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INVESTIGATION OF THE SHOOT LENGTH, NUMBER OF LEAVES, LEAF AREA, FRESH AND DRY WEIGHT OF BRANCH, ROOT, AND LEAF OF THE WHITE SEEDLESS GRAPE

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ABSTRACT

To determine impacts of infected roots of seedless white currant grape with three species of Mycorrhizal fungi (Glomus fasiculatum, Glomus intraradices, and Glomus mosseae) on growth traits (shoot 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 shoot growth, number of leaves, leaf area, dry weight of root and shoot. Inoculation with mycorrhizal fungi had a positive effect on the above-mentioned traits compared with the control group; in this case, among fungal treatment traits, the Glomus mosseae had the highest effect on the shoot length. All of three funguses had an impact on the fresh weight of leaf and reduced temperature of leaf area. There was not any difference between irrigation levels of 25 and 50% under the water stress; there was not also any difference between the average length of the shoot at irrigation levels of 50 and 75%.

1. INTRODUCTION

Being one of the main horticultural products in Iran, the grape is the first rank among fruit trees in terms of cultivation area and ranked after pistachio and date palm economically [1]. The origin of grape cultivars is a debatable issue for experts. In particular, there is no agreement on the early or second places of domestication grapevine from the wild grape, introduced the Near East Region as the early place for grape creation based on the plant geological and archaeological studies. Herbal archeological studies suggest that grape domestication has begun since the second half of the fourth

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*Corresponding author (Mohammad Aslanpour) E-mail: Aslanpour.mohammad@gmail.com millennium BC in two neighboring areas, Mezopotamia (southern Anatolia, Syria, northern Lebanon, Kurdistan, and western Iran) and south of Caspian Sea. Water scarcity is an important factor, which 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 Mycorrhizal 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 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]. Reduced atmospheric precipitation and improper distribution of rainfall, as well as water stress during sensitive grape growth periods, are the factors decreasing function and quality of grape in many regions in Iran.

2. THEORETICAL LITERATURE

Grapevine is from the Ampelidaceae family called Saramantaceae or Vitacae. This family belongs to the Rhamnales specie, which is Dialypetalae belonged to the angiosperms from the Spermatophytes. 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 [5]. The most important Iranian cultivars include white and red seedless grape, Askari, Yaghooti, Shahroodi, Shahani, Rishbaba, Peykani, Fakhri, Reshe, Sahebi, and other cultivars. All of the edible grapes in Iran belong to the Vinifera species [6].

2.1 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 [7]. 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 [8]. Drought stress affects the morphological traits of the plant such as leaf area, shoot 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 CO2, evaporation and Proline accumulation [9, 10].

2.2 DROUGHT STRESS AND PHOTOSYNTHESIS

Under water stress conditions, plant apertures are closed and therefore CO_2 concentration in Mesophile tissue is reduced; such condition disturbs dark photosynthesis reactions and the products obtained from light reactions (ATP and NADPH) are not consumed. Under such conditions, consumption of NADP+ is reduced for electron capture due to lack of ADPH oxidation; therefore, Oxygen molecule performs as an electron substitute receiver through the electron transfer chain forming superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and Hydroxyl radical (OH^-)[11].

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 [12].

2.3 MYCORRHIZAL FUNGI

The term “mycorrhizal” was introduced by Frank in 1885; this term 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 cover 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 [13, 27].

2.4 MYCORRHIZAL FUNGI IN GRAPE

Nowadays, Vinifera grape species is cultivated in regions with enough rainfall within rain fed form and due to its drought and limestone soil resistance [14]. 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 [15].

It is reported that medium drought stimulates mycorrhiza colonization. Under limited irrigation conditions (30% of water requirement), a number of arbuscular in grapevine hairy root is higher than its number under the standard irrigation conditions (60% of water requirement). Accordingly, an increase in mycorrhiza colonization and stimulation of grapevine to create deeper roots leads to drought condition, which creates higher resistance. Nevertheless, grape resists against the drought stress by using stomatal adjustments and moving leaves to prevent the contact between leaf and light beside the two mentioned factors [16].

3. MATERIALS AND METHODS

3.1 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. Half of the seedlings were inoculated in the Arbuscular

Mycorrhizal (AM) fungi suspension at the same bed and rest of them were used as the control samples.

3.2 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 05% 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. 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 constant need of 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 soil analysis

Row	Characteristic	Unit	Value	Value	Value	Optimal range
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	-	-	-

Grape shrubs were monitored completely during the growing season and irrigation regimes were done for seedlings during 3 months. Before applying water treatments, length of branches and number of leaves were measured in all treatments. At the end of the experiment, morphological traits during growth season and end of the season were measured in order to examine effects of treatments on various traits such as leaf area, fresh and dry weight of leaf and shoot growth.

