Effects of arbuscular mycorrhizal fungi (*Glomus mosseae*) on growth enhancement and nutrient (NPK) uptake of three grape (*Vitis vinifera* L.) cultivars under three different water deficit levels

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Abstract

We tested the effects of arbuscular mycorrhizal fungi (AMF) and three levels of water deficit on growth enhancement, physiological performance and nutrient uptake of three local Iranian grape cultivars. Mycorrhizal inoculation (*Glomus mosseae*) showed a significant increase in grape's growth characteristics, and variation was observed among the cultivars and field capacity percent (FC %) levels. Mycorrhiza inoculation increased significantly ($p<0.05$) the number of leaves in 'Pikani' and 'Shahroudi'. The results showed that water deficit significantly increased the chlorophyll index and decreased the stomatal conductance, leaf relative water content (RWC), leaf area index, nitrogen (N%) content, and increased proline content ($P<0.05$). Water deficit and mycorrhiza increased potassium (K%) in all cultivars and phosphorous (P%) in two cultivars ('Shahrudi' and 'Keshmeshi') significantly ($P<0.05$). By increasing the water deficit level, 'Shahrudi' and 'Keshmeshi' showed more relative drought resistance than 'Pikani'. The cultivar 'Shahrudi' showed a better symbiotic interaction with mycorrhiza and more resistance to water deficit in some traits in comparison to other two cultivars.

Keywords: field capacity, growth characteristics, mycorrhiza, NPK%, physiological performance.
Abbreviations: Arbuscular mycorrhizal fungi (AMF), field capacity percent (FC), nitrogen (N), phosphorus (P) and potassium (K), Relative water content (RWC),

Introduction

Grape, (*Vitis vinifera* L.), is one of the most important fruit crops of the world. A large proportion of vineyards are located in regions with seasonal drought and atmospheric water deficits, together with high temperatures, including Iran (Chaves et al., 2010). The global climatic changes also increase the frequency of heat waves in large parts of the world. Furthermore decrease in annual mean precipitation in many mid-latitude, and dry subtropical regions make it more erratic (Zandalinas et al., 2018). This scenario will affect crops to a greater range and number of abiotic stresses such as drought stress which is the primary cause of crop loss worldwide, reducing average yields and quality (Awasthi et al., 2014; Teskey et al., 2015). Drought stress has adverse effects on different plant growth parts by hampering key metabolic attributes including nutrient uptake and assimilation, enzyme activity, photosynthesis, protein synthesis and antioxidant metabolism (Hameed et al., 2014; FanizzaRicciardi, 2015). In recent irrigation model of viticulture, deficit irrigation emerged as a tool to mitigate the negative impact of drought on yield and quality and to save water (Chaves et al., 2010; FanizzaRicciardi, 2015; Merli et al., 2015). The effect of deficit irrigation on water savings and drought stress varies with the genotype (scion and rootstock), the environmental conditions as well as the adopted agronomic strategies (Permanhani et al., 2016). Moreover deficient irrigation and drought in viticulture

reduced the amount of sugar in the berries, and their size (Keller et al., 2016). Several studies indicated that there is wide variations in water use efficiency among grapevine cultivars (Merli et al., 2015; Permanhani et al., 2016; Medrano et al., 2018). The best-adapted grape genotypes in water restriction in semiarid climate have the leaf cooling capacity and high water use efficiency (Chaves et al., 2016). High water use efficiency genotypes avoid dehydration by closing stomata, but under hot spells, they may reach supra-optimal temperatures (Bota et al., 2016). Therefore they must possess high mesophyll resilience to heat stress (Chaves et al., 2016).

Among soil microorganisms, arbuscular mycorrhiza fungi (AMF) are capable of forming symbiotic associations with host plant roots (Trouvelot et al., 2015). AMFs are known to improve water uptake by plants and nutrient uptake at the same time (Kohler et al., 2008; Trouvelot et al., 2015). This mutualistic collaboration is based on biotrophic nutrient exchanges between the plant and the fungal companion. The host plant supplies the partner with carbon from photosynthesis (Trouvelot et al., 2015). The AMF boosts the ability of the plant through an extensive network of hyphae to absorb and accelerate water and minerals from areas unavailable to the plant root system (Hameed et al., 2014; Hashem et al., 2018). AMF communities are highly influenced by the soil characteristics, but also to a smaller

