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Research Article

Change in Growth and Photosynthetic Parameters of Lentil (*Lens culinaris* Medik.) in Response to Methanol Foliar Application and Drought Stress

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ABSTRACT

There are many reports on the role of methanol (ME) foliar application for increasing drought tolerance in C_3 plants. For this reason, we examined the effects of ME treatments on photosynthetic and growth responses of lentil to water shortage stress. This study was a factorial experiment with a completely randomized design and three replications. ME spraying was at five levels; control (0), 5, 15, 25 and 35%. Water stress was applied in three regimes of field capacity (FC); well-watered (WW, 100% of FC), moderate water-limited (MWL, 75% of FC) and severe water-limited (SWL, 25% of field capacity). ME application was carried out three times per 10 days in the growing season. We found that the ME treatments under WW and MWL conditions had a positive role in enhancing growth and photosynthetic parameters. Results demonstrated that under WW and MWL, plant height, number of pods, leaf dry weight (DW), shoot DW, root DW, chlorophyll (Chl) *a*, *b*, total Chl content (Chl *a+b*), leaf water content (LWC), maximal quantum yield of PSII photochemistry (F_v/F_m), intercellular CO_2 concentration (C_i), net photosynthetic rate (P_N) and water-use efficiency (WUE) were significantly increased compared with control. Under SWL, plant height, leaf and stem DW, F_v/F_m, C_i and P_N increased by ME treatments. The results suggest that ME foliar application can ameliorate the negative effects of water shortage stress on lentil (*Lens culinaris* Medik.).

Key words: Abiotic stress, Chlorophyll content, Gas exchange, Methanol spraying, Water-use efficiency

INTRODUCTION

Legumes such as lentil (*Lens culinaris* Medik.) are a particularly important source of protein in the diet of many people throughout the world. The protein content in lentil is about twice that of cereals, so they provide a good source of protein for humans (Oweis *et al*., 2005). Lentil is a crop grown all over the world and is compatible with different climatic conditions; from temperate to thermal and humid to arid (Erskine *et al*., 2009). Its features such as nitrification, deep rooting system and effective use of precipitation have caused this crop to play important roles in the stability of farming production systems (Erskine *et al*., 2009).

Approximately one third of the world's arable lands significantly lack water (Ganjeali *et al*., 2011). Water shortage is considered as one of the most important environmental stresses limiting photosynthesis, plant growth and crop productivity in Iran (Hosseinzadeh *et al*., 2015). General plant symptoms to water shortage are decreasing growth and productivity, accelerate leaf senescence, limit CO_2 diffusion to chloroplasts because of stomata closure and reduce photosynthesis rate (Rahbarian *et al*., 2011). The closing stomata which reduce transpiration and conserve water in plants is the first mechanism of plants against dehydration stress (Sikder *et al*., 2015). Photosynthesis is a highly complex mechanism and one of the main targets to improve lentil yield (Erskine *et al*., 2009). Increasing the concentration of CO_2 can neutralize the effect caused by water deficit stress. Thus, the use of substances that can increase the concentration of CO_2 in a plant will improve photosynthesis rate and yield under water deficit conditions (Ramadant and Omran, 2005). Foliar applied methanol is a method which increases C_3 plant CO_2 assimilation in unit area (Ramirez *et al*., 2006). Several reports suggest that photosynthetic pigments such as Chl a, b and carotenoids (Car) help to stabilize photosynthesis under drought stress (Rahbarian *et al*., 2011, Lotfi *et al*., 2015). In a study on chickpea and grapevine plants, it was observed that methanol foliar spray led to a significant increase in chlorophyll content in the studied plants

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(Ramandant and Omran, 2005, Hosseinzadeh *et al*., 2014). The LWC and WUE are key parameters for evaluation of plant tolerance to water stress (Condon *et al*., 2004, XIA *et al*., 2014). Plants that are tolerant to water deficit stress show controlled stomata function for carbon fixation; thus, WUE and LWC increases in these plants (Sikder *et al*., 2015).

