RESEARCH ARTICLE Agricultural Science Digest, Volume 41 Special Issue: 119-128 (April 2021)

Comparison of Effectiveness of Arbuscular Mycorrhiza Fungi (AMF) on *Vitis vinifera* under Low Irrigation Conditions

M. Fattahi, S. Nasrollahpourmoghadam, A. Mohammadkhani

10.18805/ag.D-253

ABSTRACT

Background: Grapevine is an important perennial crop worldwide, consumed as fresh or dried (raisins) fruit. Grapevines are exposed to a variety of abiotic stresses during their growth. Water shortage is one of the primary stressors and severely restricts the development of the grape industry. Arbuscular mycorrhizal fungi (AMF), a kind of beneficial soil microorganism, can create a symbiotic association with plant roots forming arbuscular mycorrhizas (AMs), which play a role in the regulation of plant growth.

Methods: This research was accomplished in order to investigate the effect of 4 species mycorrhizal fungi on grapevine 'Asgari' cultivar under water stress conditions. We use of 3 irrigation regime that consist: 100% (as control), 70 and 40 % of field capacity. As well as, mycorrhizal treatments include: non-use of mycorrhizal (NM) and use of mycorrhiza (M) (*Glomus mosseae, G. intraradices*, *G. etunicatum* and *G. verciform*).

Results: The results indicated that shoot and root dry weight, pigments (total chlorophyll and carotenoids), relative water content (RWC), P, Mg^{2+} and Ca^{2+} under water limitation decreased but electrolyte leakage (EL), proline and total soluble sugars (TSS) increased chlorophyll content, RWC and nutrient (P, K^{+} and Ca^{2+}) in M plants was higher than NM plants. Generally, the use of mycorrhiza fungi in this experiment reduced the harmful effects of water stress. Our results shown that *G. verciform* and *G. etunicatum* were better for symbiosis with "Asgari" grapevine under water limitation.

Key words: Drought, Glomus spp, Grapevine, Nutrient, Osmolyte.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) member of family vitaceae, is one of the most important perennial crop worldwide and its one of the most economically momentous temperate fruit trees (Krithika *et al*. 2015; Stupic *et al*. 2019; Bettoni *et al*. 2019), its fruits are processed into products such as wine and juice or used as fresh table grapes or raisins. In 2017, grapevines enveloped 7.53 million ha and produced 73.3 million tons of fruit worldwide (Bettoni *et al*. 2019; Zyprian *et al*. 2018). Grape cultivated under tropical and sub-tropical regions of the world and the capability for *V. vinifera* to tolerate inappropriate environmental conditions for superlative crops will partially be dependent on the functioning on its soil society (Holland *et al*. 2014; Kumar *et al*. 2015).

Drought stress, decreased crop quality and yield and also restrict the geographical range over which production of crop is viable (Thakur *et al*. 2010: Kumar *et al*. 2018). Abiotic stress such as drought, causes generate secondary stresses, including oxidative and osmotic stress, which have harmful effects on the plants and causing changes in the plants growth, development and metabolism (Kranner *et al*. 2010). Plant species to cope with bad environmental conditions have expand a number of physiological, biochemical and molecular mechanisms (Chen *et al*. 2011). Drought stress inhibits the plants photosynthesis, causes changes of chlorophyll contents and components and damage to the photosynthetic system (Salekjalali *et al*. 2012). Thus, it is obligatory to steer research for obtaining cultivars, which would be more tolerant to drought stress. Water limitation is one of the adverse environmental factors that can extremely extent crops dispensation in the whole world (Cattivelli *et al*. 2008; Farooq *et al*. 2009; Fathi *et al*. 2017).

Arbuscular mycorrhizal fungi (MAF) is one of the most beneficial soil microorganisms in which most of the plants can establish a symbiosis with them. MAF help plants to improve the nutritional absorption and other functions such as osmotic regulation and photosynthesis that results in tolerance enhancement against biotic and abiotic stresses (Jothi *et al*. 2005: Hashem *et al*. 2015; Vimal *et al*. 2017). Nicolas *et al*. (2015) concluded that AMF inoculation technique can be recommended for sustainable viticulture in arid and semi-arid areas. Mycorrhizal formation has been associated with enhanced growth, elevate mineral uptake from soil and increased tolerance against drought conditions (Trouvelot *et al*. 2015; Bavaresco and Fogher, 1996). The mycorrhiza is fungi-plant roots bilateral coexistence. AMF could expand root absorbing surface, elevate stomatal

Department of Horticulture Science, Shahrekord University, Shahrekord, Iran.

Corresponding Author: M. Fattahi, Department of Horticulture Science, Shahrekord University, Shahrekord, Iran.
Email: ma.fatahi67@gmail.com

How to cite this article: Fattahi, M., Nasrollahpourmoghadam, S. and Mohammadkhani, A. (2021). Comparison of Effectiveness of Arbuscular Mycorrhiza Fungi (AMF) on *Vitis vinifera* under Low Irrigation Conditions. Agricultural Science Digest. 41 (Special Issue): 119-128. DOI: 10.18805/ag.D-253.

Submitted: 01-04-2020 **Accepted:** 31-07-2020 **Online:** 10-11-2020

conductivity, transpiration rate and RWC, as well as reduced the leaf water potential and permanent wilting point (PWP) under stress condition and promote hydrological cycle in plants and induce a dynasty of defense responses mechanisms when experienced hardness (Ruiz-lozano *et al*. 1995; Li *et al*. 2002; Wu *et al*. 2009). Under water deficit plants associated with AMF show enhanced osmotic regulation and root hydraulic properties than NM ones (Ruiz-lozano *et al*. 2012). The inoculation of *pistacia vera* with AMF enhanced drought tolerance of plants by promoting nutrient uptake (Bagheri *et al*. 2012). Zhang *et al*. (2016) reported that mycorrizal hyphae beneficed soil water absorption and nutrient uptake in grassland plants also previous studies have shown that drought tolerance of plants could be improved by AMF (Lu *et al*. 2003). For instance, Zhang and He (2007) reported that AMF can modify the protective system and enhancement the drought resistance of *Artemisia ordosica* plants. Wu and Xia (2005) noticed that the ability of *A. ordosica* against drought stress was elevated by insemination with the AMF. Studies carried out by Yooyongwech *et al*. (2016) and Quiroga *et al*. (2017) showed that, when subjected to drought, mycorrhizal potato and maize plants had higher level of photosynthetic pigments than those not colonized by AMF. Thus, one of the most important strategies to use in novel viticulture under water limitation is the choice of the privileged adapted rootstock and best MAF for symbiosis.