3.3 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.

3.4 MEASURING HEIGHT OF PLANT

The height of the plant from the soil surface to nodes 8 and 9 and the end of shrub was measured separately in two steps within the one-month interval.

3.5 STATISTICAL ANALYSIS OF DATA AND APPLIED SOFTWARE

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

4. RESULTS AND DISCUSSION

4.1 ANOVA RESULTS

Table 2 shows ANOVA results of the effects of mycorrhizal fungus treatments and water stress on the growth traits of leaf and branch length.

Characteristics of leaf and branch (fresh weight, area, and number of leaves, branch length)

To examine and compare mean values of different fungal and water treatments for variables with significant effect, LSD test was performed at 5% level.

Table 2: ANOVA of leaf and branch’s characteristic

Change source	df	Mean square			
		Fresh weight of leaf	Leaf area	Average number of leaves	Average length of branch
Fungus	3	0.431**	321.335*	30.28*	328.29**
Irrigation	2	0.032^{ns}	123.216^{ns}	37.66*	575.63**
Fungus × irrigation	6	0.207^{ns}	76.692^{ns}	7.56^{ns}	45.97^{ns}
Error	36	0.095	108.946	8.66	42.01
Change (%)		15	12	16	14

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

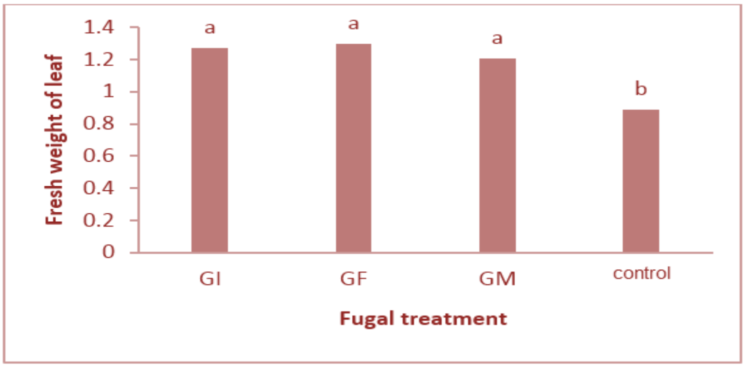

Figure 1: Comparing the average fresh weight of leaf affected by fungus treatments

4.2 FRESH WEIGHT OF LEAF

Effects of three types of inoculating mycorrhiza fungus were significant on fresh weight of leaf

*Corresponding author (Mohammad Aslanpour) E-mail: Aslanpour.mohammad@gmail.com at 1% level (Table 2). The highest fresh weight of leaf was seen in grapevine inoculated by all of three mycorrhiza funguses and this rate was significantly greater than grapevines, which was not infected with fungus (control subjects) (Figure 1).

4.3 LEAF AREA

Effects of three types of inoculating mycorrhiza fungus were significant on leaf area at 1% level (table 2). According to the effects of fungus treatments on leaf area, the highest leaf area was seen in treatments with Glomusintraradices and Glomus mosseae and the difference between experimental and control subject was significant. The significant increase in leaf area in grapes inoculated with Glomus intraradices and Glomus mosseae compared with control subjects can be attributed to expanded nutrients uptake, which leads to increase in leaf area and therefore to improved photosynthetic activity and CO2 fixation. There was not any significant difference between the fungal treatment of Glomus fasiculatum and other treatments in terms of average leaf area (Figure 2).

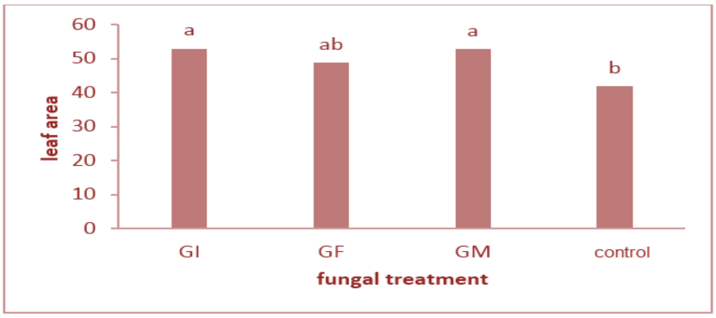

Figure 2: Comparing average leaf area affected by fungus treatments

Depending on drought severity, the plant loses less water through transpiration by decreasing the number of leaves [17]; limited leaf area is the first resistance mechanism of the plant against drought [7]. Reduction in the area and number of the leaf is a resistive mechanism of the plant against stress [18]. Declined leaf area in the control sample was seen in this research, which is in line with studies conducted by [19]. A study [20] found a significant increase in leaf area of mycorrhizal basil compared to non-mycorrhizal plants and attributed this to increased nutrients uptake. A reported that symbiosis with intraradices Glomusfungus in pepper plant leads to expanded leaf area and water uptake. A study [21] carried out on the vetch and found that mycorrhiza fungus increases leaf area, relative growth speed and growth speed of the product.