Water deficit stress produces reactive oxygen species (ROS) in plants which are responsible for oxidative stress (Lotfi *et al*., 2015). The reaction centers of PSI and PSII in chloroplasts are major sites of ROS generation (Chakraborty and Pradhan, 2011). F_v/F_m is used to determine the amount of damage to the PSII (Hosseinzadeh *et al*., 2015). The amount of F_v/F_m is a function of leaf photosynthetic activity and can be used to determine the duration of environmental stress (Bencze *et al*., 2014). Therefore, investigation of F_v/F_m is considered as an important indicator for evaluating the integrity of photosynthetic process within a leaf and provides a technique for quantifying the tolerance of plants to water stress (Lu *et al*., 2002).

The aim of this research was to study the effect of foliar application of ME on growth and photosynthetic parameters in lentil plants with different water shortage stress. We hypothesized that ME foliar application could improve growth and photosynthetic parameters of lentil under water stress.

MATERIALS AND METHODS

Experimental details

The experiment was performed under controlled condition at Khatam-Alanbia University of Behbahan in Iran. Tests were done as a factorial experiment in a fully-random format with three replications. The first treatment prepared four ratios of ME and water (W) as follows: control (100% water); 5% ME + 95% W; 15% ME + 85% W; 25% ME + 75% W and 35% ME + 65% W. The second treatment was water deficit stress as follows: WW (100% FC); MWL (75% FC); SWL (25% FC). The lentil seeds were soaked in a solution containing 40% sodium hypochlorite for 30 min for superficial sterilization and washed water to remove any remnants of sodium hypochlorite. Five seeds were sown in pots (26 cm in diameter × 24 cm in depth) filled with a mixture of sandy clay loam and this was reduced to three seedlings in each pot after emergence. Plants were kept in a phytotron chamber at 25°C and 20°C (day/night), 50% relative humidity and photoperiod of 12.5 h and 11.5 h (day/night). After applying standard irrigation (100% FC) for two weeks until lentil seeds had become green, then, water shortage in the soil was controlled by weighing and applying enough water for the daily maintenance of the desired FC. Foliar application of ME was conducted three periods during the plant-growing season and at 10 d intervals. The first foliar application was carried out 4 weeks after sowing (seedling stage) and subsequent foliar application were conducted at 6 weeks after sowing (flowering stage) and 7 weeks after sowing (podding stage). The ME solution was sprayed continuously until the droplets on a leaf reached saturation. The sprayer had a volume of 1.5 L, an attempt was made to position it 20 cm above the bushes, and foliar application was applied on the designated days at 9-10 am. The traits were measuring 1 d after third foliar application.

Measurements of growth

The shoots and roots were separated and growth traits of plant height, number of pods and DW of stems, roots and leaves were measured at the end of the experiment. The stems, roots and leaves were separately oven-dried at 80°C for 48 h and then DW was determined.

Measurements of photosynthetic pigments

Leaf Chl and Car was determined according to Lichtenthaller and Wellburn (1983) method. 0.1 g of leaves added to 4 ml of 80% acetone was grinded in a wooden mortar and the resulting solution centrifuged at 3000 rpm for 5 min. Absorbance of centrifuged extracts was measured using a spectrophotometer (Model SPEKOL 2000, analyticjena , Germany) at wavelengths of 647, 664 and 470 nm, respectively.

Measurements of LWC

For the measurement of LWC healthy and developed leaves were harvested and weighed to determine the fresh weight (FW). Thereafter, leaves were immersed in distilled water for 48 h to determine turgor weight (TW). Next, the leaves were dried in an oven at 70°C for 48 h and their DW was calculated (Karimi *et al*., 2015). LWC (%) = (FW-DW/TW-DW) × 100

Measurements of Gas-exchange parameters

C_i [μmol (CO_2) mol^{-1}], P_N [μmol (CO_2) m^{-2} s^{-1}] and E [mmol (H_2O) m^{-2} s^{-1}] were determined in fully expanded leaves using a portable infrared gas analyzer (*KR8700 system; Korea Tech Inc. Suwon.,* Korea) in conjunction with an automatic leaf chamber. Leaf chamber conditions were adjusted by the gas analyzer and leaf temperature, CO_2 concentration, PPFD and relative humidity were set to match conditions in the phytotron chamber. The instantaneous WUE was calculated automatically by the gas analyzer device. Measurements were taken from healthy and developed leaves of lentil (third and fourth leaves under uniform conditions for all plants).