extent by the host plant development stage (SchreinerMihara, 2009; Balestrini et al., 2010). Thus, this symbiotic helps the plant to grow in the challenging conditions. The effects of mycorrhizal fungi are particularly evident in the soil with low soluble phosphorus and reduced diffusion coefficient due to water stress (Smith et al., 2011). Research results have shown that arbuscular mycorrhizal fungi can increase plant tolerance to the drought by supplying water and nutrient to the host plant (SchreinerMihara, 2009; Hameed et al., 2014; Rouphael et al., 2015; Trouvelot et al., 2015; Essahibi et al., 2018; Hashem et al., 2018; Xu et al., 2018; Zandalinas et al., 2018). AMF from grape roots mainly belongs to the Glomerales group, although other's form can be found in sandy vineyard soils (Balestrini et al., 2010). Arbuscular mycorrhiza-colonized grapevine roots can exhibit more efficient water uptake and allow grapevine to cope with water stress. During drought stress, although arbuscular mycorrhiza colonization declined, the biomass and the proline content were higher in arbuscular mycorrhiza plants than in non-arbuscular mycorrhiza plants (Valentine et al., 2006).

Iran is one of the most important grape origins in the world (This et al., 2006) and there are several high-quality local cultivars with very low information about them and AMF interaction. On the other hand, dry weather and drought stress are considered as one of the leading problems of Iranian vineyards. So that, in this research, the effects of AMF colonization was studied on growth enhancement and nutrient (NPK) uptake of three important Iranian grapes cultivars under drought stress.

Results

Enhancement of growth

Mean compression of cultivar and water deficit showed that the highest and the lowest numbers of leaves were achieved by 'Pikani' at the water deficit (FC) 80% and 40%, respectively (Table 1). By increasing the drought stress level, the number of leaves reduced significantly from 43% in 'Keshmeshi' to 63% in 'Pikani' (Table 1). Table 2 shows that AMF increased the average number of leaves in both 'Pikani' and 'Shahroudi' 23% and 37% respectively., in the other hands, fresh and dry leaves weight were significantly reduced in noninoculated 'Pikani' by AMF(Table 2). Mean comparison of cultivar, water deficit and AMF interactions showed that the 'Shahroudi' had the highest number of internode, where the number of internodes decreased significantly in 40% water deficit (Table 3). The highest fresh and dry weights of the current shoot growth and the cane was observed in 'Shahroudi' AMF inoculated vines in 80% irrigation period (Table 3). Furthermore, 'Shahroudi' had the longest stem than other two cultivars in all applied treatments (Table 3). The highest fresh and dry stem, and root weight were achieved by 'Shahroudi' at the water deficit of 60% and 40% with AMF respectively. 'Shahroudi' also had the highest total weight and fresh and dry weight of roots than two other cultivars (Table 4). 'Keshmeshi' showed to have the most extended root length at the water deficit of 60% in the inoculated soil (Table 3).

Grape physiological performance

Significant differences (p≤0.05) were observed among non-mycorrhizal and mycorrhizal plants for the chlorophyll index. 'Pikani' showed the highest and lowest chlorophyll index under the water deficit of 80% and 40% (Table 1). Chlorophyll content decreased significantly in the water deficient condition (Table 1). There were no significant reductions (p≤0.05) on stomatal conductance among non-mycorrhizal and mycorrhizal plants under three different water deficit levels. Stomatal conductance was higher in both cultivars 'Shahroudi' and 'Pikani' than 'Keshmeshi' (Table 4) and it was also significantly reduced in the 40% water deficit (Fig 1). The stomatal conductance in 'Pikani'and 'Keshmeshi' cultivars which are treated with AMF shows a lower reduction than the 'Shahroodi'cultivar (Fig 1). Relative water content (RWC) was significantly different (p≤0.05) among studied cultivars and applied water deficit levels (Table 4 & 5). RWC was higher in 'Keshmeshi' than two other's cultivars (Table 4). The heights and the lowest level of proline were produced at the water deficit of 40% and 80% (Table 5). Water deficit treatments had some significant effect (p≤0.05) on some growth and physiological characteristics of the studied cultivars (Table 5).

Nutrient enhancement

The concentration of nitrogen (N%) was significantly higher (p< 0.05) at the water deficit of 80% and 60% than 40% (Table 5). AMF plants 'Shahroudi' cultivar had the highest level of phosphorous at the water deficit of 40% (Fig 2). AMF plants showed significantly higher potassium concentration (K%) than non-mycorrhizal plants. Table 1 shows the potassium concentration in leaves of study cultivars affected by three water deficit levels.

