DOI: 10.1007/s11099-015-0178-2 PHOTOSYNTHETICA 54 (2): 288-294, 2016

The effect of methanol on photosynthetic parameters of bean (*Phaseolus vulgaris* L.) under water deficit

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Abstract

Water availability is the main factor limiting crop growth and productivity in dry regions. This study was carried out in order to determine the effect of spraying methanol solution on the photosynthetic characteristics of bean plants. The main aim of our experiment was to improve plant performance under stress caused by water shortage. Two factors were involved: water-deficit stress, such as severe stress (25% of field capacity), mild stress (75% of field capacity), and no stress (100% of field capacity), and application of methanol solution spray at four concentrations: control (without spraying), 10, 20, and 30%. Methanol was applied three times at different growth stages (seedling, flowering, and podding stage) in 10-d intervals. The treatment with 20% methanol at the seedling stage resulted in increased net photosynthesis (P_N), intercellular CO_2 concentration (C_i), and decreased transpiration rate (E) under no stress and mild stress conditions. Under severe stress, 10 and 20%-methanol treatments resulted in increased C_i, maximal quantum yield of PSII photochemistry, and decreased E. At the flowering stage, methanol treatments resulted in decreased E and increased C_i under mild and severe stress. At the podding stage, 10 and 20%-methanol treatments resulted in increased P_N, C_i, and total chlorophyll content under mild stress. In conclusion, we suggested that foliar application of methanol had a positive role in enhancing photosynthetic performance.

Additional key words: chlorophyll fluorescence; gas exchange; maximal quantum yield of PSII; physiological characteristics.

Introduction

Bean is an important legume, with 25% of protein and 58% of carbohydrate content. As such, it is an essential food source in many developing countries (Dorri 2008). One of the main problems affecting production in Iran is stress caused by water deficiency. The main effect of water deficiency stress is a reduced water content in leaves that induces stomata closure and causes disruption to the development of photosynthetic apparatus in plants. This response to water deficiency results in reduced performance and limited productivity in cultivated plants (Hosseinzadeh *et al*. 2015, Sikder *et al*. 2015). Under conditions of water deficiency, water potential is reduced by aggregation of soluble materials in plant cells. This involves most adaptive compounds, such as soluble sugars, sorbitol, betaine, organic acids, amino acids, proline, and glycine, together with ions, such as K^+ and Ca^{2+} (Hu and Schmidhalter 2005). Ca^{2+} deficiency results in diminished Rubisco activity, stomatal conductance, and increased production of reactive oxygen species (ROS) leading to decreased photosynthesis in plants (Cakmak 2005). For this reason, solutions capable of mitigating the effect of water deficiency have attracted much attention. It has been shown that an elevated concentration of CO_2 can nullify the effects of stress caused by water shortage (Ramírez *et al*. 2006, Hosseinzadeh *et al*. 2014). Accordingly, application of compounds, which are able to enhance the concentration of intercellular CO_2, can improve performance under conditions of water deficiency (Makhdum *et al*. 2002, Hosseinzadeh *et al*. 2012).

In the early 1990s, it was reported that application of a methanol solution on aerial parts of cultivated plants resulted in improved performance and reduced water demands in plants (Nemecek-Marshall *et al*. 1995). Gout *et al*. (2000) reported that some cultivated plants were able to absorb methanol by leaves and convert it to CO_2, which

Received 17 May 2015, *accepted* 11 September 2015, *published as online-first* 10 October 2015.
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Abbreviations: Chl – chlorophyll; C_i – intercellular CO_2 concentration; CK – control without methanol; E – transpiration rate; F_v/F_m – maximal quantum yield of PSII photochemistry; M10 – 10% methanol treatment; M20 – 20% methanol treatment; M30 – 30% methanol treatment; MS – mild water stress; NS – no water stress; P_N – net photosynthetic rate; ROS – reactive oxygen species; RuBP – ribulose-1,5-bisphosphate; S1 – seedling stage; S2 – early flowering stage; S3 – early podding stage; SS – severe water stress; TChl – total chlorophyll content.

was then used by the plant as a source of carbon in addition to carbon from the atmosphere. Spraying methanol solution has been cited as advantageous; it reportedly enhanced saccharification of leaves resulting in raised turgor pressure and increased rate of net photosynthesis (P_N) in treated plants (Safarzade Vishgahi *et al*. 2008).