Use of mycorrhiza leads to an increase in plant dry material owing to the increase in water and nutrient uptake as well as higher leaf area. Such function of mycorrhiza develops the photosynthetic activity, fixes CO2 and increases aerial organ’s biomass [22]. It has been reported that symbiosis with mycorrhiza can develop photosynthesis through morphological changes such as increased leaf area. This research is matched with relevant studied in terms of leaf area.

4.4 AVERAGE NUMBER OF LEAVES

Effects of three types of inoculating mycorrhiza fungus were significant on leaf area at 5% level (Table 2). The largest number of leaves was seen in Glomus mosseae treatment, which was significantly different from other treatments. In other words, Glomus mosseae fungus increases the number of leaves (Figure 3). There were more leaves at 75% water requirement compared with other stress levels. In other words, water stress led to a decline in the number of leaves (Figure 4).

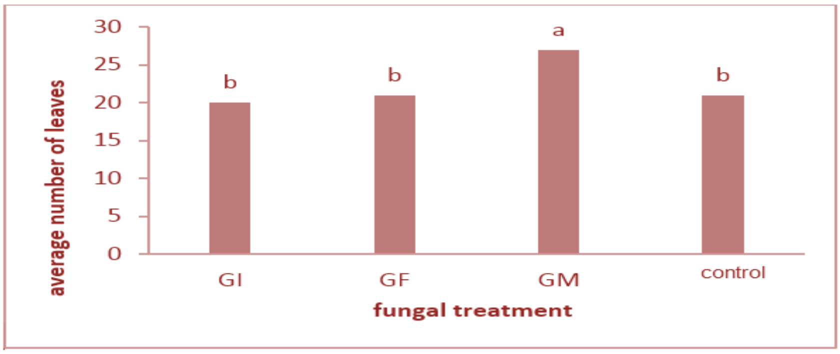

Figure3: Comparing the average number of leaves under the effect of fungal treatments**.**

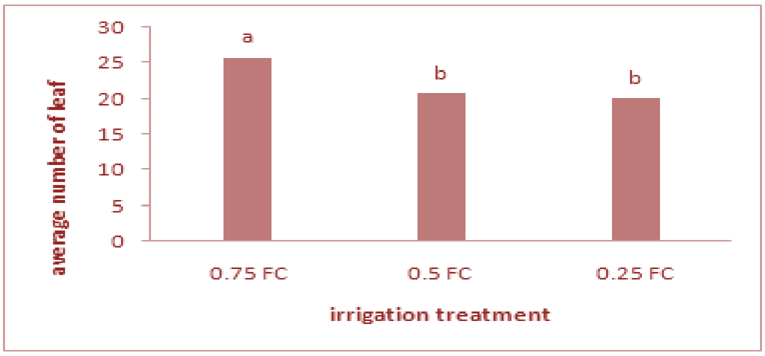

Figure 4: Comparing the average number of leaves under the effect of different irrigation levels

Vegetative growth of branch highly depends on the growth medium. As the growth phenomenon associates with vital activities when there is enough water available for the plant, water deficit leads to growth reduction due to decline in tungsten pressure of growing cells and its impact on length of cells [23]. Studies [19, 24] reported a decline in grape shrub growth and subsequent decrease in a number of leaves per shrub due to drought stress. Fungal treatment with Glomus mosseaemodified effect of drought stress by an increasing the number of leaves and improved the function by increasing nutrients uptake; this result matches with the conducted studies in this field.

4.5 AVERAGE BRANCH LENGTH

ANOVA results showed that fungal and water treatments had a significant effect on the average length of white seedless grapevine' branch at 1% and 5% levels, respectively (Table 2). According to comparisons between average lengths of branch, this variable was significantly different in Glomus mosseae and Glomus fasiculatum compared with other treatments (Figure 5). Water stress had an impact on longitudinal growth of grapevines so that average branch length showed a significant difference at stress levels of 75% and 25% (Figure 6).

