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EFFECT OF WATER STRESS ON GROWTH TRAITS OF ROOTS AND SHOOTS (FRESH AND DRY WEIGHTS, AND AMOUNT OF WATER) OF THE WHITE SEEDLESS GRAPE

Mohammad Aslanpour (Mohammad Omar Aziz) [a*], Hamed Doulati Baneh [b], Ali Tehranifar [c], Mahmoud Shoor [c]

[a] *Department of Horticulture, University of Raparin Rania, Sulaimany, IRAQ*
[b] *Horticulture Crops Research Department, West Azerbaijan Agriculture and Natural Resources Research and Education Center, AREEO, Uremia, IRAN*
[c] *Horticultural Sciences and Landscape Department, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IRAN*

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ABSTRACT

To determine effects of infected roots of seedless white currant grape cultivar with three species of Mycorrhizal fungi (*Glomus fasiculatum, Glomus intraradices*, and *Glomus mosseae*) on growth traits (branch length, number of leaves, leaf area, fresh and dry weight of branch, root and leaf) under the water stress conditions, this factorial experiment was implemented in the randomized complete block design with four treatments. The obtained results showed that the increase in drought stress led to a reduction in the factors including branch growth, number of leaves, leaf area, dry weight of root and branch. Inoculation with mycorrhizal fungi had a positive effect on the above-mentioned traits compared with control group; in this case, among fungal treatment traits, the *Glomus fasiculatum* had the highest positive effect on the dry weight of roots. All three funguses had an effect on the fresh weight of leaf. There was not any difference between irrigation levels of 25% and 50% under the water stress.

1. INTRODUCTION

Being the Iran main horticultural product with greatest cultivation area, grape is economically ranked after pistachio and date palm [1]. Water scarcity, an important factor, limits the function of fruit trees in arid and semi-arid areas. Functional assessment of fruit trees under stress conditions and application of beneficial soil microorganisms as biological fertilizers to reduce damages caused by environmental stresses are novel solutions in sustainable agriculture in arid and semi-arid regions to reduce pollutions and environmental degradation [2], [3]. The term of Mycorrhiza indicates the symbiotic association between fungi and plant roots, which is the most common symbiosis. Mycorrhiza is formed by the fungal penetration into the intercellular spaces of rootstock in which, a

*Corresponding author (Mohammad Aslanpour) E-mail: Aslanpour.mohammad@gmail.com

network of fungus hyphae form a colony over the root. The fungal coating can expand itself in the soil and facilitate the water uptake [4]. According to estimations, about 70% of microbial biomass of soils contains hyphae of the mentioned funguses. However, the use of VAM in agriculture is highly important. In general, mycorrhiza affects only on those nutrients, which have very low mobility in soil and low concentration of them exists in soil solution for plant [5, 6]. Under drought stress conditions, mycorrhizal funguses can reduce stress effects and improve growth and function of host plants in sustainable agriculture system by increasing absorption of nutrients (phosphorous, nitrogen and some micronutrients) and increasing water uptake [7, 8, 9].

2. RESEARCH NECESSITIES

Mycorrhiza funguses, in particular, Arbuscular Mycorrhizal funguses play a vital role in proving water and nutritional needs of plants and mycorrhizal symbiotic plants can endure higher concentration of heavy metals, salinity, and soil dryness and resist against different pathogens and high soil heat. In addition, another practical and significant aspect of Arbuscular Mycorrhizal funguses is their function as a biological fertilizer in agricultural lands so that they can be used instead of chemical fertilizers in order to reduce the danger of chemical fertilizers in soil texture. Hence, sustainable agriculture can be developed by spending lower cost while protecting the ecological balance of the ecosystem.

3. THEORETICAL LITERATURE

3.1 BOTANICAL CHARACTERISTICS AND CLASSIFICATION OF GRAPEVINE

Grapevine is from the Ampelidaceae family called Saramantaceae or Vitaceae. This family belongs to the Rhamnales species, which is Dialypetalae belonged to the angiosperms from the Spermatophytes. Such species include shrubs with Knitted shoots and grows upward due to their ivies. These ivies are located in front of claw-shaped leaves using them to stick to the tree or wall moving upward. Ampelography is the field of identifying genre, species, and variety of plants, which belongs to grapevines [10].