The aim of this study was, to investigate the effects of different species of AM fungi on drought tolerance of one of the most important grape cultivars in Iran. In this experiment, the effect of AMF symbiosis on morpho-physiological and nutritional characteristics of 'Asgari' grape was investigated.

MATERIALS AND METHODS

Mycorrhizal inoculum and stress conditions

Glomus mosseae, G. intraradices, *G. etunicatum* and *G. verciform* were multiplied in a sterile potted soil cropped with *Zea mays* L. between June and October 2018. Inoculum of AMF was included of spores, hyphae and mycorrhizal root pieces of corn and it was used to grape cuttings symbiosis. In this experiment, 'Asgari' grapes were used and the mycorrhizal grape cuttings were treated with inoculum (non-mycorrhizal plants were not inoculated) and they cultivated at 10 L pots. Plants were grown for three months in an experimental greenhouse in Shahrekord, Iran (32°21′ N, 50°49′ E) with middle temperatures and relative humidity of 26°C and 42% respectively. Water treatments in three levels (100, 70 and 40% of field capacity - FC) were applied in three replications (two plants in each replication) in the basis of FC. The FC was determined by the gravimetric method according to Souza *et al*. (2000).

Pigments, RLWC and Electrolyte Leakage

Contents of pigments (chlorophyll and carotenoids) in these experiments were estimated matching to Lichtenthaler (1987) method. To determine relative leaf water content (RLWC) samples (100 mg) of fresh leaf weighed [fresh weight (FW)], then placed in distilled water and shaken for 4 h and samples were weighed [as total weight (TW)], after that samples were dried at 65°C for 48 h and weighed again [dry weight (DW)]. The RWC was computed by following equation (1): (Gonzalezvilar, 2001)

$$(RLWC\ (\%) = (FW - DW) / (TW - DW) \times 100 \quad \text{.....(1)}$$

For Electrolyte leakage (EL) assay 15 freshly cut leaf discs (1 cm^2) were rinsed 3 steps (2-3 minute) with double distillation water and after that floated on 10 mL of water. Subsequently the EL in the solution was calculated after 24 h at room temperature using a conductimeter (Crison 522, Crison Instruments, S.A., Spain). Total conductivity was measured after keeping the samples in an oven (90°C) for 2 h. Results were represented as % of total conductivity.

TSS and Proline

Total soluble sugar (TSS) was estimated through Irigoyen *et al*. (1992) method. Proline content estimated based on reflex with ninhydrin according to Bates *et al*. (1973). For this purpose, one solution of proline, ninhydrin acid and glacial acetic acid (1:1:1) was prepared and incubated at 100°C for 1 hour. Subsequently reaction was seized in an iced bath and the cromophore was extracted with 4 ml toluene and its absorbance at 520 nm was registered. Proline content was assessed with a standard curve and expressed as $\mu g.g^{-1}$ FW.

Nutrient

Dried samples of leaf and root were weighed singly and ground to transit a 40 mesh sifter .The leaf and root samples were dry-ashed at 500°C, in the following plant sample solved in 10 cc hydrogen chloride (HCl 2N) and made the volume to 100 cc with distilled water. Mg^{2+}, Ca^{2+} and Fe^{2+} concentrations were measured by atomic absorption spectrometry and K^{+} content was estimated by flame photometry. The content of leaf and root P in the digest was obtained by the ammonium molybdate blue method using spectrophotometry (Chapman *et al*. 1982).

Root sampling and assessment of MAF colonization

The experiment was finished 40 days after treatments beginning and separating shoots from roots. After washing the roots with tap water the roots of 2 plants in each pot was mixed and cut into 1cm in size pieces. Mycorrhizal symbiosis evaluation were prepared *via* Phillips and Haymann (1970) method. Roots sample were boiled for 1 h in 15% KOH and then washed with water. Staining of roots pieces was accomplished in 0.05 % trypan blue and 15 min autoclave. Thereafter, roots pieces were kept in lacto glycerol. Finally, colonization of mycorrhizal was measured using 50 root segments of each sample by compound microscope (Giovannetti and Mosse, 1980).

Statistical analysis

Data were evaluated by Two-way analysis of variance (ANOVA) with SPSS 25.0.0 (*Inc., Chicago, IL, USA*). When treatments interactions were significant ($P \leq 0.05$), means

were separated using Duncan's multiple range test at $P \leq 0.05$. Principal component analysis (PCA) was evaluated by R version 3.5.2.

RESULTS AND DISCUSSION

According to the results, root dry weight (DW) in plants treated with mycorrhizae (M) plants was significantly higher than NM plants under both stress conditions. As well as, shoot/root in *G. etunicatum* and *G. mosseae* was lower than another fungi (Table 1). Under drought stress, biomass (root and shoot DW) decreased and the lowest shoot and root DW and shoot/root ratio was in MAD 60 (Table 1).

The chlorophyll a, b and total chlorophyll decreased under water limitation and the lowest level of chlorophyll a, b and total were observed at MAD 60 (FC 40). Reductions in chlorophyll a, b and total chlorophyll content in Mycorrhiza plants were lower than those without Mycorrhiza and the highest chlorophyll a, b and total chlorophyll were 2.71, 0.94 and 3.65 $mg.g^{-1}$ DW respectively in *G. versiforme*. carotenoids increased under drought stress. Higher level of Car was observed in the *G. etunucatum* (Fig 1).

Under drought stress electrolyte leakage (EL) increased but in M plants was lower than NM plants. More EL was observed on MAD 60 (90.7%). The effect of low irrigation and mycorrhiza species on RWC was significant. RWC decreased 31.2% under low irrigation conditions on compare with control but RWC was higher in M plants than NM plants, although there was no significant difference between control and *G. mosseae* (Fig 2).

Reducing irrigation (MAD 60) increased the TSS content of shoot and root, which was significantly different with control (MAD 0). An increase in TSS in the shoot and root M plants, less than NM plants. Most TSS of shoot and root on NM plants in MAD 60 was with an average of 39.22 and 46.12 $mg.g^{-1}$ DW respectively. The results indicated that interaction of treatments on shoot and root proline content was significant. No significant difference was observed between treatments in MAD 0 on shoot proline. In MAD 30 and MAD 60, the amount of shoots proline increased and the lowest shoot proline content was observed in *G. versiform* and *G etunicatum*. Under drought stress roots proline increased and proline content on NM plants was more than M plants. The highest root and shoot proline (32.2 and 66.5 $\mu g.g^{-1}$, respectively) on NM plants was observed in MAD 60 (Fig 3).