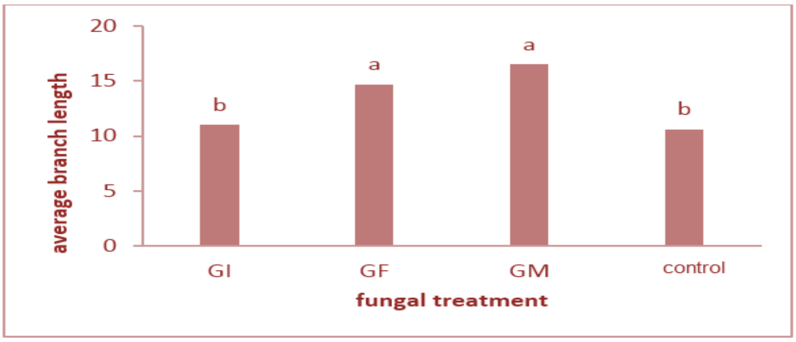

Figure 5: Comparing average branch lengths between different fugal levels

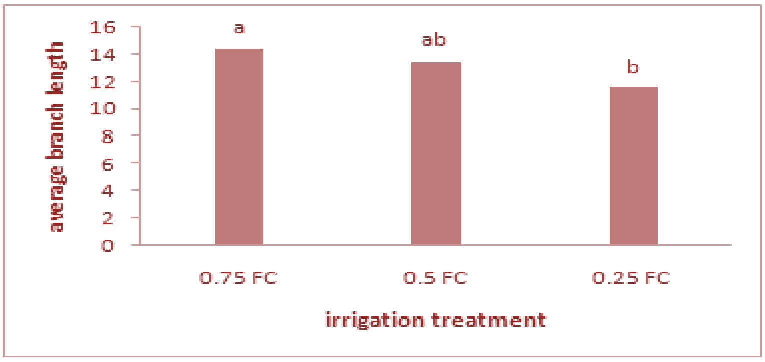

Figure 6: Comparing average branch lengths under the effect of different irrigation levels

A study [25] examined the effect of different moisture levels on growth traits including shrub height, number of nodes, leaf area, number of leaves, and dry weight of leaf in 5 grape cultivars and reported a different reduction in all of the growth parameters in cultivars after an increase in stress severity.

A study conducted on the effect of mycorrhiza fungus on growth nutrients uptake in grapevine planted in poor soils and reported higher growth of seedlings inoculated with mycorrhizal fungi to relate to control ones; this result is matched with findings of this work. In the mentioned study, two fungal species expanded the growth, which was a significant increase in growth. [16] carried out some studied and showed that symbiotic association rate between rootstock and fungus can stimulate growth rate so that mycorrhiza-inoculated root of grapevine may vary due to the fungus species and cultivar. In general, the currant white seedless cultivar of the grape has medium vegetative growth genetically; hence, inoculation with mycorrhizal fungus could increase branch growth by improving water conditions and nutrients uptake.

According to research reports, Glomus intraradices is one of the most efficient funguses in improving water uptake in Lettuce, while Glomus mosseae leads to a reduction in water uptake of lettuce; these differences are associated with different genetic regulation of Akupurins by the fungus [25]. According to a study on the effect of different arbuscular mycorrhizal funguses on the growth of Coleusforskohlii**,** shrub height of plants under the mycorrhizal fungus treatment was increased compared to control [26]. As seen in Figure 5, fungal treatment of Glomus mosseae indicates a positive effect by increasing branch length, reducing drought stress and branch growth. This result was in line with the conducted studies.

5. CONCLUSION

This paper investigated the impact of Mycorrhiza Fungi on Growth Traits (Shoot Length, Number of Leaves, Leaf Area, Fresh and Dry Weight Of branch, Root, and Leaf) of the White Seedless Grape under Drought Stress. According to the effects of fungus treatments on leaf area, the highest leaf area was seen in treatments with Glomusintraradices and Glomus mosseae and the difference between experimental and control subject was significant. The significant increase in leaf area in grapes inoculated with Glomus intraradices and Glomus mosseae compared with control subjects can be attributed to expanded nutrients uptake, which leads to increase in leaf area and therefore to improved photosynthetic activity and CO_2 fixation. There was not any significant difference between the fungal treatment of Glomus fasiculatum and other treatments in terms of average leaf area. According to research reports, Glomus intraradices is one of the most efficient funguses in improving water uptake in Lettuce, while Glomus mosseae leads to reduction in water uptake of lettuce; these differences are associated with different genetic regulation of Akupurins by the fungus fungal treatment of Glomus mosseae indicates a positive effect by increasing branch length, reducing drought stress and branch growth.

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