Water-deficiency stress and high radiation increase the rate of light inhibition (Lu *et al*. 2002, Ismaili *et al*. 2015). A study on chickpea genotypes under drought conditions demonstrated that spraying methanol solution at 30% concentration resulted in an enhanced maximal quantum yield of PSII photochemistry (F_v/F_m) in comparison with plants not treated with the methanol solution (Hosseinzadeh *et al*. 2014). Another study on cotton determined that methanol resulted in decreased leaf temperature, diminished transpiration, increased index of leaf area, and leaf durability (Makhdum *et al*. 2002). Research has shown that preservation of a chlorophyll (Chl) content under water deficiency makes a significant contribution to maintaining photosynthetic activity in plants (Flexas and Medrano 2008). Another experiment reported the elevated Chl content after spraying by a methanol solution on leaves of wheat, oat, and grape (Ramadant and Omran 2005).

There are few studies on the interaction of methanol and water stress in Iran. Our research aimed to address following objectives: (*1*) to determine whether or not spraying methanol solution can effectively reduce the adverse effects of water stress in bean and (*2*) to determine the best concentration of methanol spray for effective increase of photosynthetic parameters.

Materials and methods

Experimental details: This experiment was set up as a factorial in a fully-random format with three replications and under greenhouse conditions. Treatments in our experiment involved methanol solution spray at four different concentrations: control (without spraying, CK), 10 (M10), 20 (M20), and 30% (M30, v/v) methanol. Glycin (2 g L^{-1}) was added into all spraying solutions including the control. The addition of glycine to the aqueous solution of methanol prevented damage from methanol toxicity. The three levels of water stress were tested: control (100% of field capacity, NS), mild water stress (75% of field capacity, MS), and severe water stress (25% of field capacity, SS). Bean seeds were soaked in water for 24 h and then transplanted at a rate of four seedlings per pot. The pots were exposed to day/night temperature of 25/15°C in a growth chamber [light intensity of 1,000 μmol(photon) m^{-2} s^{-1}, relative humidity of 60–70%, CO_2 concentration of 400 ppm]. Irrigation was applied to pots for two weeks until bean seedlings had become green. Thereafter, pots were irrigated according to the above water-stress treatments. Methanol solution spray was applied three times during the plant growth season at 10-d intervals. The first spray was applied at the seedling stage (30 d after potting, S1), the second application was made at the early flowering stage (40 d after potting, S2), and the third application was done at the early podding stage (50 d after potting, S3). Photosynthetic traits were then measured 24 h after each application of methanol solution spray.

Gas-exchange parameters: Transpiration rate (E), P_N, and intercellular CO_2 concentration (C_i) were measured in attached young and fully expanded leaves of main shoots. Measurements were taken from healthy and developed leaves (second and third leaf from each plant). Environmental conditions of the leaf chamber were adjusted in order to match conditions in the growth chamber. Measurements were taken using a portable infrared gas analyzer (*KR8700 System*, *Korea Tech Inc.*, Suwon, Korea) in conjunction with an automatic leaf chamber with a leaf surface area of 6 cm^2.

Total Chl content (TChl) was measured with a portable leaf Chl meter (*CCM-200 Plus*, *Opti-Sciences Inc.*, NH, USA) on the second uppermost leaf from three plants per pot. TChl was determined from averages taken from ten readings per sample. The leaves used for measuring gas exchange were also used to specify the Chl content.