Measurement of F_v/F_m

F_v/F_m ratio was measured at room temperature (25°C), using a portable fluorometer (*Pocket PEA, Hansatech, Instruments Ltd., King's Lynn, Norfolk,* England). F_v/F_m was determined automatically as: $[F_v/F_m=(F_m–F_0)/F_m]$; where F_m and F_0 were maximum and initial fluorescence yields of dark-adapted leaves, respectively (Hosseinzadeh *et al*., 2015). The samples were removed from the phytotron chamber and placed in saturating light (3,500 μmol m^{-2} s^{-1}) after 20 min of adaptation to dark. The leaves used for measuring gas analyzer were also used to specify F_v/F_m ratio. For F_v/F_m ratio and gas analyzer parameters, an average of five records from each individual leaf was considered for each replicate.

Statistical analyses

The data presented to the mean values±standard deviation (SD) of three replicates. Statistical analyses were made by *MASTAT*-C. Statistical analyses were made by *MASTAT*-C. Level of significance was determined by the analysis of variance (ANOVA). Means were compared using the *Duncan*'s multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Morphological features

The results showed that SWL significantly decreased the morphological features of plant height, number of pods, leaf, shoot and root dry weight at all levels (Table 1). A comparison of means on the plant height indicated that this trait increased for all ME treatments over that for the control under WW and SWL. Under MWL condition, results indicated that methanol foliar application 15 and 25% levels resulted in a significant increase in plant height compared with the control (Table 1). Results for number of pods indicated that under WW and MWL, concentration level of 15% led to a significant increase in the number of pods in comparison with the control. Under SWL, level of methanol did not show significant difference compared to the control (Table 1). Numerous studies have shown that the height of a plant and number of pods is reduced by a shortage of usable water (Rahbarian *et al*., 2011). After foliar application, ME changes to formaldehyde through oxidase methanol enzyme and then changes to formate (Methanoic acid). The formate is then converted to CO_2 by the dehydrogenase formate enzyme that increases intracellular CO_2 in the plant (Hosseinzadeh *et al*., 2014). Therefore, as a carbon source, ME can play a role in developing CO_2 assimilation and net-photosynthesis (Gout *et al*., 2000). An investigation on flax, reported that spraying a solution of methanol might have stimulated growth and increased height in the treated plants by increasing cytokinin levels and cell division (Ramirez *et al*., 2006).

Table 1 shows that the ME treatments (5, 15, 25 and 35%) under both WW and SWL conditions increased the shoot DW. Under MWL conditions, ME treatments at 5, 15 and 25% concentrations resulted in a significant elevation of stem DW compared with control level. ME foliar application significantly increased the leaf DW, under WW and MWL. The 5 and 15% ME treatments significantly increased leaf DW when compared to the control group under SWL (Table 1). Comparison of data means indicated that under WW conditions the root DW showed a significant increase compared with the control. The application of ME at 5 and 15% concentrations increased root DW under the MWL conditions over that for the control, but no significant difference was observed under SWL between the ME and control treatments (Table 1). The DW of a plant is an important morphological feature and is used to determine which plants are susceptible or resistant to water deficit stress (Ganjeali *et al*., 2011). Previous studies have demonstrated that the DW of aerial organs or roots is directly related to the rate of plant photosynthesis; DW increases as photosynthesis increases (Yang and Li, 2015). ME affects the photosynthetic capacity of plants and increases their performance, especially under environmental stress (Makhdum *et al*., 2002, Hosseinzadeh *et al*., 2012). ME play an important role in increasing photosynthesis by increasing CO_2 in the mesophyll cells of the leaf; thus, increasing the DW of the plant can result from an increase in photosynthesis in the mesophyll cells (Gout *et al*., 2000).