As drought is the significant geographical characteristic of Iran, there is not any way out of this natural phenomenon, and as there is increasing consumption of energy resources, water, and nutrients, some practices such as correct exploitation of water should be done through correct farming methods like planting resistant species, recognizing the relation between water deficit, soil and growth of products at each step, assessing morphological, physiological and metabolic reactions, identifying the beneficial associations in plants in exposure to the stress, transferring resistant traits to abundant but sensitive cultivars into the land and some other cases, which develop plantation in arid regions [11].

3.2 WHITE SEEDLESS GRAPE

The white seedless cultivar has larger cultivation area compared with the red seedless grape. The white cultivar is grown rapidly and its branch length reaches to 2.5m with bright brown color. End buds of this cultivar are closed, hairy and white. Leaves are not hairy and have a dark green color with bright yellow nervure. This cultivar has thin leaves used for food (Dolma), green nodes in purple color at lower parts.

3.3 DROUGHT STRESS

Drought is defined as environmental conditions in which, soil or air prevent from enough water uptake by the plant, which leads to loss of critical function and water in plant's tissue [12]. Drought is a factor, which limits the production of agricultural products in the world leading to considerable damage to such produces. Average rainfall in Iran is lower than one-third of the world [13]. Drought stress affects the morphological traits of the plant such as leaf area, branch growth and root expansion, plant pigments, fresh and dry weight of leaf and root, physiological traits such as leaf's water potential, stomatal resistance, relative water rate of leaf, photosynthesis activity, photosynthetic adsorption of CO_2, evaporation and Proline accumulation [14-15]. In addition, drought stress leads to a water loss of plant tissue and cells, food deficit and reduction in CO2 adsorption due to closed stomata and plant starvation [16]. In some studies, drought stress led to a reduction in fresh and dry weight of leaf and roots, leaf area, leaf water potential and relative water amount of olive and grape leaf [14-15]. The plant growth is influenced by the mutual effects of several internal processes such as photosynthesis, respiration, transmission, water relations, and nutrients balance. Growth is a process of increase in the dry matter, volume, length or level of the cell. Water stress effect on cellular development is more obvious than the cell division because growing cell occurs due to turgor pressure. Hence, any water deficit leads to a growth pause [17].

Severe water deficit at grape growth step wilts the leaves and diminishes moisture in shoots. This wilting process can be seen in grapes, which are cultivated in the pot with the soil, which is wilting. Such condition is observable in hot weather and sand low-deep soils, which all parts of them reach to the wilting point under the farm conditions. This situation is rarely seen in deep soils, as the soil around the growth area does not wilt within a short time [18].

Drought stress in grapevine dries the petioles, ivy and young leaves on the shoots. Moreover, long drought stress may create necrotic spots on the margin of grape leaves; these spots can be seen in lower leaves on the branch. Leaf color also indicates the effect of drought stress so that young leaves on the shoots are green to yellow and mature leaves show gray-green color. Drought stress leads to early aging in lower leaves. Severe drought stress reduces the number of branches and leaves as well as evaporation rate [19].

3.4 MYCORRHIZAL FUNGI

The term "mycorrhizal", introduced by Frank in 1885; is composed of two words "Myco", which means fungus and "Rhiza", which means roots indicating symbiosis between the fungus and plant roots. In this system, the fungus forms the broad coverage of the filamentous called hyphae around the host plant's root. Many plants can form mycorrhizal system; 83% of Dicotyledon and 79% of Monocotyledon plants can develop a mycorrhizal system [5].

3.5 MYCORRHIZAL FUNGI IN GRAPE

Nowadays, Vinifera grape species is cultivated in regions with enough rainfall within the rainfed form and due to its drought and limestone soil resistance [20]. However, severe drought stresses in some years reduce the function rate at sensitive phonological steps such as fruit formation time. On the other hand, plantation of one-year-old seedlings in these arid regions makes problem in initial years owing to water deficit and improper soil. In addition to the use of resistant and premature cultivars, resistant bases and water management (rainwater harvesting, limited irrigation, and regional irrigation or PRDI), rootstock of grapevine is infected with mycorrhiza fungus (Arbuscular Mycorrhizal fungi (AMF)) in order to develop gardens in arid and semi-arid regions [21].