Measurement of F_v/F_m was done with a portable Chl fluorometer (*Pocket PEA*, *Hansatech Instruments Ltd.*, King's Lynn, Norfolk, England). Measurements were taken using the same leaf used for gas-exchange determination, illuminated with saturating light of 3,500 μmol(photon) m^{-2} s^{-1} after 20 min of adaptation to dark. F_v/F_m was determined automatically as $(F_m - F_0)/F_m$; where F_m and F_0 were maximum and initial fluorescence yields of dark-adapted leaves, respectively (Maxwell and Johnson 2000). All photosynthetic measurements used three plants from each treatment.

Content of leaf tissue elements: Na^+, Ca^{2+}, and K^+ concentrations of leaves and roots were determined using the method cited in Chapman and Patt (1982). Wet ash was prepared with nitric acid and the content of each element was calculated as gram per 100 g of dry mass (DM) with flame photometry (*Sherwood Scientific*, Cambridge, UK).

Statistical analyses were made by *MASTAT-C*. Level of significance of photosynthetic indices in response to spraying methanol solution and water stress was determined by the analysis of variance (*ANOVA*) test. Means were compared by the *Duncan*'s multiple range test ($p \leq 0.05$).

Results and discussion

E: The results of the first methanol spraying at the S1 stage showed that under NS and SS treatments, a significant decrease in *E* compared with CK was observed. Under MS, M20 and M30 treatments significantly decreased *E* below the CK value (Table 1). After the second foliar spraying at the S2 stage, we demonstrated that methanol applied under NS, MS, and SS conditions significantly decreased *E* below CK (Table 2). After the third foliar spraying at the S3 stage, M20 and M30 treatments significantly reduced *E* compared with CK. During the MS treatment, methanol concentrations had no significant effect, but under SS, M10 significantly decreased *E* below the CK value (Table 3).

E is the first plant parameter to be affected by drought stress. *E* responds to water deficit by stomata closure in order to preserve the water content in leaves and to

Table 1. Effects of foliar application of methanol on transpiration rate (E), net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), total chlorophyll content (TChl), and maximal quantum yield of PSII photochemistry (F_v/F_m) under water deficit stress at the seedling stage. Data are means ± SD (n = 3). Difference between the data of each column followed by *the same letter* was not statistically significant (p<0.05).

Treatments/Methanol	E [mg dm^{-2} h^{-1}]	P_N [μmol m^{-2} s^{-1}]	C_i [ppm]	TChl [μg cm^{-2}]	F_v/F_m
No water stress (100% of field capacity)					
Control	45.58 ± 3.22^{a}	3.31 ± 1.41^{b}	401.7 ± 11.4^{bc}	5.13 ± 0.45^{bc}	0.75 ± 0.03^{cd}
10%	32.51 ± 2.91^{b}	5.50 ± 1.25^{a}	498.3 ± 33.6^{a}	6.10 ± 0.54^{a}	0.78 ± 0.03^{a}
20%	23.53 ± 5.63^{d}	6.44 ± 2.12^{a}	534.4 ± 41.8^{a}	5.96 ± 0.50^{a}	0.79 ± 0.06^{a}
30%	22.03 ± 2.88^{de}	5.47 ± 1.82^{a}	524.7 ± 30.3^{a}	5.66 ± 0.38^{ab}	0.78 ± 0.04^{ab}
Mild water stress (75% of field capacity)					
Control	27.97 ± 2.91^{c}	2.62 ± 0.53^{bc}	390.7 ± 14.2^{c}	4.60 ± 0.05^{cd}	0.73 ± 0.03^{ef}
10%	27.58 ± 2.42^{c}	2.35 ± 0.87^{bcd}	414.2 ± 17.7^{bc}	5.13 ± 0.75^{bc}	0.76 ± 0.05^{bc}
20%	21.76 ± 1.93^{de}	3.30 ± 1.13^{b}	438.9 ± 13.1^{b}	4.56 ± 0.22^{cd}	0.75 ± 0.01^{cde}
30%	20.79 ± 3.70^{def}	2.89 ± 1.40^{bc}	424.9 ± 13.3^{bc}	4.30 ± 0.94^{de}	0.74 ± 0.01^{de}
Severe water stress (25% of field capacity)					
Control	23.91 ± 4.11^{d}	1.15 ± 0.38^{d}	277.1 ± 17.7^{e}	3.80 ± 0.11^{e}	0.71 ± 0.01^{g}
10%	19.15 ± 5.23^{ef}	1.83 ± 0.71^{cd}	318.0 ± 21.1^{d}	4.23 ± 0.35^{de}	0.73 ± 0.09^{ef}
20%	18.03 ± 5.93^{f}	2.39 ± 2.44^{bcd}	322.4 ± 38.0^{d}	4.06 ± 0.64^{de}	0.74 ± 0.06^{de}
30%	19.22 ± 3.51^{ef}	1.69 ± 1.68^{cd}	317.7 ± 33.1^{d}	3.76 ± 0.11^{e}	0.72 ± 0.01^{fg}