Correlations

The correlation analysis among some traits in Table 6 showed that there is a significant positive correlation between leaf relative water content and chlorophyll index, and there is a negative correlation with stomatal conductance. With the decreasing of stomatal opening in leaf, water content increased. Potassium has a negative correlation with chlorophyll index and leaf nitrogen content. Total weight has a positive relationship with nitrogen content. The root length has a positive correlation with stomatal conductance and negative correlation with RWC. Shoot length has a positive correlation with RWC, nitrogen, and veins total weight. The number of nodes increased by increasing of shoot length, total weight, leaf nitrogen content, and RWC.

Discussion

Results showed that water deficit reduced the number of leaf and internode, fresh and dry weight of leaf and stem growth in the one-year-old vine. These growth characteristics affected by water deficit levels showed less variation for 'Pikani' than other two cultivars 'Shahroudi' and 'Keshmeshi' (Table3). Water deficit can affect plant growth significantly; morphological characteristics such as leaf, shoot, and root are the most affected parts. Thus, they can

Table 1. The effects of cultivar and water deficit levels (field capacity (FC%) on number of leaves, potassium, and chlorophyll index of three Iranian grape cultivars.

Cultivar	Water deficit (FC%)	No. of leaf	Chlorophyll index	Potassium %
Shahrudi	80	19.70±2.69 [abcZ]	20.75±1.60 [bc]	0.50±0.048 [cde]
	60	19.00±2.85 [abc]	21.18±0.84 [b]	0.64±0.041 [abc]
	40	10.00±1.36 [d]	21.19±1.04 [b]	0.59±0.042 [bcd]
Pikani	80	25.20±1.60 [a]	27.08±0.64 [a]	0.39±0.061 [e]
	60	22.5±2.60 [ab]	25.10±3.10 [ab]	0.69±0.021 [ab]
	40	9.30±1.73 [d]	16.06±1.23 [c]	0.55±0.056 [bcde]
Keshmeshi	80	22.10±1.84 [ab]	22.47±0.63 [b]	0.46±0.039 [de]
	60	18.30±2.16 [b]	24.17±1.51 [ab]	0.44±0.029 [de]
	40	12.55±1.68 [c]	20.90±1.55 [cd]	0.77±0.099 [a]

[Z] Mean ± Standard Error., Different letters within columns indicate significant differences among the treatments according to Duncan Test (P < 0.05).
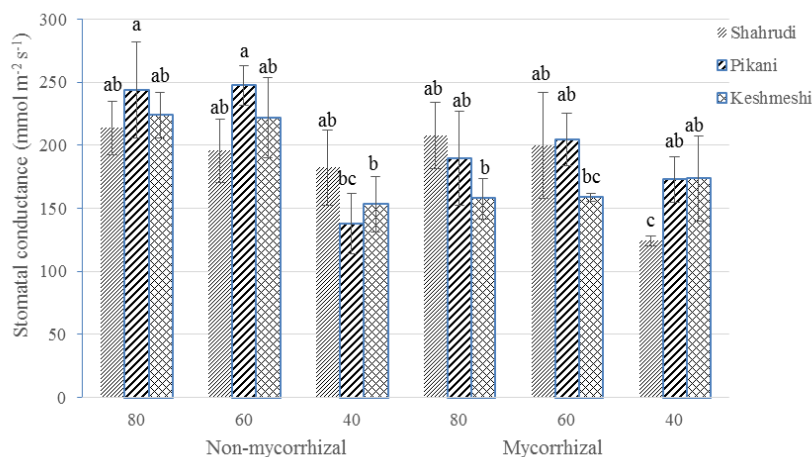

Fig 1. The effects of cultivars ('Shahroudi', 'Pikani', and 'Keshmeshi'), water deficit levels (field capacity (FC%, 80, 60, and 40), and mycorrhizal (mycorrhizal and non- mycorrhizal) on Stomatal conductance in studied grape cultivars.

Table 2. The effects of cultivar and mycorrhizal on some growth characteristics of three Iranian grape cultivars.

