

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 of roots of grape var *Sefidbidaneh* with three species of Mycorrhizal fungi namely *Glomus fasciculatum*, *Glomus intraradices* and *Glomus mosseae* on the amount of chlorophyll, soluble sugars, betaine glycine and proline under water stress, the present study was conducted as a factorial experiment based on randomized complete block design in four replications. Compared to control treatment, inoculating with Mycorrhizal fungi could have significant effects on aforementioned traits so that the amount of betaine glycine, proline and soluble sugars increased but chlorophyll decreased in control. The highest amounts of soluble sugars, betaine glycine and proline were observed at the irrigation level of 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, indicating the positive role of Mycorrhizal fungi in moderating the negative effects of water stress and in increasing of 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, Rish Baba, Peikani, Fakhri, Siah Sardasht, Sahebi and several other cultivars are important vine varieties cultivated in Iran. All edible grapes of Iran belong to *Vinifera* species (Dowlati et al., 2005). Grapes is one of the main horticultural crops of Iran so that it has the first position among fruit trees with respect to the area

of cultivation and economically is placed after pistachio and date (Nazemiyeh 1999). Causing a huge damage annually, drought is one of the most important factors limiting the crops production in the world. Environmental conditions which prevent the access of plant to adequate water to perform its vital function and repetition of this process which leads

to the dehydration of plant tissues is called drought (Levitt, 1980). When plant cells lose their swelling state and speed of transpiration is greater than the absorption, plant is dehydrated (Kozłowski and Palardy, 1997). If water stress is high, it causes a sharp decline in photosynthesis, disruption of physiological 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 other effective factors on 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 characteristics of 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, absorption of photosynthetic carbon dioxide, transpiration and proline accumulation (Sadrzadeh et al., 2007; Zarrabi et al., 2009). Moreover, it results in losing of water and plant cells and shortage of food as well as reducing of carbon dioxide absorption due to stomata closure, and finally causes the starvation of plant (Kramer and Boyer, 1995).

Mycorrhizal word is formed from two words of (Myco) means fungus and (Rhiza) means root, which represents the participation 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 Mycorrhizal system (Mostajeran and Zoe, 2006). In plants with good symbiotic relationship, Mycorrhizal fungus is an important factor to increase the resistance and growth of plant especially in adverse environmental conditions such as drought stress and food

shortage. Mycorrhizal fungi help plants to absorb fluid and non-fluid resources by forming individual and developed network. Network hyphae generally increases the receiving of fluid food resources in some plants and affects the growth of plants in different ways, and, by contrast, receives its required materials by sending its suckers into the host cell, particularly in the area under the epidermis (the cortex), (Ameriyan, 1992).

One of the most important physiological roles of proline accumulation in response to water shortage is the role it plays as a regulator of osmotic pressure and protective agent of cytoplasmic enzymes and membrane structure. In a study about the effect of drought stress on proline level, soluble and insoluble sugars of some grape varieties, drought stress led to the increase in the concentration of proline and soluble sugars, and reduction of insoluble sugars in leaf (Rabie et al., 2009; Azizi et al., 2009). Increase of proline in stress period may be the result of proteins breakdown as well as reduction of their use due to the reduction in plant growth. Under stress conditions, proline accumulation occurs in all organs of the plant, but the rate of accumulation in leaves is quicker than other organs. As the stored 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 resistance of plants to drought (Hokstira and Booting, 2001). Sugars retain the cells during drought by two mechanisms: (1) water is replaced with hydroxyl groups of sugars in other to maintain 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).