Table 2. Effects of foliar application of methanol on transpiration rate (E), net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), total chlorophyll content (TChl), and maximal quantum yield of PSII photochemistry (F_v/F_m) under water deficit stress at the flowering stage. Data are means ± SD (n = 3). Difference between the data of each column followed by *the same letter* was not statistically significant (p<0.05).

Treatments/Methanol	E [mg dm^{-2} h^{-1}]	P_N [μmol m^{-2} s^{-1}]	C_i [ppm]	TChl [μg cm^{-2}]	F_v/F_m
No water stress (100% of field capacity)					
Control	55.59 ± 8.69^{a}	7.29 ± 1.41^{bc}	397.9 ± 16.3^{cd}	6.06 ± 0.10^{d}	0.76 ± 0.01^{cd}
10%	42.52 ± 4.67^{b}	9.71 ± 1.62^{ab}	471.9 ± 17.7^{b}	7.66 ± 0.25^{bc}	0.80 ± 0.03^{a}
20%	45.02 ± 3.23^{b}	10.52 ± 2.10^{a}	568.0 ± 10.5^{a}	8.30 ± 0.47^{ab}	0.78 ± 0.01^{abc}
30%	46.80 ± 7.41^{b}	9.53 ± 2.01^{ab}	560.0 ± 11.4^{a}	8.96 ± 0.50^{a}	0.79 ± 0.05^{ab}
Mild water stress (75% of field capacity)					
Control	46.30 ± 3.41^{b}	4.60 ± 1.57^{de}	363.8 ± 10.4^{de}	4.36 ± 0.11^{e}	0.74 ± 0.05^{e}
10%	31.69 ± 1.55^{c}	4.46 ± 3.74^{de}	441.7 ± 13.5^{bc}	6.13 ± 0.35^{d}	0.77 ± 0.05^{cd}
20%	30.34 ± 1.58^{c}	6.20 ± 0.68^{cd}	468.4 ± 15.4^{b}	6.66 ± 1.57^{cd}	0.77 ± 0.01^{bcd}
30%	31.95 ± 2.24^{c}	6.02 ± 0.87^{cd}	448.1 ± 9.4^{bc}	6.33 ± 0.05^{d}	0.76 ± 0.03^{d}
Severe water stress (25% of field capacity)					
Control	42.46 ± 2.91^{b}	3.81 ± 0.47^{de}	313.5 ± 22.2^{e}	3.53 ± 0.64^{e}	0.71 ± 0.03^{f}
10%	26.45 ± 3.23^{c}	3.32 ± 2.44^{e}	376.0 ± 9.5^{d}	4.13 ± 0.62^{e}	0.72 ± 0.01^{ef}
20%	22.71 ± 1.75^{c}	5.32 ± 1.70^{cde}	431.9 ± 11.7^{bc}	4.40 ± 0.52^{e}	0.72 ± 0.01^{ef}
30%	27.77 ± 0.87^{c}	3.57 ± 1.68^{de}	349.7 ± 6.1^{d}	3.60 ± 0.13^{e}	0.73 ± 0.05^{e}

Table 3. Effects of foliar application of methanol on transpiration rate (E), net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), total chlorophyll content (TChl), and maximal quantum yield of PSII photochemistry (F_v/F_m) under water deficit stress at the podding stage. Data are means ± SD (n = 3). Difference between the data of each column followed by *the same letter* was not statistically significant (p<0.05).