Photosynthetic pigments and LWC

The results showed that photosynthetic pigments and LWC decreased significantly in response to SWL conditions (Table 2). Table 2 shows a significant increase in Chl a for the 5, 15 and 25% ME treatments under WW and MWL compared to the control. Under conditions of SWL, ME treatments showed no significant difference. A comparison of the results demonstrated that under WW conditions, treatments at concentrations of 5, 15 and 25% elevated Chl b in comparison with the control. In MWL conditions, ME foliar application at 25% concentration let to a significant increase of Chl b compared to the control. ME treatments had no significant difference in conditions of SWL (Table 2). The results shown on Table 2 demonstrate that under WW and MWL conditions, treatments of 5, 15 and 25% concentrations led to a significant increase Chl [*a*+*b*] in comparison with the control. In conditions of SWL, the use of methanol had no a significant effect on Chl [*a*+*b*]. The highest carotenoid content was observed for 25% ME treatment under WW, but this was not significantly different than the results for the 15% ME treatment under WW conditions. The lowest carotenoid content was recorded for the control group under SWL, but there was no significant difference with the results of all ME treatments under SWL (Table 2). Decreasing the water available for plants and, thus, the occurrence of water shortage stress, decreased the photosynthetic pigments (Chl a, Chl b, Carotenoids and Chl [*a*+*b*]) in green leaf tissue. This reduction was probably the result of the decrease in the size of the leaf cells (and the subsequent decrease in leaf area) and decrease in the density of chlorophyll (Sikder *et al*., 2015) along with increased production of ROS (Rahbarian *et al*., 2011). Several studies have shown that in plants under water stress, the absorption of Mg and Fe in the soil is reduced which then results in a reduction in carotenoids and Chl synthesis (Flexas and Medrano, 2008, Rahbarian *et al*., 2011). Mg is a precursor of Chl that under water shortage stress, decrease its participation for the synthesis of Chl (Zlatev and Yordanov, 2004). Fe plays a main role in the destruction of ROS and chlorophyll stability in plants (Hosseinzadeh *et al*., 2015). In experiments on chickpea plant, it was observed that the effect of methanol on root features such as root length, diameter, DW and root area is effective, which contributes to assimilation of nutrient elements, especially Mg and Fe (Hosseinzadeh *et al*., 2012). The results of root DW on lentil are consistent with the results on chickpea plant. It appears that methanol, through the absorption of iron by the prosthetic group of hemeproteins (CAT, POX and SOD) can destroy the ROS in plants (Atik, 2013, Lotfi *et al*., 2015).

Under WW condition, ME treatments were placed in a statistical group and the control was placed in a different statistical group, results showed that ME treatments led to increased LWC under WW conditions. The use of 15% ME treatment, under MWL, increased LWC to 11% compared to control and that there was no significant difference for the 25% ME treatment (Table 2). Under conditions of water shortage in soil, the LWC is an appropriate index to assess the amount of water in the leaves (Karimi *et al*., 2015). The LWC decreases under environmental stress such as drought and salinity (Wise *et al*., 1990). One method of increasing the resistance of plants to water stress is to increase the LWC (Rahbarian *et al*., 2011). Studies have reported a doubling of sugar in the leaves as the cause of increased LWC in plants treated with methanol (Gout *et al*., 2000, Downie *et al*., 2004). Methanol is metabolized after foliar application and the increasing C_i increase photosynthesis and the production of carbohydrates in the leaves (Gout *et al*., 2000, Safarzade Vishkaei, 2008).

Photosynthetic features

Results indicated that ME foliar application at 35% level resulted in a significant decrease in F_v/F_m compared with the control in all treatments of water shortage stress. ME treatments (5, 15 and 25%) enhanced the F_v/F_m ratio under WW and MWL more than that of control. The 25% ME treatment significantly increased F_v/F_m when compared to the control group under SWL (Table 3). Chl a fluorescence measurement is a suitable index for evaluating photosynthetic apparatus in plants exposed to environmental stress (Giorio 2011). Decreasing the F_v/F_m ratio is a reason for the significant effect of environmental stresses (including drought and heat) on photosynthetic efficiency caused by a decline in the transfer of electrons from PSII to PSI and light protection (Sikder *et al*., 2015). Numerous studies the destructive effects of water stress on reaction centers of PSI, oxygen-evolving complex and D_1 protein of PSII has been reported (Zlatev and Yordanov 2004, Liu *et al*., 2015). Hosseinzadeh *et al*., (2014) reports showed that ME foliar application on chickpea increased resistance to drought stress. Our results showed that the application of the ME reduced negative effects of water shortage stress in lentil.

Table 1: Effect of methanol (ME) treatments on plant height, number of pods, leaf dry weight (DW), shoot DW and root DW of lentil, grown under three levels of water-limited. Data are means±SD (*n* = 5). Difference among data of each column followed by the same letter was not statistically significant (P<0.05).

