube. 10 ml of distilled waterwas added into each sample and stirred. To each sample the amount of 2 ml ninhydrin and then 2 ml of acetic acid was added. After the above mentioned steps, samples were placed inside bainmarie at 100 ° C for 45 minutes.After cooling samples in ice water, 4 ml of toluene was added to each sample, and the solution obtained was shaken to the levelthat prolineenters the phase of toluene. Then, the samples were left for 30 minutes in stasis and then the level of upper phase light absorbance of samples was determinedwith the help of spectrophotometer Varian, model CARY-100 at 515 nm.Prolinelevel of leaf samples was calculated in micromoles per gram of leaf fresh weight from the following equation:

$$\frac{\text{Leaf proline}}{\mu\text{mol} \times \text{gW.s}} = \frac{\text{extractproline } (\mu g \times ml) \times \text{ extract content(ml)}}{115.5\ (\mu g \times \mu mol)} \times \frac{5}{\text{sample weight (g)}}$$

In the above equation, the number 115.5 is prolinemolecular weight.To determine the concentration of samples proline, standard solutions (proline) with theconcentrations of 100, 200 and 300 micrograms per ml were used.Ninhydrinrepresentwas prepared by solving the amount of 1.25 g ninhydrinin 30 ml of acetic acid and 20 ml phosphoric acid 6 M (with heating and stirring regularly) 24 hours before the test.

Measuring the amount of soluble carbohydrates

Extraction and measurement of leaf soluble carbohydrates level was performedaccording to the method used by Buysse and Merckx (1993). For this purpose, first 0.3 g of fresh leaf tissue was pulverized with 5 ml of ethanol 95% in a Chinese mortar. Then pulverized sample was poured into the test tube and was shaken strongly for 2 minutes. Thus, two solid and liquid phases were carefully separated and again were added to solid phase two times and each time with 5 ml of 70% ethanol and shakenstrongly until the liquid phase was separated. The total liquid phase was centrifuged at 3500 rpm for 10 minutes and the upper part of it was removed. From the mentioned extracts, an amount of 1.0 ml was separated and 3 mlAnthrone representwas mixed (the reaction of Anthronewith sugars in an acidic environment produced bluish green color). To complete the colorimetric, the solution was put 10 minutes at 100 ° C inside bainmarie. After cooling the samples, absorption level of their light was measured using a spectrophotometer Varian, and model CARY-100 in wavelength of625 nm and the amount of soluble sugars was calculated in mg per gram of fresh weight from the following equation:

$$\frac{\text{Leaf soluble sugar}}{\text{mg} \times \text{gW.s}} = \frac{\text{extract sugar (mg}\times\text{l)} \times \text{extract content(ml)}}{180.16\ (\text{mg}\times\text{mmol}) \times \text{sample weight (g)}}$$

To determine the amount of carbohydrate of samples, standards of pure glucose were prepared at concentrations of 250, 500, 1000, 2000 and 4000 ppm (ppm) and all the testing stages wereapplied on them.

Measuring glycinebetaine

To measure glycine betaine, Greve and Grattan method (1983) was used.

Results and discussion

Before performing data analysis, their quality including identification of outliers and normality of data distribution was investigated using KolmogroSmirno (K-S) software. The variables that were not normally distributed were normalized usingSPSS conversions inKolmogroSmirno (K-S) software .The experiment was conducted in a randomized complete block design but because the effect of block was not significant,the data were finally analyzed based on completelyrandomized design using SAS (version 9).Table 1 shows the results of variance analysis related to some traits of photosynthesis and some measured compounds. As seen,effects oftreatments on traits such as chlorophyll index and betaglycine and on the solublesugarsandproline were significant at 5 and1% levels, respectively.The effect of different levels of stress on mentioned traits was significant at 1%,suggesting that these traits were significantly affected by the treatments.The effects of the interaction between fungus treatments and stress were just significanton the amount ofproline at 5%level.