Mycorrhiza is a fungus, which exists in all type of soils and makes a connection with plants' roots. Nutrients uptake is increased in the symbiosis between this fungus and grapevine roots so that the grapevine send photosynthetic materials to the fungus. Such a connection between grapevine and fungus is called symbiosis in which, both grapevine and fungus will be symbionts. Nutrients will be transferred through tree branch-like structure into the root or Arbuscular cells, which expand the connection area between the host and fungus; hence, they are called Arbuscular Mycorrhizal Fungi (AMF). The thinnest grapevine root is 500-1000 times larger than mycorrhiza hyphae. Various studies implemented in pots and farms have shown that grape seedlings with mycorrhiza grow better under drought and improper soil conditions so that they can absorb water and other elements such as phosphorous and iron in comparison with control subjects. In addition, such samples are highly resistant against soil pathogens. It has been also proved that mycorrhizas protect and stabilize the soil texture due to connected soil grains by hyphae. [21] showed that the infection rate of the roots with the fungus, growth, and nutrient uptake rate through inoculation of grape roots with mycorrhiza might vary depending on the type of fungus and cultivar. Use of AMF leads to increase in height, dry weight of branch, dry weight of root and dry weight of branch to root ratio, and root colonization of different types of citrus. Many of citrus species show a positive response to AMF, while some species such as lime and pomelo have rapid growth compared with other species. Different citrus species and varieties respond to AMF in different methods [22].

4. MATERIALS AND METHODS

4.1 EXPERIMENTAL MATERIALS, PLAN, AND TREATMENTS

This study was conducted to improve nutritional situation, soil fertility and growth of white seedless grape under drought stress conditions; in this case, effects of inoculated one-year grape seedlings with several mycorrhiza funguses was examined on the water and nutrition relations under low irrigation conditions in the pot compared with the control group (without inoculation). This study was done during two years (2013-2014) in the form of a factorial experiment in the randomized complete block design with four treatments. The factors included inoculation with three mycorrhiza fungus species (*Glomus mosseae*, *G. fasciculatum*, and *G. intraradices*) and without inoculation (four levels), and irrigation at three levels (stress levels). The soil bed of the pot composed of wind sand and crop soil in equal amount. The white seedless grape cuttings were prepared then rooted in the wind sand using Mamarov method [32]. Half of the seedlings were inoculated in the Arbuscular Mycorrhizal (AM) fungi suspension at the same bed and the rest of them were used as the control samples.

4.2 PREPARATION OF MYCORRHIZA PLANTS

Mycorrhiza fungus inoculums (spore, mycelium, mycorrhizal roots, and soil) were taken from the Turan Biotechnology Company of Shahrood and propagated on Sorghum roots. To produce mycorrhizal seedlings, woody white seedless grape were put on the rhizogenic antiseptic rootstock sand bed, which has been mixed with Mycorrhizal fungus inoculum based on the 15:1000 ratio then sampling was done at each week in order to make sure of root colonization. Staining the root with Trypan blue 0.5% and making sure of colonization, colonization percent of roots was determined at the final step. Rooted seedlings, which were inoculated with mycorrhiza fungus at next step (end of winter), were put in 20-liter plastic pots. The seedlings were pruned as twin buds in early spring. The seedlings were pruned as twin buds in early spring. After 20-cm vegetative growth and plantation of seedlings, drought stresses were imposed as follows: the usable water for the plant was calculated based on the weight percent of agricultural capacity and wilting point then this rate was expressed as weight vale by consideration of the pot soil weight. Accordingly, the obtained usable water and stress treatments were applied. Irrigation treatments included 35%, 55%, and 75% of usable water (agricultural capacity), which were not applicable in 100% capacity due to the constant need for water. According to the surveys, the irrigation plan was implemented within 2 days, 4 days and 6 days. To determine the physiochemical situation of the soil composition used for plantation of rooted seedlings, a soil sample was sent to the laboratory. The obtained results are reported in Table 1.

Table1. Results of pots' soil analysis

Row	Characteristic	Unit	irrigation plan was implemented within			Optimal range
			2 days	4 days	6 days	
1	Depth	cm	0-30cm	30-60cm	60-90cm	-
2	Electrical conduction ($EC*10^3$)	Ds/m	1.61	-	-	<2
3	Acidity (PH)	-	7.43	-	-	5.5-6.7
4	Saturation percent (SP)	-	34	-	-	40
5	Lime percent ($Caco_3$) (T.N.V)	%	9.4	-	-	<15
6	Organic carbon percent (O.C)	%	0.16	-	-	>2
7	Total nitrogen percent (T.N)	%	0.02	-	-	>0.2
8	Available phosphorous (P_{ava})	Mg/kg	2.5	-	-	>15
9	Available potassium (K_{ava})	Mg/kg	154	-	-	>350
10	Clay percent	%	14	-	-	20-30
11	Silt percent	%	14	-	-	30-40
12	Sand percent	%	72	-	-	30-40
13	Soil texture	-	Sa.L	-	-	Loam, clay loam
14	Copper (Cu)	-	1.23	-	-	-
15	Iron (Fe)	-	5.91	-	-	-
16	Manganese (Mn)	-	4.03	-	-	-
17	Zinc (Zn)	-	0.528	-	-	-