Cultivar	Mycorrhizal	No. of leaves	Fresh leaves weight (g)	Dry leaves weight (g)
Shahrudi	Non-mycorrhizal	12.33±1.41 [bZ]	8.22±1.07 [ab]	2.10±0.25 [ab]
	Mycorrhizal	19.66±2.56 [ab]	9.42±1.14 [ab]	2.26±0.26 [ab]
Pikani	Non-mycorrhizal	17.07±2.55 [ab]	5.04±1.19 [b]	1.64±0.27 [b]
	Mycorrhizal	22.30±2.82 [a]	8.07±1.11 [ab]	2.18±0.27 [ab]
Keshmeshi	Non-mycorrhizal	18.60±2.06 [ab]	11.99±1.08 [a]	3.23±0.27 [a]
	Mycorrhizal	17.00±1.57 [ab]	9.64±1.18 [ab]	2.66±0.25 [ab]

[Z] Mean±Standard Error., Different letters within columns indicate significant differences among the treatments according to Duncan Test (P < 0.05).
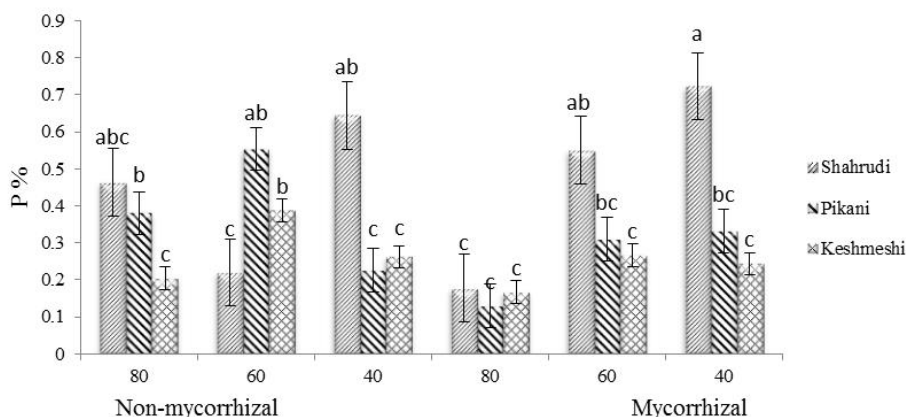

Fig 2. The effects of cultivars ('Shahroudi', 'Pikani', and 'Keshmeshi'), water deficit levels (field capacity (FC%, 80, 60, and 40), and mycorrhizal (mycorrhizal and non- mycorrhizal) on leaf phosphorus concentration (P%) in studied grape cultivars.

Table 3. The effects of mycorrhizal fungi, *Glomus mosseae,* on some of the growth parameters of three grape cultivars under water deficit levels (irrigation in three field capacity (FC%).