Table1. Analysis of variance of traits related to photosynthesis and protein synthesis

Source of variation	Degree of freedom	Mean of squares					
		Net photosynthesis	ChlorophyllinGroup8	ChlorophyllinGroup9	Solublesugar	Glycinebetaine	Proline
Fungus	3	1.220^{ns}	3.373^{ns}	15.00*	292.00**	355.00*	160.00**
Irrigation	2	9.110^{ns}	25.722^{ns}	61.99**	486.05**	2106.04**	453.50**
fungus×irrigation	6	2.891^{ns}	9.226^{ns}	8.89^{ns}	31.25^{ns}	164.71^{ns}	106.47*
Error	36	8.358	9.684	7.63	54.34	171.99	42.11
Percentage of changes		28	10.9	9.67	19.26	7.95	4.55

ns: Difference is not significant

*: Difference is significantat five percent

**: Difference is significant at one percent

Means comparison of traits related to proteins compounds and chlorophyll

A) The main effects of the different treatments of Mycorrhizal fungi

The results of means comparison of soluble sugars, beta glycine, chlorophyll and proline suggested that the highest levels of these compounds in control treatment,indicating the fungustreatments play an important role in increasing water absorption and increasing plant resistance against dehydration(Fig. 1,2, 3 and 4).

Soluble sugar

The lowest amount of protein synthesisand chlorophyll observed in treatment inoculated with *Glomusfasiculatum* fungus. There were significant differences between the amounts of soluble sugarsin control and*Glomusintraradices*and*Glomusfasiculatum* fungi,and there was not a significant difference between the control and *Glomusmosseae* fungus (Figure 1).

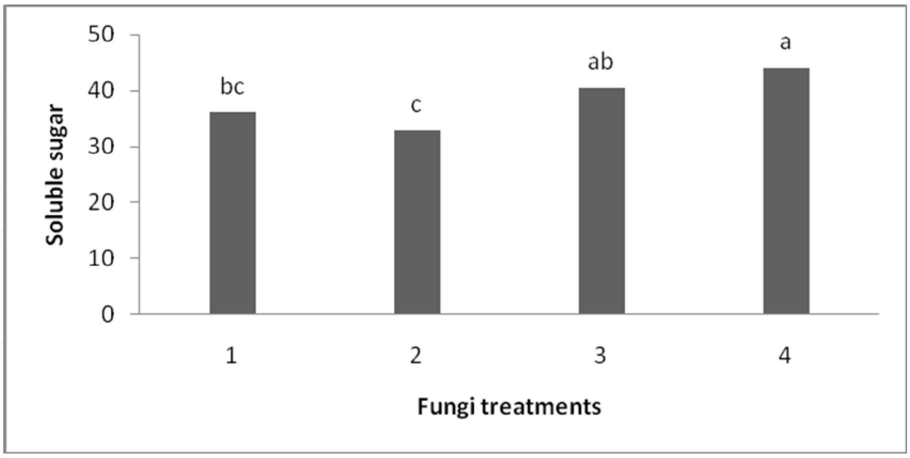

Figure1. Means comparison ofsoluble sugar amount under theinfluence of fungustreatments

It has been reported in a number of studies that the accumulation of soluble sugars is related with plants resistance against drought stress (Hokstra and Booting, 2001). Actingas an osmotic protective, sucrose strengthens cell membranes and maintains the cell Turgescence (Kripsyand Galyba, 1998). Starchis acomplexsugarthatis catalyzedby amylase enzyme in dry conditions and causes an increase the concentration of soluble sugars. Increase in the concentration of soluble carbohydrates under stress conditions is for osmoregulation and continuation of water absorption (Ghaderiet al., 2006).Using water relationsandbetternutritionthannon-Mycorrhizalplants,Mycorrhizalplantsare able to escape temporarily from the drought conditions and experienceless damage;and therefore the amount ofsoluble sugarsshows lower increase comparedto non- Mycorrhizal plants(Ruiz-Lozano, 2009).The results related to the effects ofArbuscularMycorrhiza on soluble carbohydrates in this experiment are in accordance with the results reported by other researchers(Huixing, 2005; WU et al, 2007).

Glycinebetaine

Glycine betaine level in leaves of grapes with inoculated and non-inoculated roots was different. The highest amount of glycine betaine observed in control treatment while the lowest amountwas seen in plants inoculated with threeother fungi species. Glycine betaine amount in treatment containing *Glomusfasiculatum* fungus has significantly decreased compared to control treatment. Difference between the means of glycine betaine in the three types offunguswas not significant (Figure 2).