4.3 FRESH AND DRY WEIGHT OF ROOT AND SHOOT

To examine the effect of treatments on some of the vegetative traits of grape in all water treatments at the end of the experiment, one pot was selected randomly from each experimental unit and shrubs with their roots were removed from the pot. Measuring the height of shrub (by using a ruler), each shrub was divided into three parts of leaves, shoot and roots. Then, a number of leaves, leaf area (using graph paper) and fresh and dry weight of leaves, shoots, and roots (using a digital scale with a precision of 001 / g) were measured. To determine dry weight, different organs were put on the oven under 70°C for 72 hours.

4.4 MEASURING DRY WEIGHT OF LEAF

After drying leaves under the 70°C of the oven, the dry weight of them was measured using a digital scale with 0.01-gram accuracy.

4.5 DRY WEIGHT OF ROOTS AND SHOOTS

After drying roots and shoots under the 70°C of the oven for 48 hours, the dry weight of them was measured using a digital scale with 0.01-gram accuracy.

4.6 FRESH WEIGHT OF ROOTS, SHOOTS, AND LEAVES

Fresh weight of roots, shoots, and leaves was measured using a digital scale with 0.01-gram accuracy.

4.7 STATISTICAL ANALYSIS OF DATA AND APPLIED SOFTWARE

Before data analysis, normal distribution of data was examined using the Kolmogorov-Smirnov test (K-S) through SPSS® Software. Variables with non-normal distribution were standardized using suitable conversions.

The SAS® software was employed for analysis of variance (ANOVA) and a comparison of the measured traits. Means were compared using Duncan's multi-domain test. Moreover, Excel software was used to plot charts.

5. RESULTS AND DISCUSSION

ANOVA Results of effects of mycorrhizal fungus treatments and water stress on the growth traits of roots and shoots- characteristics of roots and shoots (fresh and dry weight as well as the amount of water) (Table 2)

Table2. ANOVA of roots and shoots' characteristics

Change source	df	Mean square					
		Fresh weight of shoot	Dry weight of shoot	Fresh weight of root	Dry weight of root	Roots' amount of water	Shoots' amount of water
Fungus	3	18.766ns	5.253*	1620.02**	145.489*	879.02*	9.33^{ns}
Irrigation	2	313.08**	55.493*	5026.94**	603.147**	2149.3**	110.36**
Fungus × irrigation	6	32.451^{ns}	8.037 ^{ns}	478.08^{ns}	100.880*	18.07**	712.67**
Error	36	38.163	13.730	245.574	35.420	203.88	17.25
Change percentage		19	15	12	8	17	10

ns: lack of significant difference ** and * indicate significant difference at 1% and 5% levels, respectively

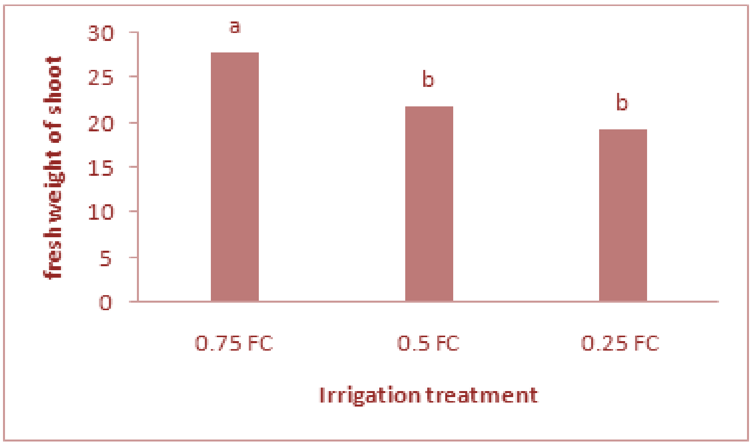

Figure1. Comparing the average fresh weight of shoots affected by different irrigation levels.