Treatments/ME	Plant height [cm]	Number of pods	Leaf DW [g plant^{-1}]	Shoot DW [g plant^{-1}]	Root DW [g plant^{-1}]
Well-watered (100% field of capacity)					
Control	31.87±1.45^{b}	5.0±0.57^{cde}	0.263±0.32^{f}	1.213±0.21^{b}	0.473±0.10^{de}
5%	34.83±2.33^{a}	7.3±1.15^{a}	0.306±0.55^{ab}	1.533±0.09^{a}	0.626±0.14^{a}
15%	36.27±1.64^{a}	7.0±1.15^{ab}	0.316±0.78^{a}	1.557±0.32^{a}	0.653±0.10^{a}
25%	34.77±3.05^{a}	6.0±0.57^{abc}	0.296±0.14^{de}	1.520±0.28^{a}	0.566±0.05^{b}
35%	35.33±2.19^{a}	6.0±0.50^{abc}	0.297±0.22^{de}	1.505±0.10^{a}	0.530±0.15^{bc}
Moderate water-limited (75% field of capacity)					
Control	26.97±0.55^{d}	4.3±0.57^{de}	0.210±0.19^{f}	0.916±0.06^{d}	0.406±0.13^{fg}
5%	28.77±3.13^{cd}	5.6±1.15^{bcd}	0.230±0.26^{e}	1.137±0.09^{bc}	0.473±0.18^{de}
15%	29.17±2.62^{c}	6.0±1.52^{abc}	0.246±0.05^{de}	1.270±0.45^{b}	0.483±0.22^{cd}
25%	29.70±1.56^{c}	4.6±1.00^{cde}	0.241±0.64^{de}	1.183±0.19^{bc}	0.453±0.24^{def}
35%	28.75±0.91^{cd}	4.4±0.57^{de}	0.231±0.12^{e}	0.918±0.19^{d}	0.420±0.08^{efg}
Severe water-limited (25% field of capacity)					
Control	21.27±2.12^{f}	2.6±0.28^{f}	0.173±0.08^{h}	0.703±0.23^{e}	0.393±0.18^{g}
5%	23.33±1.54^{e}	3.6±0.50^{ef}	0.193±0.10^{fg}	0.923±0.52^{d}	0.406±0.10^{fg}
15%	23.67±1.15^{e}	4.0±1.00^{ef}	0.196±0.12^{fg}	1.013±0.44^{cd}	0.416±0.05^{efg}
25%	24.63±1.59^{e}	4.0±1.15^{ef}	0.190±0.19^{gh}	1.020±0.26^{cd}	0.393±0.21^{g}
35%	23.32±1.01^{e}	3.5 ±0.86^{ef}	0.191±0.05^{gh}	0.925±0.21^{d}	0.383±0.15^{g}

Table 2: Effect of methanol (ME) treatments on chlorophyll (Chl) a, b, total chlorophyll content (Chl (a+b)]), carotenoids (Car) and leaf water content (LWC) of lentil leaves, grown under three levels of water-limited. Data are means±SD (*n* = 5). Difference among data of each column followed by *the same letter* was not statistically significant (P<0.05).