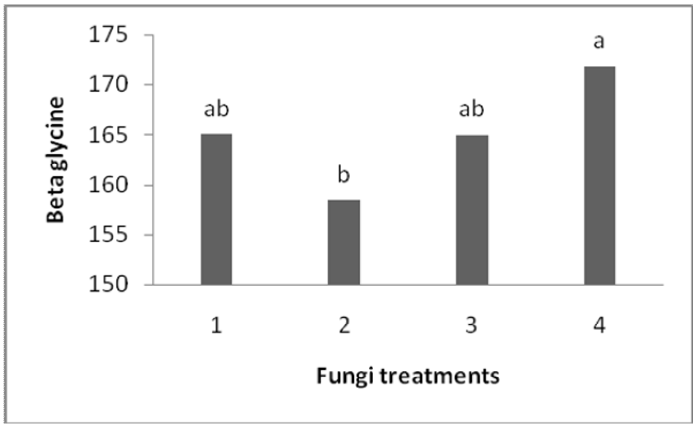

Figure2-Means comparison ofbeta glycineamount under the influence of fungi treatments

It has been shown in some studies that improving photosynthesis by glycine betaine in stressed plants is related to increase inphotochemical efficiency of photosystem II (Sakamoto and Murata, 1998). It was reported in a study on comparison of glycine betaine accumulation in the leaf of three trees of pear, grape and jujube under drought stress that the amount of glycine betaine under high stress increased just in pear but the two other plants did not show any increases(Chow et al., 2004). These results are consistent with our findings about the effects of stress on increasing the amount of glycine betaine in white seedless grapes. The results of this research are consistent with studies conducted by Tale Ahmad et al. (2010) and Naidu et al. (2003)that studied the accumulation level of glycine betaine under drought stress in wheat and cotton plants (*Gossypiumbarbadense L.*).

Proline

Gradual increase in proline under drought stress in non-Mycorrhizal treatments with average stresses is doubled, which shows that the effects of Mycorrhizause on moderating ofdehydration stresswas significant. These results showed that the production of these osmotic regulators is a common response to drought stress conditions.

Prolineamount in treatments of *Glomusintraradices* and *Glomusfasiculatum* fungi showed significant decrease compared to *Glomusmosseae* fungus and control treatments (Figure 3).

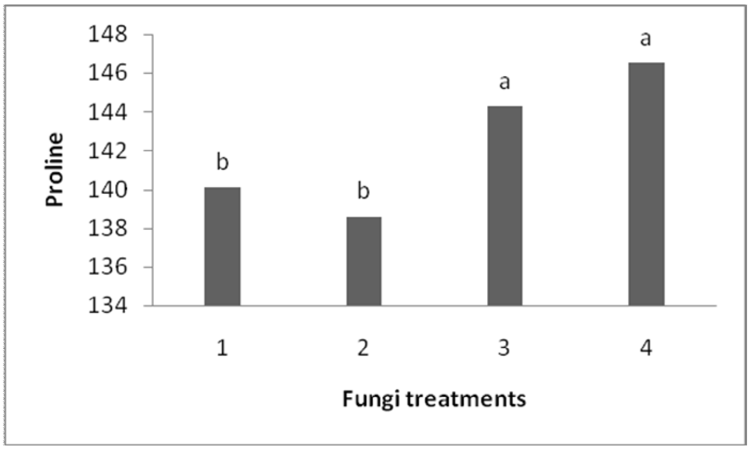

Figure3. Means comparison of proline amount under different treatments of fungus

Increase of proline concentration in plants that are under stress is an adaptation plants use to overcome the stress conditions (Bayer, 2007; Manivannan et al., 2007). The means of leaf proline amounts in the treatment combinationsindicate the different response of plants in symbiosis with fungi species,so that a significant increase was observed for fungi

Glomusmosseae, *G. intraradices*, *G. fasiculatum*, *G. claroideum*, and *Acaulosporalongula* in irrigation treatment of 50% of field capacity in hyssop plant;Our resultsare consistent with the research reported by Soleimani(2014) on hyssop plant.

Chlorophyll

In general, improving the nutritional and environmental conditions increasesthe ability of plant for production of chlorophyll in the leaves and thus production ofhigher energy. Increase in the amount of chlorophyll as the result of inoculation with Mycorrhiza can be due to the absorption of phosphorus from the soil by plant (Smith and Reed, 2008).The amount of chlorophyll index in *Glomusmosseae* fungus showed significant increase in comparison to the control treatment, but different types of fungi did not show significant difference from each other (Figure 4).