5.1 FRESH WEIGHT OF SHOOT

ANOVA results showed that water treatment had a significant effect on the fresh weight of the white seedless grapes' shoot (Table 2). Average fresh weight of shoot was significantly reduced at stress levels of 50% and 25% in comparison with 75% of farming capacity (Figure 1).

5.2 DRY WEIGHT OF SHOOT

ANOVA results showed that water treatment had a significant effect on the dry weight of the white seedless grapes' shoot at a level of 5% (Table 2). Water stress had an effect on the dry weight of grapevines' shoots so that the average dry weight of shoot at a stress level of 25% was significantly lower than 75% level (Figure 2).

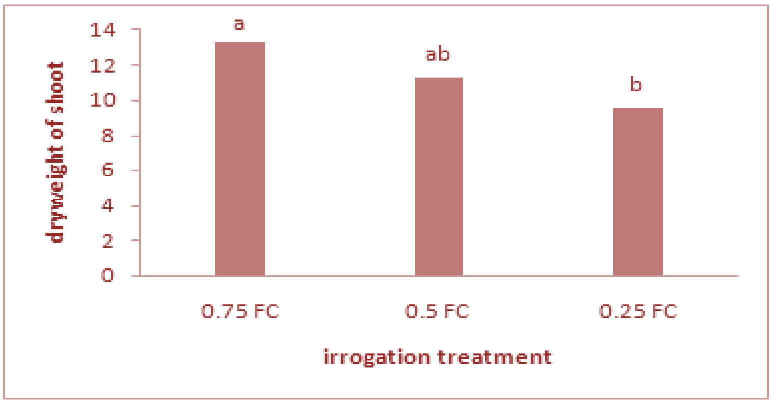

Figure2. Comparing the average dry weight of shoots affected by different irrigation levels

5.3 FRESH WEIGHT OF ROOT

ANOVA results showed that fungal and water treatment had a significant effect on the fresh weight of the white seedless grapes' root at 1% level (Table 2). Highest fresh weight of root in grapevines inoculated with three types of mycorrhiza funguses was significantly higher than grapevines without fungal infections (control subjects) (Figure 3).

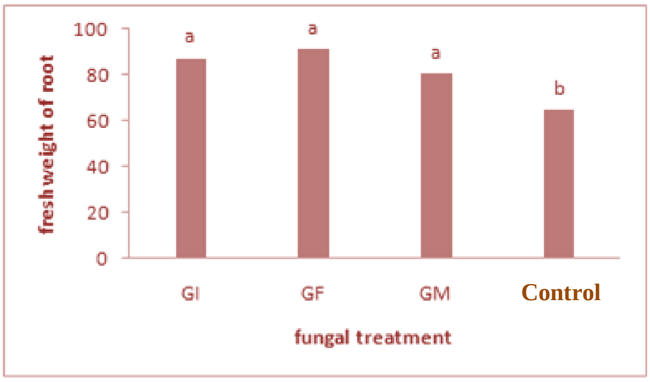

Figure3. Comparing the average fresh weight of roots affected by fungal treatments

Average fresh weight of roots at a 75% level of water requirement was significantly different two other levels (Figure 4).

Considering the effect of drought stress on reduction in a dry matter of plants, water scarcity decreases nutrients uptake, transfer, and consumption at each growth step leading to lower carbon storage and dry matter [23]. If plant height and number of leaves are reduced under drought stress conditions, dry weight of aerial organs will be reduced. Reduction in leaf growth leads to a decline in carbon and energy consumption in aerial organs as well as the higher contribution of assimilated materials, which distribute in roots where the high potential of water and minerals uptake is possible;

therefore, root to aerial organ ratio is increased and water stress removes low-depth roots while expands deep roots. Aerial organs compete with roots to uptake photosynthetic materials under the drought stress conditions and this may affect the growth of these organs [24].