Sugars are a major factor in vitrification (being glassy), a process that forms biological glasses in 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 in the enhancement of proline and 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 compounds was observed in *Film Seedless* (Rezai, 2007). Adjustment of osmotic potential through Arbuscular Mycorrhizae is probably one of the most important reasons for improving the ability of host plant to grow under water stress. Plants reduce the cell osmotic potential by collecting higher amounts of organic products, such as proline and glycine betaine, and carbohydrates such as sucrose, mannitol and inorganic ions; leading to the retention of higher 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 CO₂ concentration is reduced in mesophyll tissue. Following the reduction in CO₂ concentration, dark reactions of photosynthesis are disrupted and obtained products of light reactions, which include ATP and NADPH, are not consumed. Of the important factors reducing yield 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 evident due to calcareous soil of the vineyards. *White seedless* grape is one of the commercial varieties spread in many parts of Iran due to its great growth in clay soil. On this basis, 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 of grape 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 fungus on the improvement of growth, 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 of Nakhjavani Urmia in years 2013 and 2014 as factorial experiment based on randomized complete block design with four replications. The first factor included inoculation with three Mycorrhizal fungi (*Glomus mosseae*, *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 in necessary number and then placed in sand medium for rooting. In the same medium, inoculation of half seedlings with suspension of Arbuscular Mycorrhizal (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 producing Mycorrhizal seedlings of grape, its hardwood cuttings were put in rooting medium that was mixed with Mycorrhizal fungi Inoculum with the ratio of 15 ppm. To ensure root colonization, sampling from root was performed weekly. After staining the root with Trypan 05% and being ensure about colonization in the last stage, the percentage of roots 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 each pot was prepared for determining the moisture content and physicochemical characteristics. During the growing season, complete cares were taken from grapes plants. 20-liter plastic pots were used for

cultivation of seedling. Two-bud pruning was done. Two months after full establishment of seedlings in pot and development of the green corona, irrigation treatments were applied with daily weighing of pots and adding consumed water due to evaporation and transpiration. In fact, decrease in weight of each of the pots showed the amount of consumed 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 glycine betaine 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).

Measuring the amount of leaf 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% Solfosalicylic 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 phases of 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 water was 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 level that proline enters 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 determined with the help of spectrophotometer Varian, model CARY-100 at 515 nm. Proline level of leaf samples was calculated in micromoles per gram of leaf fresh weight from the following equation:

Leaf proline	extract proline (µg×ml)× extract content(ml)	5
= _____ × _____		
µmol × gW.s	115.5 (µg×µmol)	sample weight (g)

In the above equation, the number 115.5 is proline molecular weight. To determine the concentration of samples proline, standard solutions (proline) with the concentrations of 100, 200 and 300 micrograms per ml were used. Ninhydrin reagent was prepared by solving the amount of 1.25 g ninhydrin in 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 performed according 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 shaken strongly 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

ml Anthrone reagent was mixed (the reaction of Anthrone with 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 of 625 nm and the amount of soluble sugars was calculated in mg per gram of fresh weight from the following equation:

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

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 were applied on them.

Measuring glycine betaine

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 Kolmogor-Smirno (K-S) software. The variables that were not normally distributed were normalized using SPSS conversions in Kolmogor-Smirno (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 completely randomized 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 of treatments on traits such as chlorophyll index and betaglycine and on the soluble sugars and proline were significant at 5 and 1% 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 significant on the amount of proline 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	Chlorophyllin Group 8	Chlorophyllin Group 9	Soluble sugar	Glycine betaine	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 significant at 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).

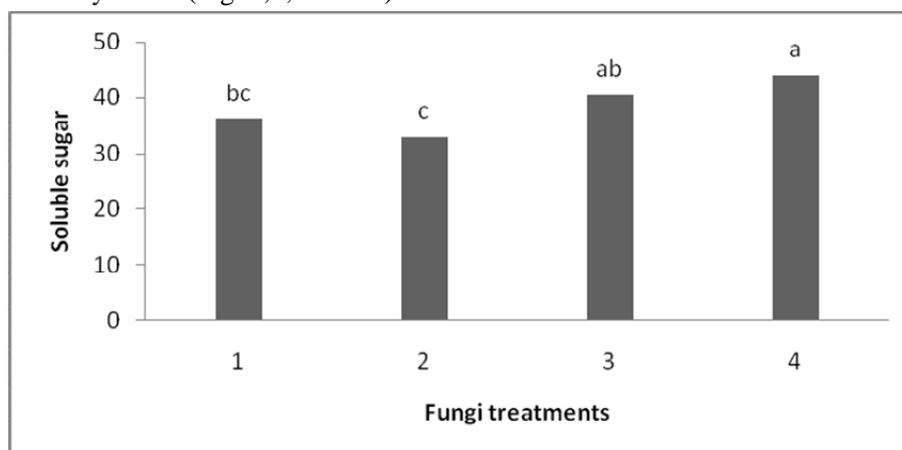


Figure1. Means comparison of soluble sugar amount under the influence of fungustreatments