Treatments /ME	Chl a [mg g^{-1}(FW)]	Chl b [mg g^{-1}(FW)]	Car [mg g^{-1}(FW)]	Total Chl [Chl (*a*+*b*)]	LWC [%]
Well-watered (100% field of capacity)					
Control	2.79±0.45^{cd}	1.780±0.38^{d}	1.247±0.15^{cde}	4.57±0.78^{bc}	0.633±0.02^{b}
5%	3.93±0.13^{b}	2.673±0.20^{a}	1.373±0.25^{bc}	6.60±0.25^{a}	0.704±0.05^{a}
15%	4.08±0.51^{b}	2.343±0.15^{b}	1.540±0.03^{ab}	6.42±0.06^{a}	0.705±0.05^{a}
25%	4.98±0.22^{a}	2.153±0.03^{bc}	1.707±0.10^{a}	7.13±0.92^{a}	0.711±0.01^{a}
35%	2.31±0.19^{de}	1.867±0.04^{cd}	1.327±0.43^{cd}	4.18±0.62^{cd}	0.700±0.06^{a}
Moderate water-limited (75% field of capacity)					
Control	1.91±0.22^{e}	1.270±0.02^{fgh}	1.093±0.08^{ef}	3.18±0.24^{e}	0.629±0.03^{b}
5%	3.06±0.05^{c}	1.420±0.41^{efg}	1.163±0.01^{de}	4.48±0.32^{bc}	0.643±0.05^{b}
15%	3.09±0.11^{c}	1.570±0.22^{def}	1.213±0.09^{cde}	4.66±0.15^{bc}	0.702±0.01^{a}
25%	3.48±0.06^{bc}	1.663±0.15^{de}	1.200±0.08^{cde}	5.14±0.08^{b}	0.688±0.02^{ab}
35%	2.08±0.28^{e}	1.357±0.44^{efgh}	1.080±0.11^{ef}	3.44±0.64^{de}	0.628±0.05^{b}
Severe water-limited (25% field of capacity)					
Control	1.16±0.06^{f}	1.040±0.10^{h}	0.876±0.15^{g}	2.20±0.44^{f}	0.610±0.08^{c}
5%	1.19±0.19^{f}	1.103±0.08^{gh}	0.943±0.25^{fg}	2.30±0.22^{f}	0.611±0.01^{c}
15%	1.22±0.05^{f}	1.110±0.34^{gh}	0.883±0.22^{g}	2.33±0.56^{f}	0.610±0.03^{c}
25%	1.20±0.15^{f}	1.117±0.22^{gh}	0.933±0.22^{fg}	2.31±0.79^{f}	0.608±0.05^{c}
35%	1.13±0.35^{f}	1.053±0.94^{h}	0.863±0.38^{g}	2.18±0.19^{f}	0.604±0.02^{c}

Table 3: Effect of methanol (ME) treatments on maximal quantum yield of PSII photochemistry (F_v/F_m), intercellular CO_2 concentration (C_i), net-photosynthesis rate (P_N), transpiration rate (E) and water-use efficiency (WUE) of lentil leaves, grown under three levels of water-limited. Data are means±SD (n = 5). Difference among data of each column followed by *the same letter* was not statistically significant (P<0.05)

Treatments/ME	F_v/F_m	C_i [µmol (CO_2) mol^{-1}]	$_N$[µmol (CO_2) m^{-2} s^{-1}]	E [mmol (H_2O) m^{-2} s^{-1}]	WUE
Well-watered (100% field of capacity)					
Control	$0.686±0.045^{e}$	$344.7±28.2^{cde}$	$7.87±1.15^{cd}$	$89.46±8.7^{a}$	$65.73±6.12^{c}$
5%	$0.796±0.023^{c}$	$375.3±14.5^{ab}$	$10.05±2.25^{b}$	$68.53±7.1^{b}$	$83.10±6.18^{ab}$
15%	$0.831±0.015^{b}$	$377.3±12.3^{a}$	$11.48±2.03^{a}$	$65.13±3.5^{bc}$	$87.90±4.21^{a}$
25%	$0.858±0.025^{a}$	$389.7±08.2^{a}$	$12.15±1.10^{a}$	$56.57±6.1^{cd}$	$78.63±5.26^{b}$
35%	$0.614±0.054^{gh}$	$321.0±10.2^{fgh}$	$9.50±0.43^{fgh}$	$73.64±9.3^{b}$	$68.07±2.12^{c}$
Moderate water-limited (75% field of capacity)					
Control	$0.650±0.023^{f}$	$338.0±13.4^{def}$	$7.38±1.02^{de}$	$50.59±6.3^{de}$	$47.17±3.67^{c}$
5%	$0.721±0.038^{d}$	$358.5±21.8^{bc}$	$8.90±0.86^{bc}$	$51.13±2.1^{de}$	$54.57±6.62^{ab}$
15%	$0.776±0.086^{c}$	$350.4±25.4^{cd}$	$9.84±1.18^{b}$	$49.02±3.8^{de}$	$58.80±4.87^{a}$
25%	$0.741±0.021^{d}$	$345.3±19.0^{cde}$	$10.11±1.23^{b}$	$47.40±1.1^{de}$	$51.20±3.15^{b}$
35%	$0.602±0.025^{h}$	$315.0±14.4^{gh}$	$8.09±1.12^{cd}$	$51.47±1.3^{de}$	$47.90±4.04^{c}$
Severe water-limited (25% field of capacity)					
Control	$0.634±0.015^{fg}$	$304.2±17.4^{hi}$	$4.75±0.54^{g}$	$47.81±4.4^{de}$	$36.77±2.24^{h}$
5%	$0.635±0.032^{fg}$	$324.4±19.5^{fg}$	$6.07±0.35^{f}$	$42.25±2.4^{e}$	$41.83±1.19^{gh}$
15%	$0.642±0.051^{f}$	$330.8±26.1^{efg}$	$6.31±1.03^{ef}$	$41.71±5.1^{e}$	$40.10±2.16^{h}$
25%	$0.675±0.068^{e}$	$333.2±09.2^{defg}$	$6.32±0.23^{ef}$	$41.46±4.3^{e}$	$40.70±1.25^{h}$
35%	$0.604±0.033^{h}$	$296.1±10.3^{i}$	$5.84±0.22^{fg}$	$46.50±1.6^{de}$	$38.83±1.06^{h}$