Results comparison of shoot and root nutrient are shown in Table 2. The effect of drought and mycorrhiza on Ca^{2+}, K^{+}, Mg^{2+} and P in shoot and root was significant. The nutrient content in root and shoot was significantly higher under irrigated conditions and inoculated with mycorrhyzae. Generally, the Ca^{2+}, K^{+}, Mg^{2+} and P content of shoots and

Table 1: Root and shoot dry weight (g) of grapevine 'Asgari' symbiosis with mycorrhizal fungi (*G. mosseae*, *G. verciform, G. etunicatum*, *G. intraradices*,) under different levels of dryness conditions (MAD).

		Shoot DW (g)	Root DW (g)	Shoot/Root
Non Mycorrhiza		6.33 b	18.40 d	0.35 a
G. mosseae		7.48 a	27.61 b	0.28 bc
G. versiforme		7.61 a	24.39 c	0.32 ab
G. etunicatum		8.33 a	32.21 a	0.26 c
G. intraradices		8.12 a	22.42 c	0.36 a
	MAD0	8.80 a	26.25 a	0.35 a
	MAD30	7.99 b	26.53 a	0.31 ab
	MAD60	5.93 c	22.24 b	0.28 b
Non Mycorrhiza	*MAD0*	7.84 b-d	23.18 c-e	0.34 b-e
	MAD30	6.90 c-e	20.07 e	0.34 b-d
	MAD60	4.25 f	11.95 f	0.36 bc
G. mosseae	*MAD0*	8.72 a-c	34.27 a	0.26 d-f
	MAD30	7.18 b-e	29.35 ab	0.24 ef
	MAD60	6.53 de	19.22 e	0.34 b-e
G. versiforme	*MAD0*	8.48 a-d	20.02 e	0.42 ab
	MAD30	7.48 b-d	27.26 bc	0.27 c-f
	MAD60	6.87 c-e	25.90 b-d	0.27 d-f
G. etunicatum	*MAD0*	9.94 a	30.21 ab	0.33 b-e
	MAD30	8.26 a-d	33.64 a	0.25 d-f
	MAD60	6.80 c-e	32.79 a	0.21 f
G. intraradices	*MAD0*	9.04 ab	23.59 c-e	0.39 ab
	MAD30	10.13 a	22.31 c-e	0.46 a
	MAD60	5.20 ef	21.36 de	0.25 ef

Comparison means by Duncan's test ($P < 0.05$) were shown for the significant main effect. Different letters indicate significant differences among data within the same factor. The vertical bars indicate the standard error.

Table 2: Comparison of mean of root and shoot nutrient (Ca^{2+}, K^{+}, Mg^{2+} and P *mg.g^{-1} dw*) of grapevine 'Asgari' symbiosis with mycorrhizal fungi (*G. mosseae*, *G. verciform, G. etunicatum*, *G. intraradices*) under different levels of dryness conditions (MAD).

		Root				Shoot			
		Ca	K	Mg	P	Ca	K	Mg	P
	MAD0	10.18 b-d	3.13 a-d	2.25 de	1.10 e-h	10.51 c	4.49 c	2.34 c-e	1.41 c-e
Non Mycorrhiza	*MAD30*	9.90 cd	2.28 cd	2.67 a-d	1.29 d-g	10.55 c	4.67 c	2.31 de	1.07 e-g
	MAD60	8.13 e	2.09 d	1.74 f	0.77 h	9.783 d	2.74 d	1.81 e	0.83 g
	MAD0	10.4 a-c	4.06 a	2.41b-e	2.23 a	11.09 bc	10.70 a	2.79 a-d	2.03 ab
G. mosseae	*MAD30*	10.66 ab	2.99 a-d	2.77a-c	1.67 b-d	11.31 b	6.66 b	3.37 a	1.62 c
	MAD60	10.31 bc	2.52 b-d	3.00 a	0.97 gh	10.96 bc	4.28 c	2.78 a-d	0.98 fg
	MAD0	10.95 a	3.52 ab	2.69 a-d	1.77 a-c	11.93 a	6.96 b	3.11 ab	2.34 a
G. versiforme	*MAD30*	10.21 b-d	2.85 b-d	2.79 ab	1.51 c-f	10.86 bc	4.51c	2.91 a-d	1.71 bc
	MAD60	9.92 cd	2.56 b-d	2.40 b-e	1.30 d-g	10.57 c	3.99 cd	2.54 b-e	1.37 c-f
	MAD0	9.65 d	2.89 b-d	2.84 ab	1.62 b-d	11.3 b	4.48 c	2.89 a-d	2.35 a
G. etunicatum	*MAD30*	10.26 bc	2.95 b-d	2.76 a-c	1.28 d-g	10.58 c	4.13 cd	2.71 a-d	1.48 cd
	MAD60	9.96 cd	3.30 a-c	2.18 ef	1.07 f-h	10.61 c	4.03 cd	2.51 b-e	1.18 d-g
	MAD0	10.10 b-d	2.99 a-d	2.98 a	2.02 ab	10.75 bc	4.59 c	3.08 a-c	1.61 c
G. intraradices	*MAD30*	10.16 b-d	2.44 b-d	2.44 b-e	1.54 c-e	10.81 bc	3.39 cd	2.19 de	1.39 c-e
	MAD60	9.94 cd	2.20 d	2.33 c-e	0.95 gh	10.51 c	3.29 cd	2.36 b-e	1.07 e-g

Comparison means by Duncan's test ($P < 0.05$) were shown for the significant main effect. Different letters indicate significant differences among data within the same factor. The vertical bars indicate the standard error.

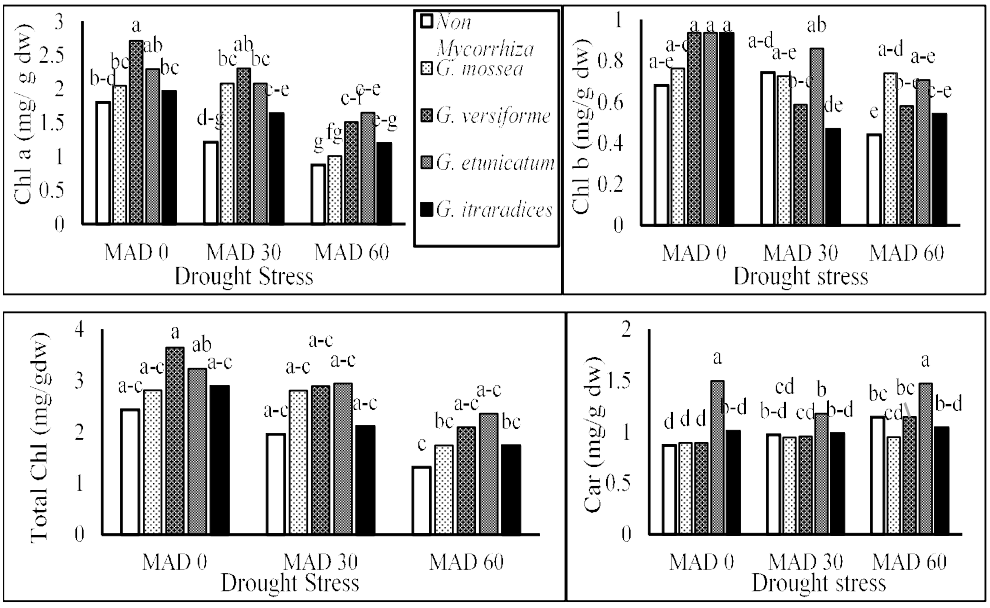

Fig 1: Effect of different irrigation levels (MAD) and mycorrhiza (*G. mosseae*, *G. verciform, G. etunicatum*, *G. intraradices*,) on the Chlorophyll a, b, total and Car of 'Asgari' grape.