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). Acting as an osmotic protective, sucrose strengthens cell membranes and maintains the cell Turgescence (Kripsyand Galyba, 1998). Starch is a complex sugar that is catalyzed by amylase enzyme in dry conditions and causes an increase in 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 relations and better nutrition than non-Mycorrhizal plants, Mycorrhizal plants are able to escape temporarily from the drought conditions and experience less damage; and therefore the amount of soluble sugars shows lower increase

Soluble sugar

The lowest amount of protein synthesis and chlorophyll observed in treatment inoculated with *Glomus fasciculatum* fungus. There were significant differences between the amounts of soluble sugars in control and *Glomus intraradices* and *Glomus fasciculatum* fungi, and there was not a significant difference between the control and *Glomus mosseae* fungus (Figure 1).

compared to non-Mycorrhizal plants (Ruiz-Lozano, 2009). The results related to the effects of Arbuscular Mycorrhiza on soluble carbohydrates in this experiment are in accordance with the results reported by other researchers (Huixing, 2005; WU et al, 2007).

Glycine betaine

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 amount was seen in plants inoculated with three other fungi species. Glycine betaine amount in treatment containing *Glomus fasciculatum* fungus has significantly decreased compared to control treatment. Difference between the means of glycine betaine in the three types of fungus was not significant (Figure 2).

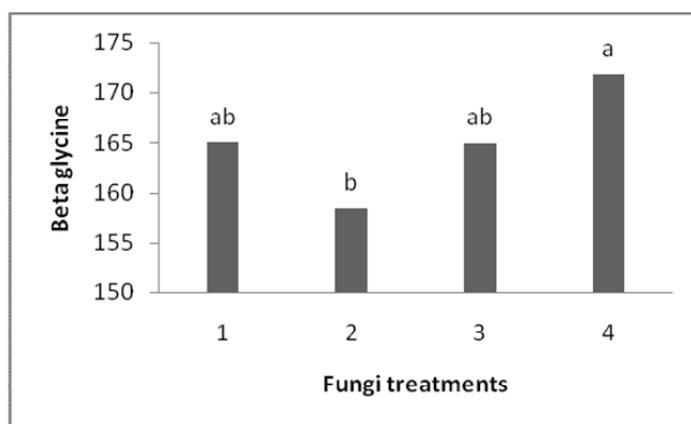


Figure2-Means comparison of beta glycine amount under the influence of fungi treatments

It has been shown in some studies that improving photosynthesis by glycine betaine in stressed plants is related to increase in photochemical 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 (*Gossypium barbadense L.*).

Proline

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

Proline amount in treatments of

Glomus intraradices and *Glomus fasciculatum* fungi showed significant decrease compared to *Glomus mosseae* fungus and control treatments (Figure 3).

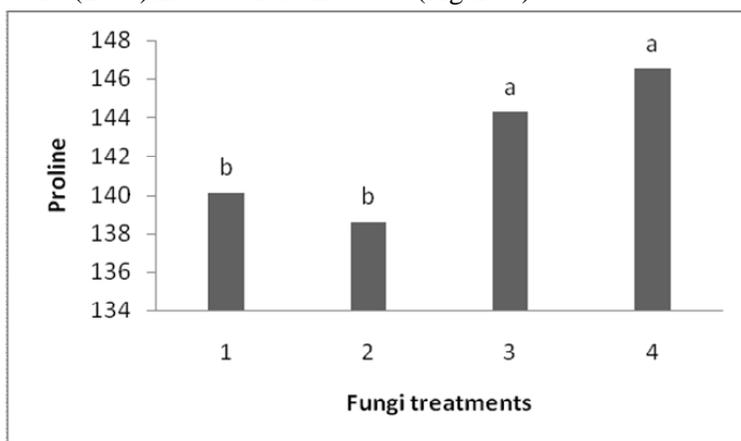


Figure3. Means comparison of proline amount under different treatments of fungus

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 combinations indicate the different response of plants in symbiosis with fungi species, so that a significant increase was observed for fungi

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

Chlorophyll

In general, improving the nutritional and environmental conditions increases the ability of plant for production of chlorophyll in the leaves

and thus production of higher 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).