	Cultivar	Water deficit (FC%)	Node number	Fresh current season shoot weight (g)	Dry current season shoot weight (g)	current season shoot length (cm)	Root length (cm)	Dry root weight (g)
Non-mycorrhizal	Shahrudi	80	25±4.46 [a-dZ]	7.5±0.92 [bcd]	2.40±0.37 [bcd]	25.4±3.38 [ab]	31.8±2.49 [c]	10.94±2.31 [b]
		60	17.6±2.66 [d]	5.6±0.94 [b-e]	1.66±0.37 [b-e]	27.2±2.63 [ab]	43.4±6.69 [b]	11.04±1.45 [ab]
		40	19.6±1.21 [cd]	6.7±1.26 [b-e]	2.68±0.65 [bc]	27.8±4.33 [ab]	42.8±6.51 b	9.88±0.37 [bc]
	Pikani	80	24.25±2.87 [a-d]	5.5±0.77 [cde]	2.18±0.52 [bcd]	20±2.79 [d-g]	35.5±2.5 [bc]	7.675±1.17 [cde]
		60	31.4±1.25 [ab]	4.7±0.83 [de]	1.34±0.40 [ef]	21.4±2.23 [c-g]	52.4±6.50 [a]	6.48±0.84 [de]
		40	27.25±2.46 [abc]	4.6±0.31 [de]	1.63±0.09 [def]	18.75±2.36 [efg]	50.25±14.9 [ab]	8.22±1.56 [bcd]
	Keshmeshi	80	25.4±5.24 [a-d]	7.5±1.93 [bc]	2.74±0.97 [b]	25.4±4.14 [ab]	29.4±2.15 [c]	10.34±0.95 [bc]
		60	23±2.32 [bcd]	6.1±0.46 [b-e]	2.18±0.20 [bcd]	27.1±2.51 [ab]	38±4.14 [b]	11.2±1.32 [ab]
		40	17±1.70 [d]	4.2±0.20 [ef]	1.50±0.14 [def]	18.8±3.6 [abc]	28.6±2.61 [c]	7.96±0.55 [cde]
Mycorrhizal	Shahrudi	80	33.6±2.09 [a]	8.5±0.93 [a]	2.96±0.30 [a]	28.2±4.82 [ab]	29.8±2.41 [c]	12.58±2.74 [a]
		60	28.4±2.50 [abc]	7.7±1.45 [b]	2.30±0.54 [bcd]	32.3±3.46 [a]	43.6±5.51 [b]	11.74±2.02 [a]
		40	21.4±2.06 [cd]	4.0±0.87 [e]	1.10±0.19 [efg]	20.2±3.95 [efg]	40.1±2.9 [b]	12.98±2.04 [a]
	Pikani	80	32±4.43 [ab]	7.1±2.15 [bc]	2.53±0.73 [bc]	24±2.54 [b]	34±4.88 [bc]	7.95±1.09 [cde]
		60	31.2±1.43 [ab]	7.4±2.06 [b]	2.22±0.71 [bcd]	26.7±5.61 [abc]	37.3±6.60 [bc]	8.84±1.16 [bc]
		40	17.25±1.31 [d]	2.3±0.51 [f]	0.58±0.11 [g]	14.75±1.05 [g]	35±7.32 [b]	7.62±0.82 [cde]
	Keshmeshi	80	31±1.87 [ab]	7.0±0.52 [bc]	2.38±0.28 [bcd]	25.6±4.05 [b]	30.2±3.38 [c]	10.58±0.54 [b]
		60	25.2±1.91 [a-d]	5.0±0.41 [cde]	1.38±0.18 [ef]	26±1.25 [abc]	47.6±7.20 [b]	11.12±1.97 [ab]
		40	20.75±1.93 [cd]	4.6±0.64 [def]	1.25±0.16 [fg]	23.75±4.73 [bc]	39.5±5.67 [b]	8.3±0.58 [bcd]

[Z] : Mean±Standard Error., Different letters within columns indicate significant differences among the treatments according to Duncan Test (P < 0.05).

Table 4. The mean comparison of total fresh weight, the fresh and dry weight of roots, leaf Stomatal conductance and relative water content (RWC) in pot growing three Iranian grape cultivars under water deficit condition and mycorrhizal fungi incubation.

cultivar	Total fresh weight (g)	Fresh root weight (g)	Dry root weight (g)	Stomatal conductance (mmol m^{-2} s^{-1})	RWC
Shahrudi	84.40 ±4.05 [a]	35.63±2.17 [a]	11.52±0.75 [a]	208.33±11.4 [a]	82.22±0.92 [b]
Pikami	69.78±2.84 [b]	27.84±1.35 [b]	7.78±0.48 [c]	205.15±11.7 [a]	83.24±0.72 [ab]
Kehsmeshi	74.89±3.65 [ab]	30.50±1.36 [b]	9.97±0.49 [b]	167.90±9.4 [b]	85.16±0.69 [a]

Mean±Standard Error., Different letters within columns indicate significant differences among the treatments according to Duncan Test (P < 0.05).

Table 5. The Effect of water deficit on some growth characteristics of three Iranian grape cultivars.

Water deficit FC%	Fresh leaves weight	Dry leaves weight	Total fresh weight	Nitrogen %	Relative water content	Proline (mg g^{-1}dw)
80	10.14±0.82 [a]	2.99±0.19 [a]	84.11±4.58 a	2.47±0.12 [a]	84.82±0.84 [a]	0.32±0.05 [b]
60	7.54±0.77 [b]	1.78±0.18 [b]	77.80±3.05 ab	2.28±0.13 [a]	84.36±0.58 [a]	0.43±0.03 [a]
40	3.37±0.68 [c]	1.13±0.16 [c]	67.95±2.90 b	1.92±0.08 [b]	81.28±0.90 [b]	0. 44±0.04 [a]

[z]Mean±Standard Error. Different letters within columns indicate significant differences among the treatments according to Duncan Test (P < 0.05).