roots of plants that symbiosis with *G. versiform* was more than other mycorrhyzae species. The quantity of P was higher in plants treated with mycorrhyzae (M) over untreated plants (NM). The higher shoot P was found in MAD 0 in *G. versiform* (2.34 g.kg^{-1}) and *G. etunicatum* (2.35 g.kg^{-1}) fungus but the most root P was observed under MAD 0 and *G. mosseae* (2.23 g.kg^{-1}) fungi (Table 2). The shoot Fe^{2+} was influenced by the effect of treatments. Water limitation decreased shoot Fe^{2+} concentration and symbiosis plant with mycorrhiza had the more Fe^{2+} content compared to control (Fig 4).

PCA-Biplot where samples of each clone are grouped in the plot of the first and second principal components: Dim1 versus Dim2. We could not find different clones clearly distinguished for mycorrhiza species and NM and M plants were separated along Dim1 and Dim2. On the other hand we can find different clones clearly for drought stress level (Fig 5).

Altogether, the results shown that the symbiosis rate of *G, etunicatum* with 'Asgari' roots was more than other species of fungi (Fig 6). Also, the symbiosis plants with *G.*

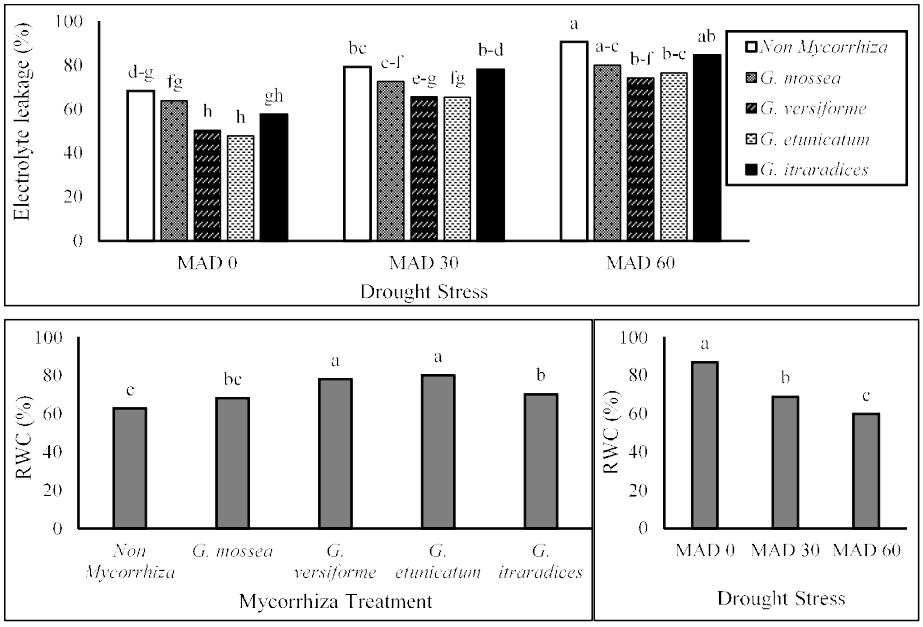

Fig 2: Effect of different irrigation levels on the basis of moisture content between field capacity and permanent wilting point (MAD) and mycorrhiza species (*G. mosseae*, *G. verciform, G. etunicatum*, *G. intraradices*,) on the Electrolyte leakage and RLWC of 'Asgari' grape.

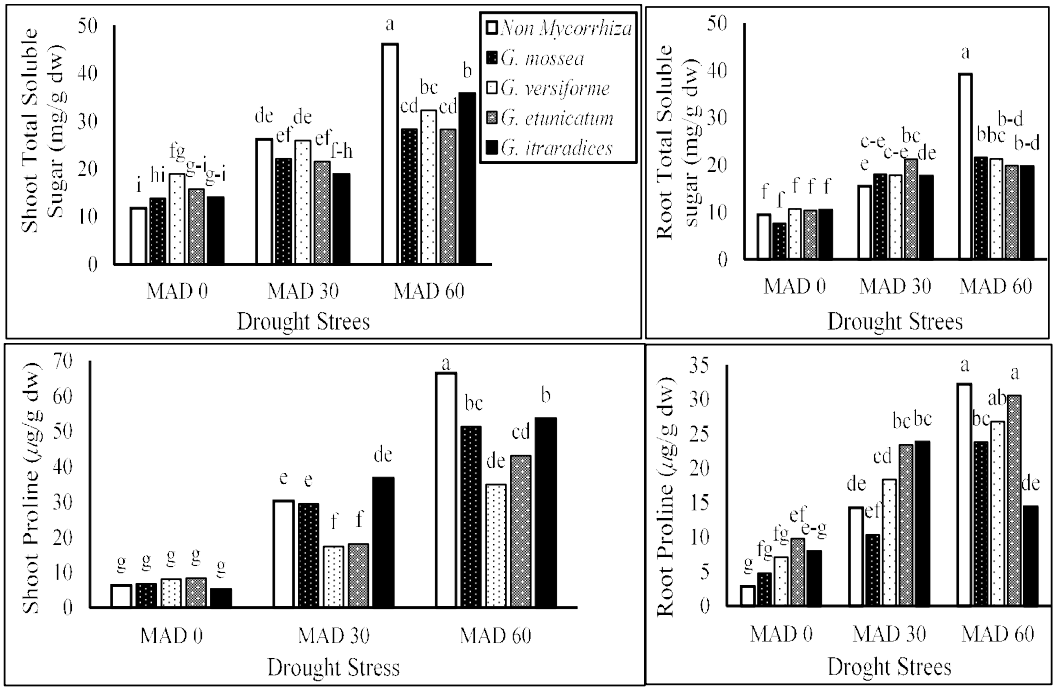

Fig 3: Effect of different irrigation levels on the basis of moisture content between field capacity and permanent wilting point (MAD) and mycorrhiza species (*G. mosseae*, *G. verciform, G. etunicatum*, *G. intraradices*,) on the shoot and root osmolyte (total soluble sugar and proline) of 'Asgari' grape.

etunicatum were better than the other of the fungi in terms of shoot and root DW, chlorophyll and carotenoids, RWC and root Mg^{2+} and P content under drought stress conditions.