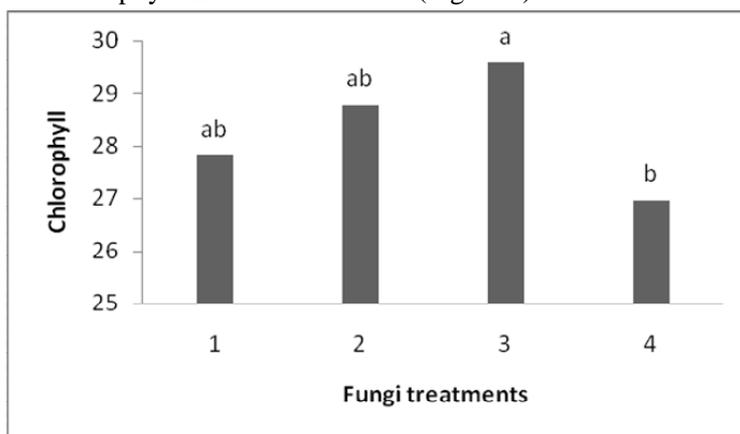


Figure 4. Means comparison of chlorophyll amount under different treatments of fungus

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

In a study on Pinot Blank grape grafting on three sensitive, 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 leaf increased 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 moisture stress

The results of means comparison of protein compounds and chlorophyll in 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 glycine

and proline. So that the maximum amounts of soluble sugars, betaine glycine and proline were found in 25% level of stress. In this level of irrigation, plant is severely dehydrated so one of 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 mentioned traits, so that the lowest amount of chlorophyll was observed at stress level of 25% of field capacity.

Soluble sugar

The amount of soluble sugars did not show significant difference at 25 and 50% levels of field capacity, while the 75% level showed significant difference from the two mentioned levels (Fig 5).

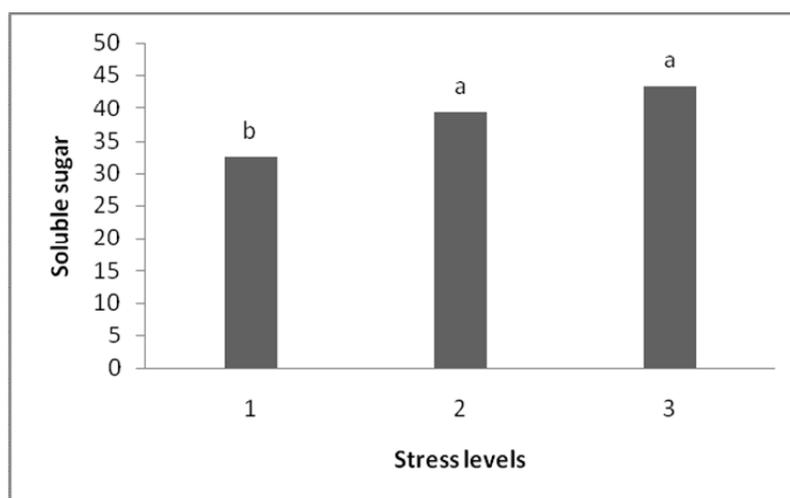


Figure 5. Means comparison of soluble sugar under different levels of moisture stress

Reduction in the amount of soluble sugars in *Rashavariety* at the beginning of the stress (55% water requirement) may indicate this fact that this variety uses other mechanisms to deal with moderate drought because sugars levels increased as stress levels increased but did not have a significant difference from the amount observed in control (Dowlati et al., 2014).

In general, nowadays the existence of direct relationship between the accumulation of sugar and drought tolerance in all plants is in doubt, so that it has been reported in crops such as sorghum (Niyoton et al., 1986) and maize (Thakur and Ray, 1980) and even in some grape varieties that the concentration of soluble carbohydrates decreased as a result of intensified water 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 inoculated with *G. claroideum* (396.7 mg dry weight) at 60% of field capacity, while the lowest

concentration was obtained in the leaves of plants inoculated with *G. fasciculatum* (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 *Rashavariety* but increased in the variety of *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 to 25% of water requirement led to an increase in the amount of this compound. Concentrations of glycine betaine in leaf showed a gradual increase as the result of increase in water stress.