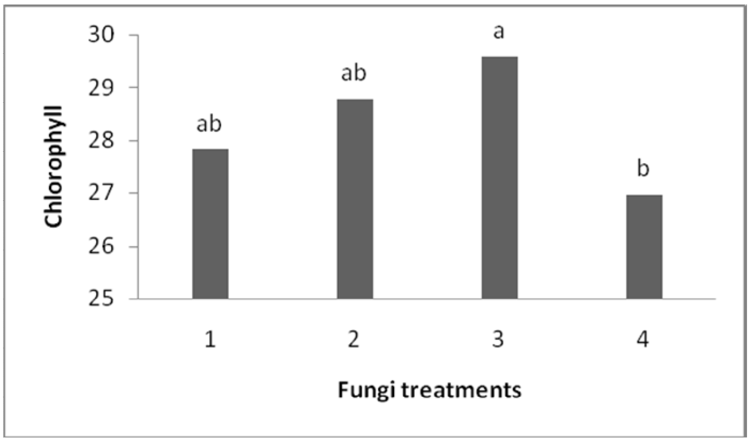

Figure4. Means comparison of chlorophyll amount underdifferent treatments of fungus

Wu and Ziya (2006) reported that the chlorophyll content in Tanjrin seedlings treated with fungi under irrigation was 23% higher thanthe seedlings without fungus treatment.

In a study on Pinot Blank grape grafting on threesensitive,semi-sensitive and resistant rootstocks to lime soil and inoculating the roots of seedlings with Mycorrhizal fungi,it was reported that the amount of iron and chlorophyll concentration of leafincreased in plants treated with Mycorrhiza (Bavarsko and Foqher, 1996). The results of this study are in agreement with our study.

B) The main effects of different levels of moisturestress

The results of means comparison of protein compounds andchlorophyllin different levels of moisture stress are presented in Figures 5 to 8. Study of these figures showed the enormous effect of stress on these traits especially betaine glycine and proline. So that the maximum amounts of soluble sugars, betaine glycine and proline were found in 25% level of stress. In thislevelof irrigation, plant isseverelydehydratedsooneof the strategies of plant to combat dehydration in this situation is sugars and protein synthesis for increasing the cell pressure and water absorption(Dowlati et al, 2014).The amount of betaine glycine and proline at stress level of 25%has increased significantly compared to two other levels. The trend of changes in chlorophyll index did not follow the changes observed in the mentionedtraits, so that the lowest amount of chlorophyll was observedat stress level of 25% of field capacity.

Soluble sugar

The amount of soluble sugars did not show significant difference at25 and 50% levels of field capacity, while the 75% level showed significant difference from the two mentioned levels (Fig 5).

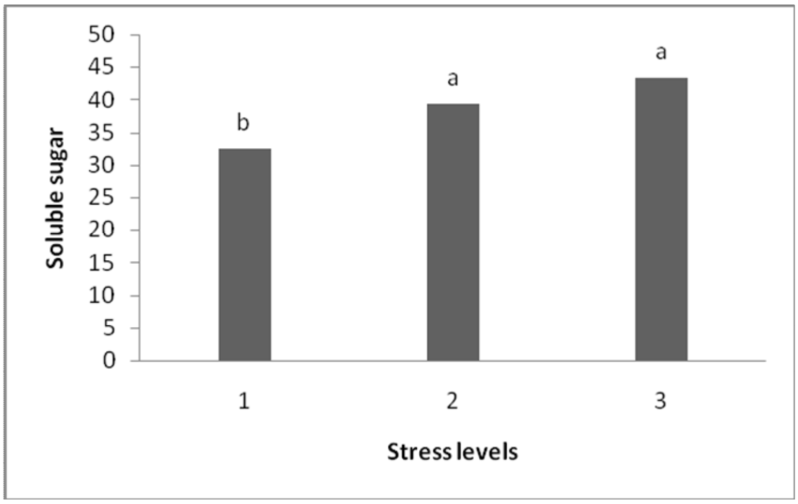

Figure5. Means comparison of soluble sugar under different levels of moisture stress

Reduction in the amount of soluble sugars in *Rasha*varietyat the beginning of thestress(55% water requirement) maybeindicate this factthat this variety uses other mechanismsto deal withmoderate droughtbecause sugars levels increasedas stress levels increasedbutdid not have a significant differencefrom theamount observed in control (Dowlati et al,2014).