Biomass was higher in M plants, which could be owing to effects of mycorrhizal symbiosis on P absorption and other minerals such as K^+, Ca^{2+}, Mg^{2+} (Abdel-Salam *et al*. 2018) furthermore in M plants, water uptake and RWC was improved. Biomass reductions under drought stress conditions has been reported in previous studies on almond (Fathi *et al*. 2017) and peach (Rieger *et al*. 2003) fruit trees. Drought condition may reduce cell division, leaf area and chlorophyll content and finally, decreased in fresh and dry weight (Nadeem *et al*. 2014).

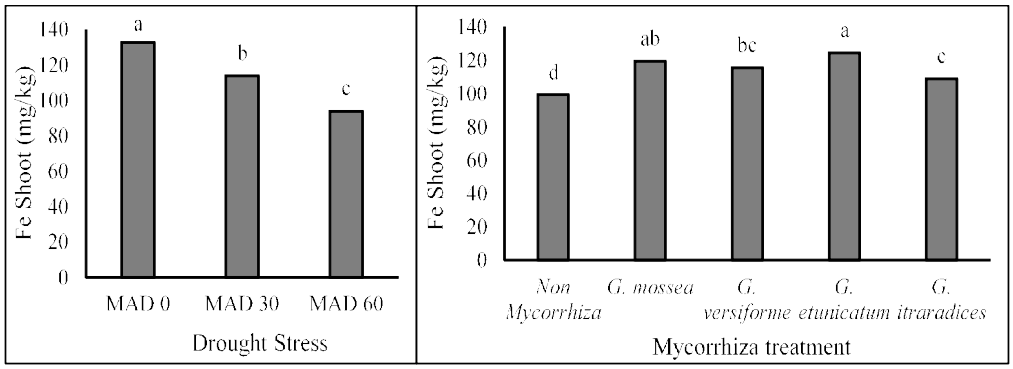

Fig 4: Effect of different irrigation levels on the basis of moisture content between field capacity and permanent wilting point (MAD) and mycorrhiza species (*G. mosseae*, *G. verciform, G. etunicatum*, *G. intraradices*,) on the shoot Fe of 'Asgari' grape.

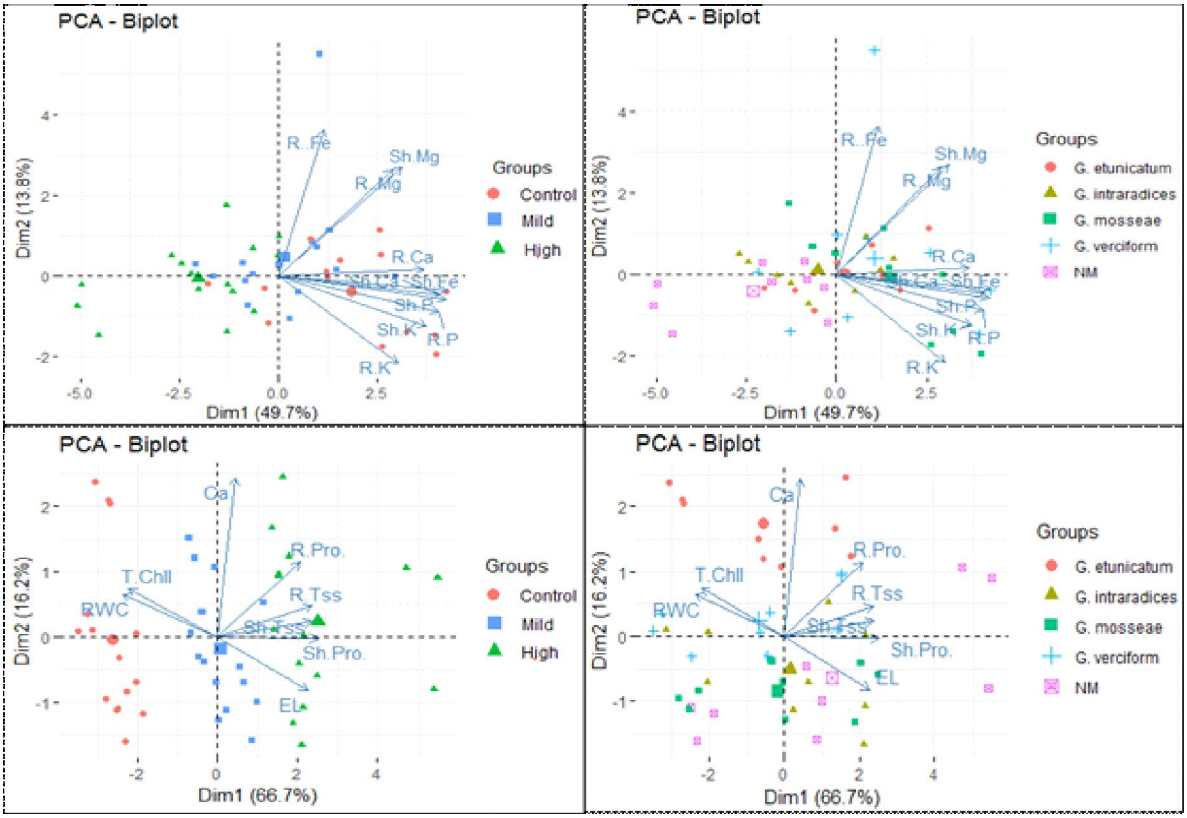

Fig 5: Principal component analysis (PCA) Biplot from characteristic of Leaf (L) and Root (R) in 'Asgari' grape symbiosis with mycorrhiza (*G. mosseae*, *G. verciform, G. etunicatum*, *G. intraradices*,) under irrigation levels (MAD).

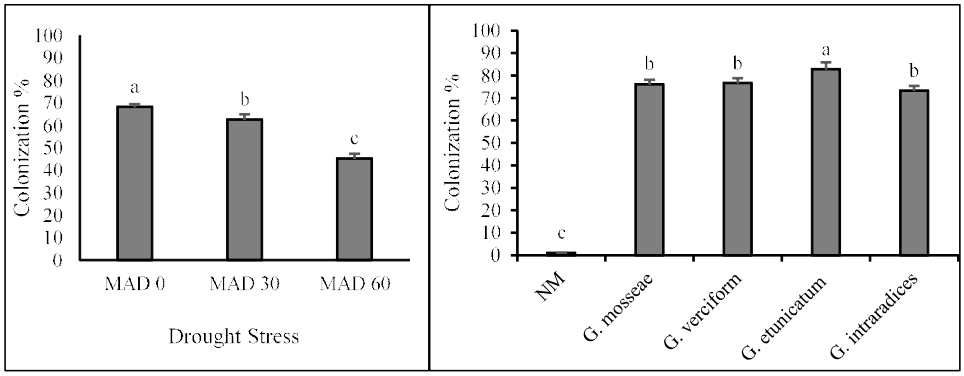

Fig 6: The effect of different irrigation levels on colonization percent of 'Asgari' grape.