Table 6. Pearson correlation among some physiological, NPK and growth characters in pot growing three Iranian grape cultivars under water deficit condition and mycorrhizal fungi incubation

	Chlorophyll index	Stomatal conductance	RWC	Nitrogen	Potassium	Phosphore	Proline	Total weight	Root length	Shoot length	Node number
Chlorophyll index	1										
Stomatal conductance	-0.10	1									
RWC	0.28**	-0.31**	1								
Nitrogen	-0.13	0.05	0.16	1							
Potassium	-0.30**	0.02	-0.05	-0.25*	1						
Phosphor	-0.07	0.08	-0.06	0.16	0.08	1					
Proline	0.09	-0.03	-0.10	0.04	0.02	0.03	1				
Total weight	-0.06	-0.12	0.13	0.25*	0.02	0.05	-0.04	1			
Root length	-0.04	0.25*	-0.27**	-0.04	0.08	0.16	-0.04	-0.20	1		
Shoot length	0.06	-0.06	0.24*	0.28*	0.06	-0.02	-0.07	0.23*	-0.04		
Node number	0.08	0.16	0.21*	0.42**	0.11	0.10	-0.10	0.31**	0.10	0.28**	1

**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.

play key roles in plant adaptation to drought stress (Farooq et al., 2009). In respond to water deficit, plants usually reduce the leaf number and leaf area to decrease the water budget for the cost of reduced performance. Research has been shown that drought stress reduced the number of leaf vines and ultimately reduced growth (FanizzaRicciardi, 2015), which the current study also confirmed that absorbing enough water by the plant is one of the main factors in responding to drought. Roots are the only part to obtain water from the soil, the root growth such as; length, density, size, weights, etc. are critical for plants to overcome drought stress (Farooq et al., 2009). In this study, we observed that 'Pikani' had the longest root at the water deficit of 60% (Table 3). Reducing the aerial parts weight by the plant is an obvious response to the drought stress. In addition, it has been noted that under drought stress, plants reduce root weight (Farooq et al., 2009; FanizzaRicciardi, 2015). The results of this experiment also indicated that with increasing the water deficit level, a reduction in aerial parts and root weight was observed. The results indicated a potential for 'Pikani' to be used for selecting drought-tolerant rootstock in grape.

The results of the current study are indicating that the highest number of leaves produced by AMF vines (Table 2). The most substantial fresh and dry stem weight, the highest number of internode, and the most root length was observed in water deficit of 60% with AMF (Table 3). Also, the highest root dry weight was recorded at water deficit of 40% with AMF (Table 3). Enhancement of vegetative growth by AMF treatment has already been reported by researchers in some species, including grape (Caravaca et al., 2005; Wu et al., 2006; Smith et al., 2011; Trouvelot et al., 2015). The ability of the AMF treatment to enhance the growth in some grape rootstocks and cultivars has been reported, although the response to the treatments among rootstocks and cultivars was reported differently (Schreiner, 2005; Eftekhari et al., 2012; Trouvelot et al., 2015). Also in this study, respond to AMF symbiosis was different among studied cultivars, whereas 'Shahroudi' showed a better symbiosis than other two cultivars (Table 2). AMF symbiosis in agricultural systems is the most well-known type of relationship. In this mutual symbiosis, the host plant consumes the minerals and water obtained by fungus, and the host plant provides carbohydrates consumed by the fungus (Smith et al., 2011; Rouphael et al., 2015). In addition to providing nutrients, some other potential benefits of AMF colonization of host plants include growth and increased resistance to pathogens and other environmental stresses (Smith et al., 2011; Trouvelot et al., 2015).

The stomatal conductance decreased with increasing drought stress in 40% of field capacity and without AMF. At all three levels of water deficit, stomatal conductance was more in AMF than without AMF. The effect on stomatal conductance showed 'Shahroudi', and 'Pikani' has a higher stomatal conductance than 'Keshmeshi'. The results showed that the stomatal conductance decreased with increasing drought stress and the correlation analysis show that when the stomatal conductance decrease leaf relative water content was increased. Plants have specific mechanisms to deal with drought conditions to increase water-use efficiency. Closing leaf stomatal is the initial response of plants to drought stress. Mechanism of stomatal opening and closing in many plants, as well as grapes, are classified

into two types of isohydric and anisohydric(HugaldeVila, 2014). In the group of isohydric, the stomata are closed in potential water reduction, while in the other group stomatal are not closed in medium stress condition. Several studies showed that grape varieties with anisohydric properties can tolerate medium stress conditions and return faster to the original state (Pou et al., 2012). Gradual reduction of photosynthesis and stomatal conductance with increasing drought stress even under stress is one of the characteristics of plants adapted to drought (Farooq et al., 2009; FanizzaRicciardi, 2015). AMF colonization accelerates nutrient uptake and most possibly upsurges photosynthetic rates in grapevine (Mortimer et al., 2005; Merli et al., 2015). However, available data on AMF to increase the photosynthetic rate in grapevine is limited and confusing (Trouvelot et al., 2015). Results of this study show that stomatal conductance rates are lower in AMF treatment compared to those with no AMF (Fig 1).