In general, nowadays the existence ofdirect relationship between the accumulation of sugar and drought tolerance in all plants is in doubt, so that it hasbeen reported in crops such as sorghum (Niyoton et al., 1986) and maize (Thakur and Ray, 1980) and even in some grape varieties thatthe concentration of soluble carbohydrates decreased as a result of intensifiedwater stress.

Obtained observations in hyssop plant showed that increase in the levels of water stress resulted in the increase of soluble carbohydrates accumulation in leaf.

The highest concentration of soluble carbohydrates was achieved in the leaves of plants inoculatedwith *G. claroideum* (396.7 mg dry weight) at 60% of field capacity,while the lowest concentration was obtained in the leaves of plants inoculatedwith *G. fasiculatum* (166.3 mg g dry weight) at 80% of field capacity. The lowest amount of soluble carbohydrates accumulation was found in symbiosis with *G.mosseae* species (Soleimani et al., 2014).

In the study on the effect of drought on sugars of grape, Ghaderi et al (2006) reported that the level of soluble sugars reduced in *Rasha*variety but increased in the varietyof *Khoshnav*, which confirms our results about seedless white grape.

Glycine betaine

Imposing stress at 55% level of water requirement did not bring about a significant change in the amount of glycine betaine but increase of stress level up to25% of water requirementled to an increase in the amount of this compound. Concentrations of glycine betaine in leaf showed a gradual increase as the result ofincrease in water stress.

These results suggest that species of Mycorrhizalplayed a crucial role in the adjustment of water stress (Fig. 6).

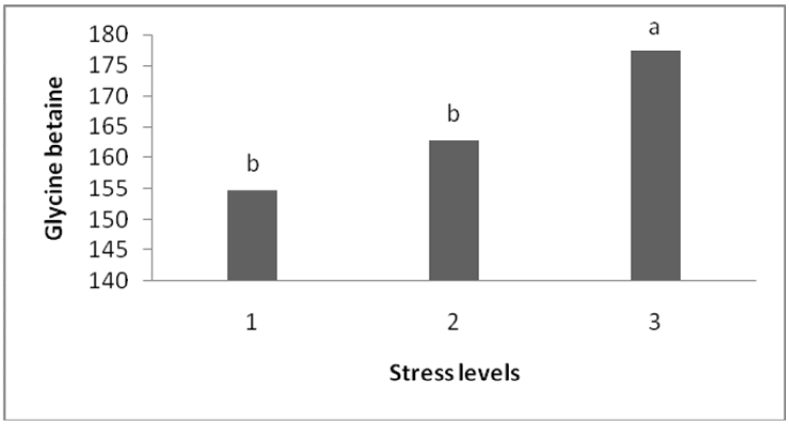

Figure 6. Means comparison of beta glycine under different levels of water stress

The highest concentration of this organic solution was due to the severe drought stress. There are reports about the existence of relationship between the accumulation of glycine betaineand tolerance of plants to osmotic stress such as drought and salinity (Lu et al., 2001, Joao et al., 2004). The concentration of glycine betainein the leaf of hyssop plant showed a gradual increase as water stress increased. The highest concentration of the organic solutionobtainedin the intense drought stress. Under drought stress conditions, the highestlevelof glycine betaineobtained in the leaf ofplants inoculatedwith *A. longula* and *G.intraradices*. Accumulation of this osmolyte in leaf may provide the possibility of continuing water absorption for cell through reducing the osmotic potential and cell water potential (Soleimani, 2014). Glycine betaineprotectsthe cells from stress by maintaining the osmotic balance with the environment and the stability of proteins, enzymes and membranes.

Proline

Prolineis an amino acid that increase in its concentrationis the most common response plant shows against stress (Suriyan and Chalermpol, 2009) (Figure 7).