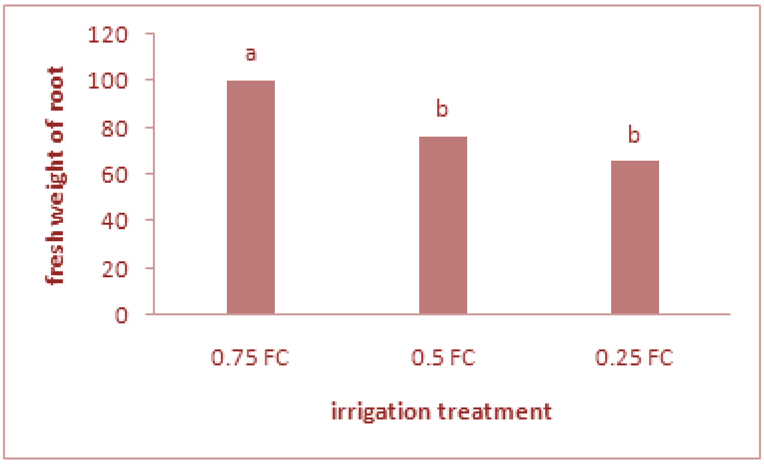

Figure4. Comparing the average fresh weight of roots affected by different irrigation levels

Leaf growth is more sensitive related to root growth under drought stress conditions. In similar water potential, reduction in leaves growth is higher than roots, because root cells are capable of keeping more cell turgor in comparison with leaf cells. On the other hand, it may be affected by the generation of Abscisic acid hormone by roots under the stress conditions. This hormone has an inhibitory feature for aerial organs while it develops the growth process of roots [24]. [18] investigated the effect of different moisture levels on four grape cultivars (Fakhri, Ghezel, raisins, and Khalili) and reported a decline in dry matter and branch length owing to the increase in stress level.

A study was conducted to examine the effect of mycorrhizal colonization on growth parameters of onion (*Allium cepa L.)* under different irrigation levels; the total dry matter was increased in mycorrhizal plants compared with non-mycorrhizal ones; in addition, increased irrigation gaps led to declining in biomass [25].

All three species of funguses had a significant effect on the fresh weight of leaf and fresh and dry weight of root so that these variables experience a significant difference and higher growth. It seems that the reason for such difference depends on the penetration of fungal hyphae and using a higher volume of soil and penetration in deeper depths as well as nutrients uptake, which leads to activation of biochemical and hormonal reactions, increase in weight of roots and leaves, and weaker effect of drought stress.

5.4 DRY WEIGHT OF ROOT

ANOVA results indicated the significant mutual effect of fungus and water stress on the dry weight of white seedless grapes' root at 5% level (table 2). There was a high probability of mutual effects of fungus and stress on dry weight of roots of grapevine infected with mycorrhiza funguses at all of the water treatments compared with grapevines without funguses; however, there was not any significant difference in dry weight of control grapevines and samples inoculated with *Glomus intraradices* at water treatment of 75%. Increase in water stress up to 25% of plant's water need led to the higher effect of funguses on the increase in dry weight of roots in comparison with control subjects. at 75% level of water need treatment, GF fungus had the highest effect on the increase in dry weight of root compared to other funguses, while this effect was seen at 50% and 25% water treatments for *Glomus mosseae* and *Glomus intraradices* funguses, respectively.

Accordingly, a specific species may have a different effect on the increase in root weight at each water stress level. Increase in water stress level led to a decline in dry weight of roots of all types of control grapevines and sample infected by mycorrhiza funguses. However, grapevines infected with Glomus intraradices fungus had the highest weight of root under the severest drought stress indicating the efficient function of this fungus in colonization under drought conditions (Figure 5).

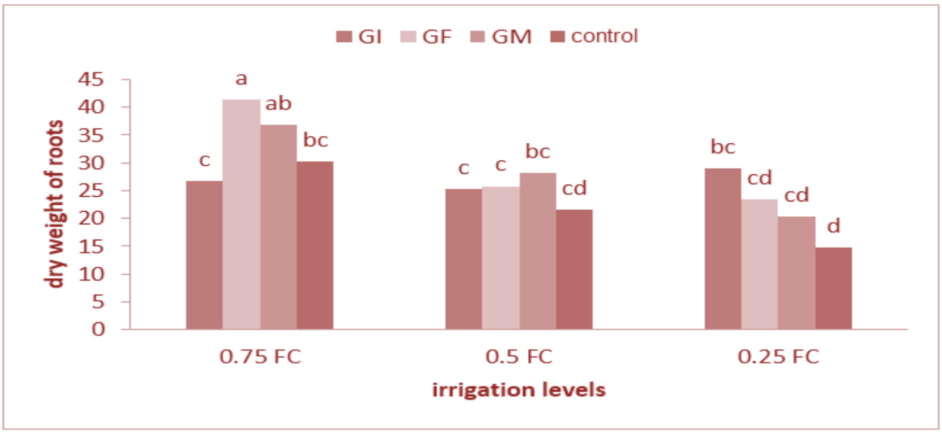

Figure5. Comparing the average dry weight of roots affected by fungal treatments and different water stress levels