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

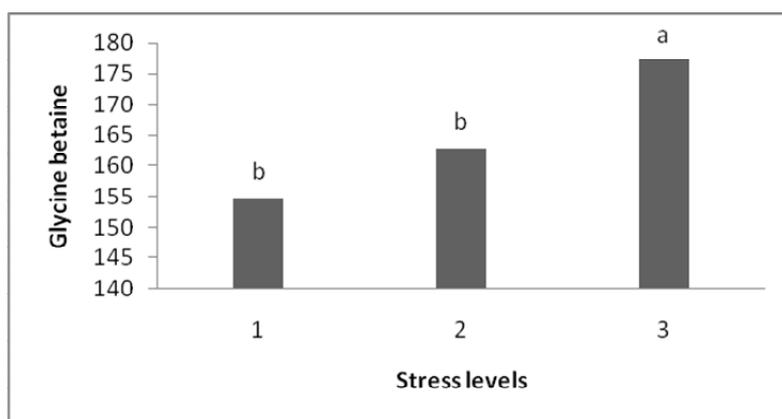


Figure 6. Means comparison of beta glycine under different levels of water stress

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 betaine and tolerance of plants to osmotic stress such as drought and salinity (Lu et al., 2001, Joao et al., 2004). The concentration of glycine betaine in the leaf of hyssop plant showed a gradual increase as water stress increased. The highest concentration of the organic solution obtained in the intense drought stress. Under drought stress conditions, the highest level of glycine betaine obtained in the leaf of plants inoculated with *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 betaine protects the cells from stress by maintaining the osmotic balance with the environment and the stability of proteins, enzymes and membranes.

Proline

Proline is an amino acid that increases in its concentration is the most common response plant shows against stress (Suriyan and Chalermopol, 2009) (Figure 7).

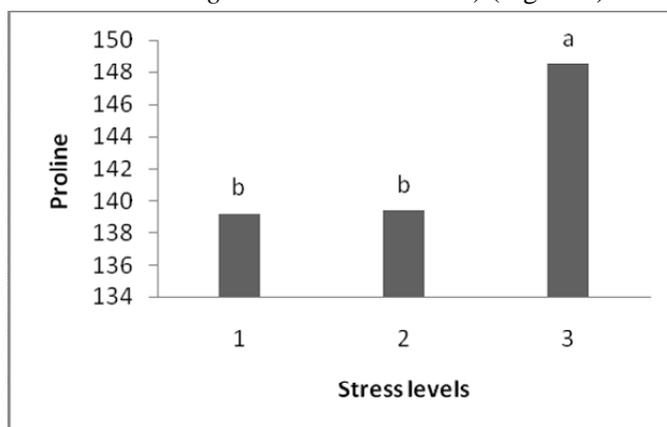


Figure 7. Means comparison of proline under different levels of moisture stress

There are several reports in relation to the role of Mycorrhiza on proline and soluble sugars levels under drought stress conditions. Some researchers believe that Mycorrhiza results in the increase of proline and soluble sugars in the leaf of host plants since accumulating of these compounds in cells causes a 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 also believe that Mycorrhizal under drought stress conditions decreases the amount of proline and soluble sugars in the leaf of host plants related to non-Mycorrhizal plants. Studying the

effect of stress on pepper plant (*Capsicum annuum L.*) showed that the amount of proline increased in plant (Koc et al., 2005). Furthermore, study on sunflower under drought stress showed that increase in the activity of Glutamil-gamma kinase caused an increase in proline level during stress (Manivannan et al., 2007). Proline content of marigold (*Calendula officinalis L.*) showed increase by 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 of vegetative growth of savory plant under drought stress conditions

compared to non-inoculated plants, but reduced the amount of proline in leaf. In general, the application of Mycorrhizal fungus caused an increase in resistance of savory plant to drought stress (Ismailpour et al. 2013). In the present study, proline concentration increased under the influence of stress; our results in this relation are consistent with the results obtained on sugarcane (*Saccharum officinarum L.*) (Suriyan and Chalermopol, 2009).

Chlorophyll

The amount of leaf chlorophyll affected by water stress so that by increasing the intensity of drought, the amount of chlorophyll decreased so that the lowest amount of chlorophyll was recorded in severe drought stress (25% of water requirement) (Figure 8).