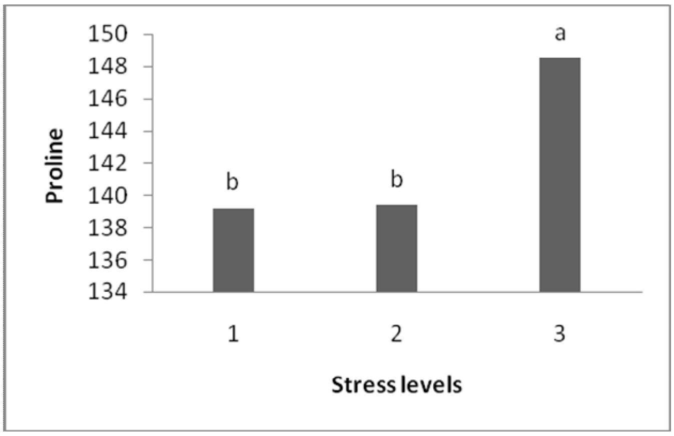

Figure 7.Means comparisonof proline under different levels of moisture stress

There are several reports in relation to the role of Mycorrhiza on proline and soluble sugars levels underdrought stress conditions. Some researchers believe that Mycorrhizaresults in theincrease ofproline and soluble sugars in the leaf of host plants since accumulating of these compounds in cells causesa decrease in leaf water potential and protects the plant from damage of drought stress (Subramanian et al, 1997; Wu et al, 2007; Khalafallah& Abo -Ghalia, 2008) Some researchers alsobelieve that Mycorrhizal under drought stress conditionsdecreases the amount of proline and soluble sugars in the leaf ofhost plants related tonon-Mycorrhizal plants.Studying the effect of stress on pepper plant (*Capsicum annuum L.*) showed that the amount of prolineincreased in plant (Koc et al., 2005). Furthermore, study on sunflower under drought stress showed that increase in the activity of Glotamil–gamma kinase caused an increase inproline level during stress(Manivannan et al., 2007). Proline content of marigold (Calendula officinalis L.) showed increaseby increasing drought stress up to relatively intense level of stress, but showed decrease after that level (Jafarzadeh et al. 2013). In response to drought stress, osmoregulation processes were activated in savory plants (*Saturejahortensis L.*) and proline level increased in leaves. Inoculation of savory plants with Mycorrhizal fungi increased significantly the indices ofvegetative growth of savory plant under drought stress conditions compared to non-inoculated plants, but reduced the amount ofproline in leaf. In general, the application of Mycorrhizafungus caused an increase in resistance of savory plant to drought stress (Ismailpour et al. 2013). In the present study,prolineconcentration increased under the influence of stress;our results in this relation areconsistent with the results obtained on sugarcane (*Saccharumofficinarum L.*) (Suriyan and Chalermpol, 2009).

Chlorophyll

The amount ofleaf chlorophyllaffected bywater stressso thatby increasing the intensity of drought,the amount of chlorophylldecreasedso that the lowest amount ofchlorophyllwas recorded insevere drought stress(25% of water requirement) (Figure8).

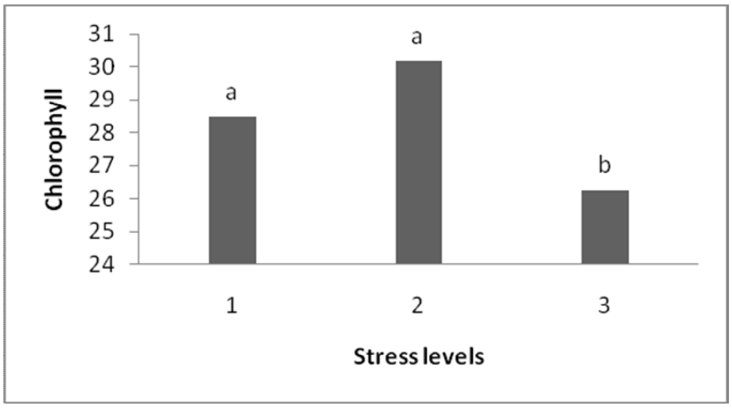

Figure 8. Means comparison of chlorophyll under different levels ofwater stress

Drying of leaf tissues not only preventsthe production of chlorophyll, but it alsocauses the degradationofavailable chlorophyll. Drought is responsible for breaking down of chloroplasts and reducing of chlorophyll level. Drought also decreases the formation ofnew Plastids, chlorophyll a and b,and changes theratio of chlorophyll a to b (Heidari Sharif Abad, 2000 and Hasani, 2003). Persistenceof photosynthesis and maintenance ofleaf chlorophyll under stress conditions are physiological indices of drought tolerance (SiyoseMarde and Ahmadi, 2004). Reduction of leaf chlorophyll due to the lack of soil moisture has been reported in grape varieties by Ghadri et al (2006) and Rezai (2007) which is in agreement with our results.