Considering the effect of drought stress on reduction in a dry matter of plants, water scarcity decreases nutrients uptake, transfer, and consumption at each growth step leading to lower carbon storage and dry matter [23]. Inoculation with Arbuscular Mycorrhizae fungi had a significant effect on the increase in vegetative indicators of the plant under drought stress conditions. In this case, the largest number of leave, dry weight and area of leaf, shrub height, number of branches, dry weight of shoot, and length and dry weight of root were seen in inoculation process of *Satureja hortensis L.* with *G. versiformis* while the lowest number of these traits were obtained for non-mycorrhizal treatment. The obtained results, in this case, are in line with a study on *Capsicum annuum L.* conducted by [26].

The work [27] reported an increase in dry matter accumulation in symbiotic Nigella sativa L. with mycorrhizal funguses of *G.* intraradices during the 10-day growth period and this rise reached to the peak (24.71% rise related to the control sample) 89 days after growing. Figure 5 shows the positive effects of mycorrhizal fungus of *Glomus fasiculatum* under the mutual effect of fungus and irrigation, which is matched with previous works. Water deficit leads to a decline in cell size, cell division, cell wall composition, plant size and dry and fresh weight of the plant, which are general growth indicators. The initial sign of water shortage is reduced turgor and therefore cell growth and development, particularly in shoots and leaves. Cell growth is the most sensitive process, which is influenced by water stress. The decline in cell growth leads to a reduction in organ size; hence, the first observable effect of water shortage on the plant can be seen in the limited size of leaves or plant height [24]. Mycorrhizal colonization increases drought resistance. As the mycorrhizal colonization changes root's characteristics such as root length and structure and as root can improve nutrients uptake, mycorrhizal plants have more optimal growth related to non-mycorrhizal plants under drought stress [28-31].

5.5 ROOTS' WATER LEVEL

ANOVA results indicated the significant mutual effect of fungus and water stress on the water level of white seedless grapes' root at 1% level (table 2). There was a high probability of mutual effects of fungus and stress on dry weight of roots of grapevine infected with mycorrhiza funguses at all of the water treatments compared with grapevines without funguses; however, there was not any significant difference in dry weight of control grapevines and samples inoculated with *Glomus intraradices* and GF at water treatment of 75%. Increase in water stress up to 25% of plant's water need led to a lower effect of funguses on the increase in water level of roots in comparison with control subjects. At 75% level of water need treatment, GF fungus had the highest effect on the increase in water level of root compared to other funguses, while this effect was seen at 50% and 25% water treatments for GM and *Glomus intraradices* funguses, respectively.

Accordingly, a specific species may have a different effect on the increase in roots' water weight at each water stress level. Increase in water stress level led to a decline in water level of roots of all types of control grapevines and sample infected by mycorrhiza funguses. However, grapevines infected with *Glomus intraradices* fungus had the highest water amount of root under the severest drought stress indicating the efficient function of this fungus in colonization under drought conditions. There was not any significant difference at the 5% level (Figure 6).

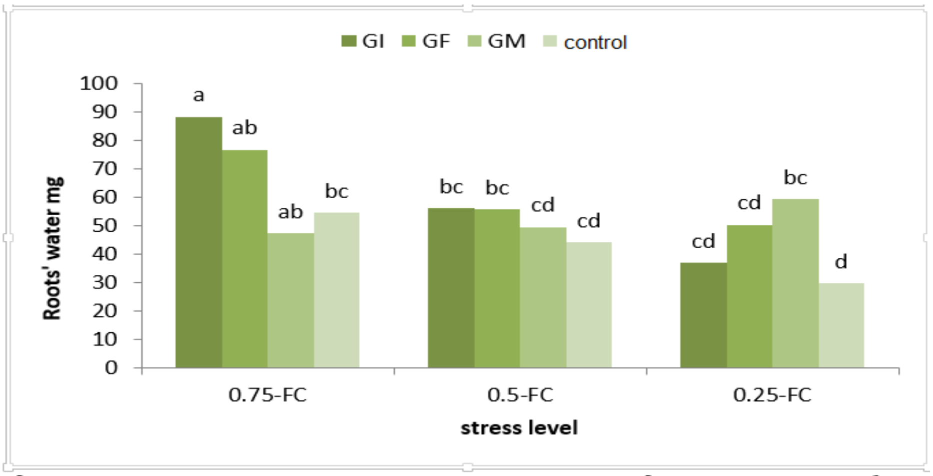

Figure6. Comparing average roots' water level affected by fungal treatments and different water stress levels