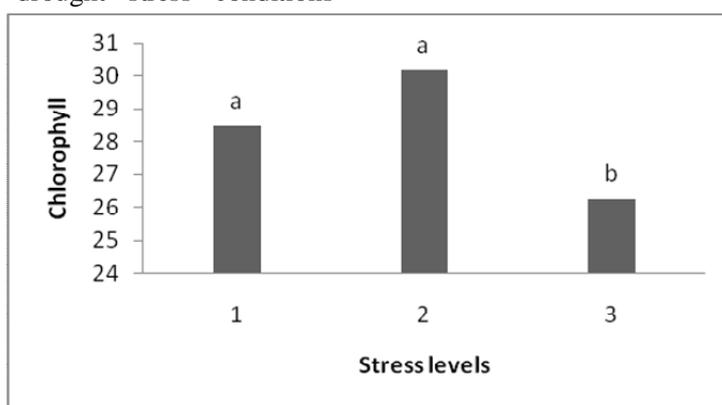


Figure 8. Means comparison of chlorophyll under different levels of water stress

Drying of leaf tissues not only prevents the production of chlorophyll, but it also causes the degradation of available chlorophyll. Drought is responsible for breaking down of chloroplasts and reducing of chlorophyll level. Drought also decreases the formation of new Plastids, chlorophyll a and b, and changes the ratio of chlorophyll a to b (Heidari Sharif Abad, 2000 and Hasani, 2003). Persistence of photosynthesis and maintenance of leaf chlorophyll under stress conditions are physiological indices of drought tolerance (Siyose Marde 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 comparison show that in each stress level, the highest amount of proline synthesis observed in control treatment, indicating the positive role of Mycorrhizal fungi in moderating the negative effects and increasing water absorption under stress conditions. As can be seen, the highest rate of change is seen in stress level of 25% that shows the vital role fungi play in

extreme drought conditions. The difference between the amounts of proline in control and fungi 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).

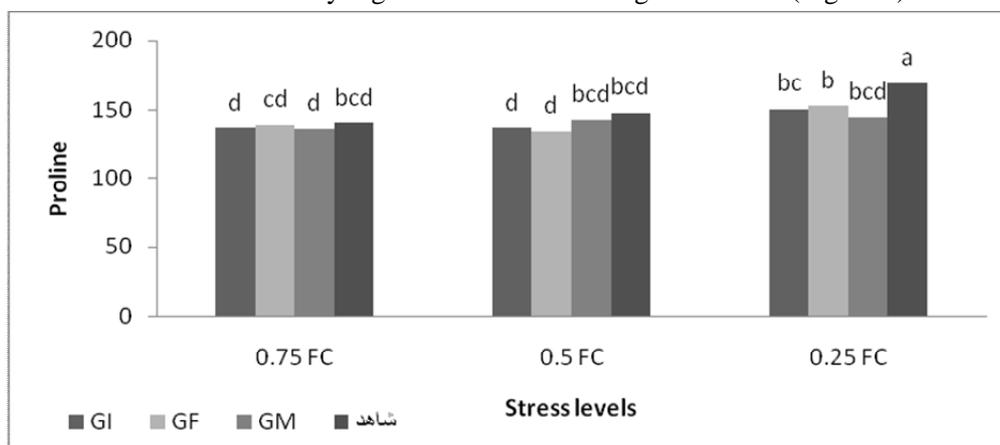


Figure 9. Means comparison of proline under different levels of interaction of fungi treatments and moisture stress levels

Unlike non-Mycorrhizal plants, Mycorrhizal plants are usually able to temporarily survive the drought stress using water relations and better nutrition and encounter less damage; therefore their proline level and soluble sugars show less increase compared to non-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 plants than non-Mycorrhizal ones under drought stress. Ruiz-Lozano also stated that under conditions that photosynthesis is limited, Mycorrhiza act as a strong competitor competing with root for receiving carbohydrates, therefore the amount of 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, therefore plants are less affected by stress.

These results are consistent with the results reported by Porcel & 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 observed by increasing the intensity of stress, but a significant reduction was observed in the amount of mentioned traits under the severe drought stress, so that the lowest amount was obtained at 25% of field capacity. Unlike chlorophyll, traits of proline, glycine betaine and soluble sugars showed the maximum amount in irrigation level of (25%). Among fungi treatments, *G. fasciculatum* showed greater effect on the traits 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, which shows the positive role of Mycorrhizal fungi in moderating the negative effects and enhancing the absorption of water under stress conditions.

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