Plants inoculated with mycorrhizae had significantly enhanced chlorophyll content in leaves than control. These results are in agreement with previous studies (Giri and Mukerji, 2004; Sannazzaro *et al*. 2004; Colla *et al*. 2008). M-mediated increased Mg^{2+} and Fe^{2+} uptake, which they are essential for biosynthesis of chlorophyll. In host plants, chlorophyll might be less degraded because the plants were in better conditions in terms of moisture and mineral nutrition. The contents of carotenoids significantly increased under water limitation. Carotenoids content are correlated with the capacity of light-protecting mechanisms and they are good known for their antioxidant activity, inhibiting peroxidation of lipids, fixing membranes and as well as serious role in the assembly of the light harvesting complex and in the radiationless scattering of surplus energy. (Demmig-Adams and Adams, 1996; Munne-Bosch and Alegre, 2000). Therefore, the results of this experiment determined that mycorrhizal plants provided stronger photoprotective system against drought condition compared with non-inucolated plants by make more leaf carotenoids concentration.

It is observed from the mean values of Fig (2) that there is a decrease in the average RWC of the plant subjected to water shortage as compared to controlled conditions. Significant differences in RWC were observed between the different treatments tried. The improvement of RWC in M plants might be due to an improvement of the water absorption by M root system (Ruiz-Lozano *et al*. 1995). Moreover, increased water transport could also be attributed to improvement in P nutrition as well as the higher content of water soluble compounds in plant tissues could be a reason for higher plants RWC (Ruiz-Lozano *et al*. 2000 and 2012; Bagheri *et al*. 2012). The results showed that due to low irrigation, EL increased on compare with control. Plant membranes are exposed to changes often associated with the increment in permeability and integrity loss under drought stress condition (Blokhina *et al*. 2003). In that's why, the capability of cell membranes to control the rate of ion movement in and out of cells is used as a damage test to a great range of tissues.

The proline content of plant shown in the Fig (3). The proline content of the plants increase from normal ratio when they are subjected to drought stress. Some experiment were conducted that alterations in osmolyte content were verified (Sircelj *et al*. 2005; Silva *et al*. 2009; Rodrigues *et al*. 2010). Accumulation of prolin and TSS (as compatible organic solute) in response to water deficit cause to memorize cell turgor and reduced water potential of plant. Hence, mechanisms of drought tolerance is adjusted by this factors. (Hasegawa *et al*. 2000; Silva *et al*. 2009; Farooq *et al*. 2009). The increases in amount of TSS may happen as a result of the Inhibition of growth and by the water deficit intensity arising from starch degradation (Pimentel, 1999). The accumulation of compatible organic solute in root may due to the translocation of photo assimilates from leaves to roots and/or reduction in growth (Pimentel, 1999).

Decreasing of mineral nutrition's (Ca^{2+}, K^{+},Mg^{2+}, P and Fe^{2+}) under drought stress may be due to reduced root growth, because root growth, mineral transfer and mineral solubility in soils has an inverse relationship with water deficit, Also water scarcity has a negative effect on the minerals absorption and their transfer from soil to plant (Tsabarducas *et al*. 2015). The results of our experiment showed that under MAD 30 and 60 condition, P, Mg^{2+} and K^{+} contents of shoot were reduced whilst these nutrients in roots and shoot of M are more than NM plants. AM formation is widely believed to protect host plants from the harmful effects of water stress. Because, AM raise the nutrient uptake *via* expanding the exploration of the soil hole place (Auge, 2004). AM hyphae adhere to soil components by glomalin (a special glycoprotein) and amend contact with the soil solution under drought stress and these hyphae Increase the availability of roots to smaller pore spaces (Davies, 1992; Auge, 2004; Wu *et al*. 2009). In this study, associated of AM increased shoot and root P content of grape under water stress and AM plants are much more efficient in P up taking than NM plants leading to recovery in plant growth (Fitter and Hay, 2002; Desnos, 2008). K^{+} content in M plants was more than NM plants and the results illustrated the positive role of AM symbiosis in Ca^{2+}, Mg^{2+} and shoot Fe^{2+} uptake. t has been illustrated in previous study that under low micronutrients level conditions such as stress, uptake by AMF hyphae is increased (Liu *et al*. 2000).

The mycorrhiza impose the secretion of phosphatase enzymes for P hydrolysis, which accelerates organic P compounds' hydrolysis, resulting in better plant productivity under P deficiency conditions (Rashid *et al*. 2016). Maycorrhiza symbiosis helps the plant to expand into the rhizosphere and reach unexplored soil areas, having the opportunity to uptake more water and minerals. Also, the MAF has the ability to produce more enzymes and organic acids to make minerals available and expedites hyphae growth, which elevates the root efficacy in minerals' absorption from the soil (Owen *et al*. 2015; Rashid *et al*. 2016). Maximum root colonization was registered in plants inoculated with AM strains followed by *G. etunicatum*. These variety between funguses may related to the genetically controlled physiological characters, which play an important role in the absorption of nutrients from the soil and also their transfer to the root (Schubert *et al*. 1990).

CONCLUSION

In general, AM formation enhanced water stress tolerance of grape 'Asgari' at least in part via the increased absorbtion of slowly diffusing mineral ions such as PO_4^{2-} and Fe^{2+} and prepare more osmotic regulation which can be associated with K^{+} accumulation in host plants. Nevertheless, AMF-mediated improvement of both growth rate and drought tolerance in plants is not just a nutritional process and some other mechanisms are also involved. Also the results indicated that chlorophyll a, b, total, proline decrease in NM plants was more than the M plants. Generally, the use of mycorrhiza in this research reduced the damaging effects of drought stress on the physiological and nutrition characteristics of grapevine, which in between the *G.*

verciform and *G. etunicatum* were better than the *G. mosseae* and *G. intraradices*. Therefore the role of various species of AMF in plants is different and some of plants may respond better to certain types of fungi.

REFERENCES

Abdel-Salam, E., Alatar, A., El-Sheikh, M.A. (2018). Inoculation with arbuscular mycorrhizal fungi alleviates harmful effects of drought stress on damask rose. Saudi Journal of Biological Sciences. 25: 1772-1780.