The results of RWC of leaf tissue showed that 'Keshmeshi' had more RWC (85.16%) in leaf tissue than the other two cultivars. With increasing drought stress level, relative water content decreases (Table 5). According to Flexas et al. (2004) photosynthesis in some species such as grape decreases progressively with increasing drought stress level without a reduction in the RWC. Thus, the cultivars that hold the RWC higher under drought stress can be introduced as a cultivar more tolerant to drought stress (Flexas et al., 2004).

The results of the proline measurement in the fresh leaves showed that with increasing drought the proline level increased, where at the water deficit of 40%, the leaf had the maximum proline content (Table 4). Valentine et al. (2006) reported an accumulation of proline with increasing drought stress level in grapevine. Proline is an essential amino acid for plants that increase under drought stress and is not toxic in high concentrations. Proline help plant to uptake water under the drought condition by lowering the water potential of the roots, it could perform as a sink for energy to adjust redox capabilities under drought stress (Ankita et al., 2017).

Leaf nutrient analysis results showed that with increasing drought stress the leaf nitrogen content reduced where the lowest amount of nitrogen (1.92%) was observed at the water deficit of 40% (Table 5). Drought stress also increased the leaf phosphorus content in 'Shahroodi' with and without AMF. Research showed that an increase in water deficit causes a reduction in the content of the nutritive element, particularly for nitrogen and phosphorus(Farooq et al., 2009). Water stress in non-irrigated vines reduced leaf chlorophyll and nitrogen level consequently. Water flow accompanies the soil-root-shoot pathway is important for the vine to uptake nutrient from the soil (Gonzalez-Dugo et al., 2010; Keller et al., 2016). As shown in Table 1; AMF and water deficit increased the leaf potassium content significantly (p≤0.05). Increased potassium was higher in 'Keshmeshi' than other two cultivars. Potassium is one of the most cations, which is required by plants that accumulate during osmotic stress to regulate osmotic pressure and stomatal. The symbiosis of AMF with some plant species under water stress conditions increases the amount nutrition elements significantly (Smith et al., 2011; Trouvelot et al., 2015). However, the results showed that AMF have the only positive effect of phosphor absorption in water deficient condition. It seems that an increase in the

absorption of nutrients by AMF plants is due to the development of mycelium associated with the internal root tissues and an additional uptake system supplemental to the plant root, which allow to the root to access to larger volumes of soil that are inaccessible to feeder roots without AMF.

Conclusion

Water deficit can affect grapevine growth significantly; morphological characteristics such as leaf, shoot, and root are the most affected parts. By increasing the water deficit level, a reduction in aerial parts and root weight was observed. The number of leaves obviously increase in vines inoculated by AMF. 'Shahroudi' and 'Pikani' has a higher stomatal conductance than 'Keshmeshi' and show an isohydric behavior. Finally, we can conclude that mycorrhiza inoculation can demolish the effects of drought stress and can be used as an anti-stress agent in vineyards. However, the response to the mycorrhiza varied among the studied cultivars.

Materials and methods

Plant materials

Three important cultivars of grapes including 'Shahrudi', 'Pikani', and 'Keshmeshi' were chosen. This cultivar is mainly cultivated in the vineyard of Shahrood and Sabzevar areas, The main grape producing areas, in north and northeast of Iran.

Experiment location

The field and laboratory tests were done at the Shahrood University of Technology, located within the Bastam region (36˚ 25′ 05″ N and 54 58′ 35″) during 2014-2016 seasons.