Data analysis in Table 3 shows that in conditions of WW and SWL, ME treatment led to a significant increase in C_i compared with the control treatments. Under MWL conditions, C_i increased in the plants treated with 5% ME. In comparison with ME treatments, 35% ME decreased C_i under WW, MWL and SWL (Table 3). Comparison of data indicated an increase in P_N for the 5, 15 and 25% ME treatments under WW over that for the control. All ME treatments had higher evaluations for P_N under MWL and SWL compared to control treatments, except for the treatment 35% ME (Table 3). Lower rates of C_i and P_N are the most important effects of water shortage stress affecting plants. Researchers have attributed these lower rates of C_i and P_N under MWL conditions to stomata closure and under SWL conditions to destruction of biochemical reactions (Rahbarian *et al*., 2011, Karimi *et al*., 2015). In comparison with CO_2, ME is formed of relatively smaller molecules and as such, it is more easily absorbed and used by plants (Ahmadpour *et al*., 2015). After foliar application, ME is rapidly metabolized to CO_2 which increases C_i and P_N in the plant (Gout *et al*., 2000). Similar results were reported by Nemecek-Marshall *et al*., (1995) and Hosseinzadeh *et al*., (2014) showing that the effect of ME foliar application on some plants is an increase in C_i which has a positive effect on photosynthesis.

ME treatments showed lower E compared with control under WW conditions, but under MWL and SWL, there was no significant difference between ME and control treatments (Table 3). Decline of E in water stress is a mechanism to protect a plant against water loss (Gates 1968). In the present study, MWL and SWL decreases transpiration compared to WW conditions. Plants with more efficient mechanisms to reduce evaporation and transpiration are better able to tolerate water stress and maintain more water in the leaves, which increases growth and improves cellular processes (Matos *et al*., 1998, Johnson *et al*., 2002). The decrease in E after ME application under WW relates to increased C_i and better retention of water in the leaves; thus, the plant is not required to open its stomata to provide CO_2.

All ME treatments showed the higher WUE under WW compared to control, except for 35% ME. Under MWL, the presence of ME treatments (5 and 15%) induced a significant increase in WUE compared with control, but under SWL, different ME treatments did not affect WUE (Table 3). Under water shortage stress, water consumption by the plants decreased, which could relate to a potential decrease in water in the root zone and decreasing capability of the plant for water uptake (Johnson *et al*., 2002). Different studies have shown that ME foliar application increases saccharification in the leaves and maintains a more negative osmotic potential in the cells, so that the absorption of water from the soil increases along with WUE by the plant (Downie *et al*., 2004, Hosseinzadeh *et al*., 2014).

Conclusion

The results of this study demonstrated that under WW and MWL conditions, ME foliar application could improve growth and photosynthetic parameters. Under SWL, plant height, leaf and stem DW, F_v/F_m, C_i and P_N increased by ME treatments. The 15 and 25% ME were more effective than the other treeatments. Increasing the methanol from 25% to 35% ME decreased Chl a, Chl (*a*+*b*), F_v/F_m and C_i, probably from the toxic effects of methanol at high concentrations. This study confirmed that water shortage stress significantly lowered all growth and photosynthetic traits. Nevertheless, the foliar application of ME did ameliorate the negative effects of water shortage stress.

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