Auge, R.M. (2004). Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science. 84: 373-381.

Bagheri, V., Shamshiri, M.H., Shirani, H., Roosta, H.R. (2012). Nutrient uptake and distribution in mycorrhizal pistachio seedlings under drought stress. Journal Agriculture Science Technology. 14: 1591-1604.

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

Bavaresco, L., Fogher, C. (1996). Lime-induced chlorosis of grapevine as affected by rootstock and root infection with arbuscular mycorrhiza and *Pseudomonas fluorescens*. Journal of Grapevine Research. 35(3): 119-123.

Bettoni, J.C., Bonnart, R., Shepherd, A., kretzschmmar, A.A., Volk, G.M. (2019). Cryopreservation of grapevine (*Vitis* spp.) shoot tips from growth chamber-sourced plants and histological observations. Journal of Grapevine Research. 58: 71-78. DOI: 10.5073/vitis.2019.58.71-78.

Blokhina, O., Virolainen, E., Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress. Journal Annals Botany. 91: 179-194.

Cattivelli, L., Rizza, F., Badeck, F.W., Mazzucotelli, E., Mastrangelo, A.M., Francia, E., Mare, C., Tondelli, A., Stanca, A.T. (2008). Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Journal Field Crops Research. 105: 1-14.

Chapman, H.D., Pratt, P.F. (1982). Methods of Analysis for Soils, Plants and Waters. Division of Agriculture. University of California. Berkeley CA. PP 4034.

Chen, S., Cui, X., Chen, Y., Chunsun, G., Miao H., Haishun, G., Chen, F., Liu Z., Guan, Z., Fang, W. (2011). *Cgdreba transgenic* chrysanthemum confers drought and salinity tolerance. Environmental and Experimental Botany. 74: 255- 260.

Colla, G., Rouphael, Y., Cardarelli, M., Tullio, M., Rivera, C.M., Rea, E. (2008). Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. Biology and Fertility of Soils. 44: 501-509.

Davies, F., Potter, J.R., Linderman, R.G. (1992). Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development on pepper plants independent of plant size and nutrient content. Journal of Plant Physiology. 139: 289-294.

Demmig-Adams, B., Adams, W.W. (1996). Xanthophyll cycle and light stress in nature: Uniform response to excess direct sunlight among higher plant species. Planta. 198: 460-470.

Desnos, T. (2008). Root branching responses to phosphate and nitrate. Current Opinion of Plant Biology. 11: 82-87.

Farooq, M., Kobayashi, N., Wahid, A., Ito, O., Basra, S.M.A. (2009). Strategies for producing more rice with less water. Advances in Agronomy. 101: 352-388.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Asra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. Agronomy Sustainable Development. 29: 185-212.

Fathi, H., Imani, A., Amiri, M.E., Hajilou, J., Nikbakht, J. (2017). Response of almond genotypes/cultivars grafted on GN15 'Garnem' rootstock in deficit-irrigation stress conditions. Journal of Nuts. 8(2): 123-135.

Fitter, A.H., Hay, R.K.M. (2002). Environmental physiology of plants. 2nd Edition. Academic Press. London. PP. 120-128.

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

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

Hasegawa, P.M., Bressan, R.A., Jian-Kang, Z., Bohnert, H.J. (2000). Plant cellular and molecular responses to high salinity. Annual Review. Plant Physiology and Molecular Biology. 51: 463-599.

Hashem, A., Abdallah, E.F., Alqarawi, A.A., Aldubise, A., Egamberdieva, D. (2015). Arbuscular mycorrhizal fungi enhances salinity tolerance of *Panicum turgidum* Forssk by altering photo synthetic and antioxidant pathways. Journal of Plant Interaction. 10: 230-242.

Holland, T.C., Bowen, P., Bogadanoof, C., Hart, M.M. (2014). How distinct are arbuscular mycorrhizal fungal communities associating with grapevines. Biology and Fertility of Soils. 50: 667-674. DOI: 10.1007/s00374-013-0887-2.

Irigoyen, J.J., Emerich, D., Sanchie, W., Diaz, M. (1992). Water stress induced changeing concentrations of prolin and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Physiologia Plantarum. 84: 67-72.

Jothi, G., Babu, R.S., Rajendren, G. (2005). Biomanagment of nematodes by mycorrhiza - A review. Agricultural Reviews. 26: 249-260.

Kumar, M., Kumar, A., Mandal, N.P. (2018). Evaluation of Recombinant Inbreed Lines (RIL) population of upland rice under stress and non-stress conditions for grain yield and drought tolerance. Indian Journal of Agricultural Research. 52: 119-125.

Kumar, R., Ahmad, A., Dular, R.K., Chahal, D. (2015). Knowledge and adoption of improved grape cultivation practices in Haryana. Indian Agricultural Science Digest. 35: 31-35

Kranner, I., Minibayeva, F.V., Backett, R.P., Seal C.E. (2010). What is stress? Concepts, definitions and applications in seed science. New phytologist. 188: 655-673.

Krithika, V., Naik, R., Pragalyaashree, P. (2015). Functional properties of grape (*Vitis vinifera*) seed extract and possible extraction techniques - A review .Agricultural Reviews. 36: 313-320.

Li, H.Y., Shu, H.R., Liu, R.J. (2002). The basis of biochemistry and molecular biology for the defense responses induced by VAM fungi. Natural Science. 33(1): 107-111.

Lichtenthaler, R.K. (1987). Chlorophylls and carotenoids - pigments of photosynthetic bio membranes. In: Methods in Enzymology. [Colowick, S.P., Kaplan, N.O (ed.)] Academic Press, San Diego, New York, Berkeley, Boston, London, Sydney, Tokyo, Toronto. Vol. 148. Pp. 350-382.

Liu, A., Hamel, C., Hamilton, R.I., Ma, B.L., Smith, D.L. (2000). Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza. 9: 331-336.

Lu, J.Y., Mao, Y.M., Shen, L.Y., Peng, S.Q., Li, X.L. (2003). Effects of VA mycorrhiza fungus inoculated on drought tolerance of wild jujube (*Zizyphus spinosus* Hu) seedlings. Acta Horticulture Sinica. 30(1): 29-33.

Munne-Bosch, S., Alegre, L. (2000). Changes in carotenoids, tocopherols and diterpenes during drought and recovery and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta. 207: 925-931.

Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A., Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnology Advances. 32: 429-448.