5.6 SHOOTS' WATER LEVEL

ANOVA results indicated the significant mutual effect of fungus and water stress on the water level of white seedless grapes' shoot at 5% level (Table 2). There was a high probability of mutual effects of fungus and stress on dry weight of shoots of grapevine infected with mycorrhiza funguses at all of the water treatments compared with grapevines without funguses; however, there was not any significant difference in dry weight of control grapevines and samples inoculated with *Glomus intraradices* and GF at water treatment of 75%. Increase in water stress up to 25% of plant's water need led to a lower effect of funguses on the increase in water level of shoots in comparison with control subjects. At 75% level of water need treatment, GF fungus had the highest effect on the increase in water level of shoot compared to other funguses, while this effect did not show any difference between funguses at 50% and 25% water treatments. Figures 6 and 7 demonstrate this result, which was obtained in this research.

Increase in water stress level led to a decline in water level of shoots of all types of control grapevines and sample infected by mycorrhiza funguses. However, grapevines infected with GF fungus had the highest water amount of shoot under the severest drought stress indicating the efficient function of this fungus in colonization under drought conditions (Figure 6).

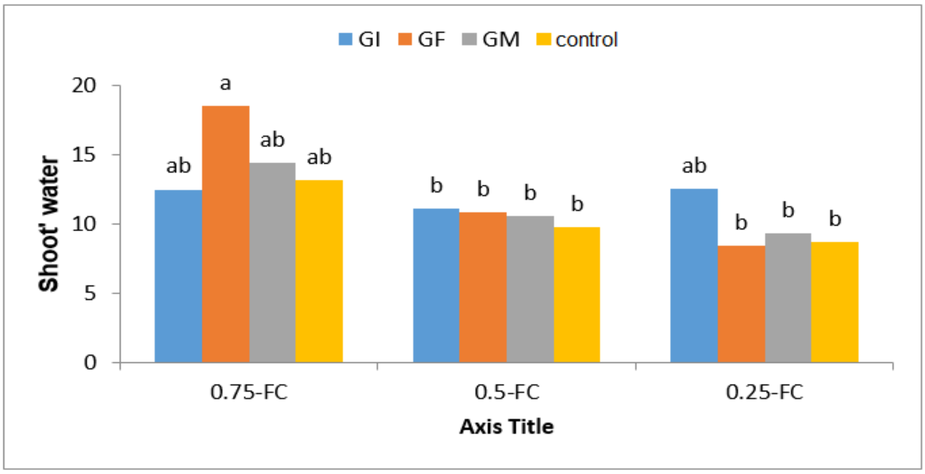

Figure7. Comparing average shoots' water level affected by fungal treatments and different water stress levels.

6. CONCLUSION

In this paper, the effect of mycorrhizal fungi on the absorption rate of macro and microelements in the leaf of grape cv. *sefid bidaneh* under drought conditions were investigated. Conducted studies on the performance of mycorrhizal funguses indicate that there is a significant mutual effect of fungus and water stress on the water level of white seedless grapes' shoot. In other words, it can be concluded that the roots inoculated with the fungus lead to increase in water and minerals (particularly, phosphorous) uptake owing to more optimal penetration pf hyphae in the soil; the adsorbed materials are sent to leaves to be converted to osmolyte.

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Dr.Mohammad Aslanpour is a Lecturer at Department of Horticulture University of Raparin, Rania, Sulaimany, Iraq. He concentrates on Grapevine and Horticulture researches.

Dr. Hamed Doulati Baneh is associated with Horticulture Crops Research Department, West Azerbaijan Agriculture and Natural Resources Research and Education Center, AREEO, Uremia, Iran. His research focuses on Plant Physiology, Plant Biotechnology, Horticulture, and SSR.

Professor Dr. Ali Tehranifar is Professor at Horticultural Sciences and Landscape Department, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. His researches concentrate on Vegetable Growth and Fruit Productions.

Dr. Mahmoud Shoor is an Associate Professor at Horticultural Sciences and Landscape Department, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. His researches spotlight on Ecological Horticulture.