C) The interaction between stress levels and fungus treatments

The results of this comparisons show that in each stress level, the highest amount of proline synthesis observedin control treatment,indicating thepositive role of Mycorrhizal fungi in moderating the negative effectsand increasing water absorption under stress conditions. As can be seen, the highest rate of change is seen in stress level of 25% that shows thevital role fungi play in extreme drought conditions. The difference between the amounts of proline in control andfungi treatments is not statistically significant at the 75% stress level but the higher levels of stress increased the difference between control and fungi treatments (Figure 9).

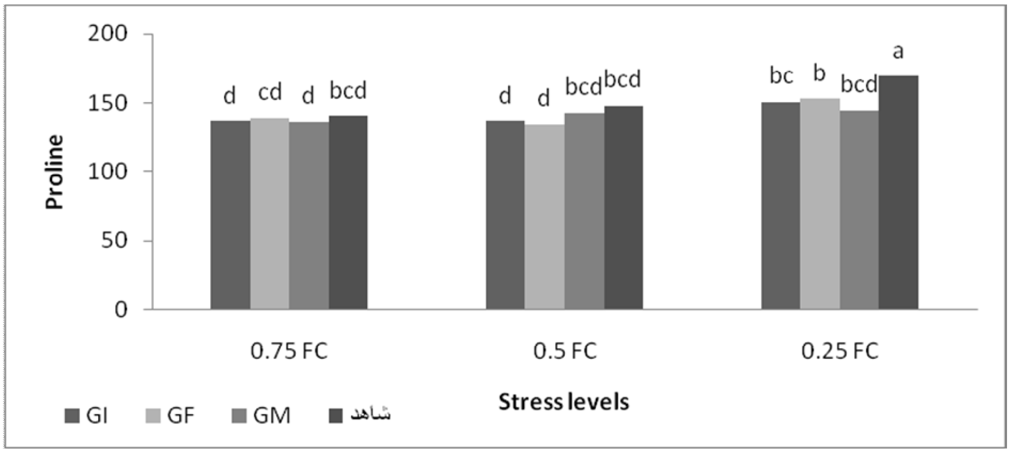

Figure 9. Means comparison of prolineunder different levels of interaction of fungi treatments and moisture stress levels

Unlike non-Mycorrhizalplants,Mycorrhizalplants are usually able to temporarily survive the drought stress usingwater relationsandbetter nutrition and encounterless damage; therefore their proline level and soluble sugars show less increase compared tonon-Mycorrhizal plants(Davies et.al, 1992; Ruiz-lozano & Azcon, 1996; Porcel & Schellenbaum et al, 1998;) Ruiz-Lozano(2004) reported that symbiotic relation with Mycorrhizal fungi significantly affected the accumulation of soluble carbohydrates in tobacco plant under drought conditions, so that lower amount of fructose and glucose accumulates in leaf of Mycorrhizal plantsthan non-Mycorrhizalones under drought stress. Ruiz-Lozano also stated that under conditions that photosynthesis is limited,Mycorrhiza act asa strong competitor competing with rootfor receiving carbohydrates, therefore the amount proline in root of Mycorrhizal plants increases.

By increasing proline in root,Mycorrhiza probably causes a decrease in water potential in the root cell and increases water absorption,thereforeplants are less affected bystress.

These results are consistent with the results reported byPorcel& and Ruiz-Lozano(2004).

CONCLUSION

Generally, the species of *G. fasciculatum,G. mosseae* and *G. intraradices* each could affect the specific traits and show their effects. Chlorophyll content, soluble sugars, betaine glycine and proline showed the highest amount at 75% of field capacity and water stress of 75% and 50% so that a gradual decrease was observedby increasing the intensity of stress, but a significant reduction was observed in the amount of mentioned traitsunder the severe drought stress, so that the lowest amount was obtained at 25% of field capacity. Unlike chlorophyll, traits ofproline, glycine betaineand soluble sugarsshowed the maximum amount in irrigation level of (25%). Among fungi treatments, *G.fasiculatum* showed greater effect on thetraits of soluble sugars and glycine betaine. In the interaction between fungi and irrigation in all levels of stress, the greatest amount of proline synthesis was observed in control,whichshowsthe positive role of Mycorrhizal fungi in moderating the negative effects and enhancing the absorption of water under stress conditions.

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