Nicolas, E., Meastre-Valero, J.F., Alarcon, J.J., Pedrero, F., Vicente-Sanchez, J., Bernab, A. (2015). Effectiveness and persistence of arbuscular mycorrhizal fungi on the physiology, nutrient uptake and yield of Crimson seedless grapevine. Journal of Agriculture Science. 153: 1084-1096. DOI: 10.1017/s002185961400080x

Owen, D., Williams, A.P., Griffith, G.W., Withers, P.J.A. (2015). Use of commercial bio-inoculants to increase agricultural production through improved phosphorus acquisition. Appl Soil Ecology. 86: 41-54.

Phillips, J.M., Haymann, D.S. (1970). Improved procedures for cleaning roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society. 55: 158-161.

Pimentel, C. (1999). Relaçoes hidricas em dois híbridos de milho sob dois ciclos de deficiência hídrica. Pesquisa Agropecuária Brasileira. 34: 2021-2027.

Quiroga, G., Erice, G., Aroc, R., Chaumont, F., Ruiz-Lozano, J. M. (2017). Enhanced drought stress tolerance by the arbuscular mycorrhizal symbiosis in a drought-sensitive maize cultivar is related to a broader and differential regulation of host plant aquaporins than in a drought-tolerant cultivar. Frontier in Plant Science. 8: 1056. DOI: 10.3389/fpls.2017.11056.

Rashid, M.A., Mujawar, L.H., Shahzad, T., Almeelbi, T., Ismail, I.M.I., Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. Microbiology Research. 183: 26-41.

Rodrigues, B.M., Souza, B.D., Nogueira, R.M., Santos, M.G. (2010). Tolerance to water deficit in young trees of jackfruit and sugar apple. Revista Ciencia Agronomica. 41: 245-252.

Rieger, M., Lo Bianco, R., Okie, W.R. (2003). Responses of *Prunus ferganensis*, *Prunus persica* and two interspecific hybrids to moderate drought stress. Tree Physiology. 23: 51-58.

Ruiz-Lozano, J.M., Azcon, R., Gomez, M. (1995). Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. Applied Environmental Microbiology. 61: 456-460.

Ruiz-Lozano, J.M., Porcel, R., Azconm, R., Aroca, R. (2012). Contribution of arbuscular mycorrhizal symbiosis to plant drought tolerance: state of the art. In: Responses to Drought Stress: From Morphological to Molecular Features. [Aroca, R., (Ed.)]. Heidelberg: Springer-Verlag. pp: 335-362.

Ruiz_Lozano, J.M. Azcon, R. (2000). Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity. Mycorrhiza, vol. 10, pp. 137-143.

Salekjalali, M., Haddad, R., Jafari, B. (2012). Effects of soil water shortages on the activity of antioxidant enzymes and the contents of chlorophylls and proteins in barley. American-Eurasian Journal of Agriculture and Environmental Science. 12: 57-63.

Sannazzaro, A., Ruiz, A., Alberto, E., Menendez, A. (2004). Presence of different arbuscular mycorrhizal infection patterns in Lotus glaber growing in the Salado River Basin. Mycorrhiza. 14: 139-142.

Schubert, A., Mazzitelli, M., Ariusso, O., Eynard, I. (1990). Effects of vesicular-arbuscular mycorrhizal fungi on micro propagated grapevines: Influence of endophyte strain, P fertilization and growth medium. Journal of Vitis. 29: 5-13

Silva, E.C., Nogueira, R.J.M.C., Vale, F.H.A., Melo, N.F., Araujo, F.P. (2009). Water relations and organic solutes production in four umbu tree (*Spondias tuberosa*) genotypes under intermittent drought. Brazilian Journal of Plant Physiology. 21: 43-53.

Sircelj, H., Tausz, M., Grill, D., Batic, F. (2005). Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. Journal of Plant Physiology. 162: 1308-1318.

Souza, C.C., Oliveira, F.A., Silva, I.F., Amorim Neto, M.S. (2000). Evaluation of methods of available water determinaton and irrigation management in “terra roxa” under cotton crop. Revista Brasileira de Engenharia Agrícola e Ambiental. 4: 338-342.

Stupic, D., Bauer, N., Jagic, M., Lucic, A., Mlinarec, J., Malenica, N., Karoglankontic, J., Maletic, E., Leljak-Levanic, D. (2019). Reproductive potential of the functionally female native *Croatian grapevine* ‘Grk bijeli’. Journal of Vitis. 58: 61-70. DOI: 10.5073/vitis.2019.58.61-70.

Thakur, P., Kumar, S., Malik, J.A., Berger, J.D., Nayyar, H., (2010). Cold stress effects on reproductive development in grain crops: An overview. Environmental and Experimental Botany. 67: 429-443.

Trouvelot, S., Bonneau, L., Redecker, D., Tuinen, D., Adrian, M., Wipf, D. (2015). Arbuscular mycorrhiza symbiosis in viticulture: a review. Agronomy for Sustainable Development. 35: 1449-1467. DOI: 10.1007/s13593-015-0329-7.

Tsabarducas, V., Chatzistathis, T., Therios, I., Koukourikou-Petridou, M., Tananaki, C. (2015). Differential tolerance of 3 self-rooted *Citrus limon* cultivars to NaCl Stress. Plant Physiology and Biochemistry. 97: 196-206.

Vimal, S.R., Singh, J.S., Arora, N.K., Singh, S. (2017). Soil-plant-microbe interactions in stressed agriculture management. Soil Science Society. 27: 177-192.

Wu, Q.S., Xia, R.X. (2005). Effects of AM fungus on drought tolerance of citrus grafting seedling trifoliate orange/cara. Chinese Journal of Applied Ecology. 16(5): 865-869.

Wu, Q.S., Zou, Y.N. (2009). Mycorrhizal influence on nutrient uptake of citrus exposed to drought stress. Philippine Agriculture Scientist. 92: 33-38.

Yooyongwech, S., Samphumphuang, T., Tisarum, R., Theerawitaya, C., Chaum, S. (2016). Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments *via* soluble sugar and free proline. Scientia Horticulturae. 198: 107-117. DOI: 10.1016/j.scienta.2015.11.002.

Zhang, H.S., He, X.L. (2007). Effect of AM fungal on the protective system in leaves of Artemisia ordosica under drought stress. Biotechnology Bulletin. 3: 129-133.

Zhang, T., Yang, X., Guo, R., Guo, J. (2016). Response of AM fungi spor population to elevated temperature and nitrogen addition and their influence on the plant community composition and productivity. Scientific Reporter. 6: 24749. DOI: 10.1038/srep24749.

Zyprian, E., Eibach, R., Trapp, O., Schwander, F., Topfer, R. (2018). Grapevine breeding under climate change: Applicability of a molecular marker linked to veraison. Journal of Vitis. 57: 119-123. DOI: 10.5073/vitis.2018.57.119-123.