Vine cultivation

One-year-old vines *Vitis vinifera* cv. 'Shahrudi', 'Pikani', and 'Keshmeshi' were purchased from a commercial supplier (Agro-industry, Joeyn, Iran) and five vines (pots) were used per treatment. The plants were established in eight-liter pots filled with sterilized loam-sand. For the AMF treatments, 100g of live mycorrhizal inoculum (substrate, *Glomus mosseae,* Toran Biotech, Shahrood-Iran) placed under the root of vines in each pot before planting. Vines were grown as a tunnel greenhouse for three months, considering as inoculation establishment time. During this time, all treatments received a similar horticultural practice such as irrigation, nutrient, etc. Vines were pruned to keep a single shoot per vine.

Treatments

After 12 weeks following transplantation and inoculation, the vines were gone under-water deficit for two months based on the field capacity (FC); FC 80% as a control, FC 60% as a moderate water deficit, and FC 40% as a severe water deficit. The mean weight of the soil moisture was calculated from five pots, then water deficit level considered as FC% moisture percentage and the amount calculated as gram water (H_2O). To achieve the desired moisture content or FC% in all treatments, the calculated amount water (g) was added to each pot (treatment). During water deficit time, only the water supply was varied among the treatments, and all other practical practices such as nutrient supply remained the same for all treatments.

Measurement of growth characteristics and physiological parameters

At the end of the experiment period, growth characteristics such as; shoot length, root length (ruler mm), the number of leaves, the number of internodes, leaf area index, fresh and dry weights of the different parts were recorded. To measure the fresh and dry weight an analytical scale with the precision of 0.001g was used. Plants pulled out from the pot, and after cleaning the root, they were divided into different parts (root, shoot, leaf). After measuring the fresh weight, different parts are placed into the oven at 65˚C for 48h to record the dry weight. Chlorophyll index was measured by a SPAD 502 set (Minolta Camera Co., Osaka, *Japan).* An SC-1 Porometer (Decagon Devices, WA, USA) was used to measure stomatal conductance. Chlorophyll index *and* stomatal conductance were measured seven weeks after the water deficit induced on leaves number 8 and 9 from each vine during 11 am to 1 pm before irrigation applied*.*

Leaf relative water content (RWC) was obtained based on the methods of (YamasakiDillenburg, 1999). Single leaves were first detached from the stem and then weighed to record fresh mass (FM) at the harvest point. To determine the turgid mass (TM), leaves was floated in deionized water inside a sealed Petri plate for 24h in low light density condition at 4˚C, then leaf surface wiped gently with the tissue paper from the water. To obtain the dry mass (DM), leaf samples were located in a pre-heated oven at 70°C for 48h. An analytical scale with a precision of 0.0001g was used for doing the measurements. FM, TM and DM Values were used to determine RWC using the following equation;

$$RWC(\%) = [(FM-DM)/(TM-DM)]\times100$$

The proline concentration of the freshly harvested leaves was determined using a spectrophotometer (Perkin Elmer, Lambda EZ201, CA, USA) at 520nm based on the ninhydrin method explained by (Bates et al., 1973).

Measurements of nitrogen (N), phosphorus (P) and potassium (K)

The dried leave samples were powdered using a pestle and mortar. Grounded samples were placed at 550°C for 6 h. The ash was mixed up with 2 M hot HCl, then filtered into a 50 mL flask and made up to 50 mL with deionized water. Phosphorus was determined in these sample solutions using a UV/visible spectrophotometer (Perkin Elmer, Lambda EZ201, CA, USA) at 470nm. Potassium was determined based on Atomic Emission Spectrometry's method using a flame photometer set (Corning 400, Sheffield, UK). Using the semi-automatic Kjeldahl set (Vapodest 20S, Gerhardt, Königswinter, Germany) total N was determined in 0.1g dried weighted samples (ChapmanPratt, 1982).

Statistical analysis

The experiment was laid out as a completely randomized design (CRD) with three factorial combination and five replications of each treatment combination, which are completely randomized in greenhouse condition. The three factors were including: first- the three grape cultivars ('Shahrudi', 'Pikani', and 'Keshmeshi'), second- arbuscular mycorrhizal inoculation and non-inoculation, and third-three different levels of water deficit based on field capacity (FC); FC 80% as a control, FC 60% as a moderate water deficit, and FC 40% as a severe water deficit. Data were analyzed by analysis of variance (ANOVA), mean treatment differences among the treatments were tested at $P < 0.05$ by Duncan test. SAS, version 9.3 statistical software (SAS Institute, Cary, NC, USA) was used for data analyzing. A Pearson correlation among traits was done with SPSS 17.

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