Treatments/Methanol	E [mg dm^{-2} h^{-1}]	P_N [μmol m^{-2} s^{-1}]	C_i [ppm]	TChl [μg cm^{-2}]	F_v/F_m
No water stress (100% of field capacity)					
Control	90.26 ± 11.10^{a}	8.37 ± 0.92^{de}	513.6 ± 13.3^{b}	6.16 ± 0.15^{bc}	0.80 ± 0.02^{b}
10%	88.87 ± 9.56^{a}	12.98 ± 1.22^{a}	634.2 ± 21.1^{a}	7.30 ± 0.41^{b}	0.82 ± 0.01^{a}
20%	66.87 ± 12.40^{b}	12.57 ± 3.47^{ab}	626.7 ± 26.9^{a}	8.40 ± 0.26^{a}	0.84 ± 0.05^{a}
30%	71.05 ± 7.41^{b}	10.83 ± 2.23^{bc}	595.6 ± 26.0^{a}	6.50 ± 0.11^{b}	0.82 ± 0.01^{a}
Mild water stress (75% of field capacity)					
Control	70.36 ± 16.70^{c}	6.40 ± 3.42^{ef}	443.7 ± 17.4^{de}	4.86 ± 0.52^{de}	0.77 ± 0.01^{cd}
10%	48.86 ± 11.60^{c}	8.81 ± 0.71^{d}	511.8 ± 13.5^{bc}	6.50 ± 0.15^{b}	0.78 ± 0.02^{bcd}
20%	49.82 ± 9.35^{c}	9.58 ± 2.53^{cd}	518.1 ± 11.6^{b}	6.50 ± 0.47^{b}	0.79 ± 0.03^{bc}
30%	53.43 ± 13.30^{c}	8.36 ± 1.22^{de}	493.6 ± 22.6^{bcd}	5.10 ± 0.11^{cd}	0.77 ± 0.01^{bcd}
Severe water stress (25% of field capacity)					
Control	46.22 ± 12.44^{cd}	4.14 ± 2.88^{f}	415.1 ± 8.3^{e}	3.83 ± 0.35^{e}	0.76 ± 0.05^{d}
10%	31.21 ± 10.42^{e}	5.51 ± 3.45^{f}	456.7 ± 11.1^{bcde}	4.16 ± 0.25^{de}	0.76 ± 0.01^{d}
20%	38.29 ± 6.10^{de}	5.81 ± 2.51^{f}	487.8 ± 10.7^{bcd}	4.93 ± 0.33^{de}	0.77 ± 0.01^{d}
30%	37.43 ± 1.51^{de}	6.33 ± 2.88^{ef}	446.1 ± 8.6^{cde}	4.33 ± 0.10^{de}	0.76 ± 0.05^{d}

prevent water loss (Gates 1968). Studies have shown that plants with an efficient mechanism for reducing E better tolerated conditions of water deficiency. Such plants possess better growth potential because they are able to preserve more water (Sicher *et al*. 2015). The results of our study indicated that conditions of MS and SS resulted in the significant decrease of E in comparison with the NS conditions. It seemed that lower E was associated with the application of methanol by increasing C_i. As stomata closure is the first response to water shortage in plants, it is followed by a reduction of C_i. Therefore, spraying methanol compensated the CO_2 deficiency and met the plant's demand for photosynthesis; the plant had no need to open stomata in order to provide CO_2 (Hosseinzadeh *et al*. 2014). A study on wheat genotypes reported that spraying methanol solution on leaves played an important role in reducing transpiration (Farzad *et al*. 2007).

***P*$_N$**: The results of the S1 application demonstrated that under NS conditions, methanol concentrations significantly increased P_N over CK, whereas the MS and SS treatments did not significantly affected P_N (Table 1). The S2 application showed that under the NS treatment, M20 increased P_N significantly compared with CK. Under the MS and SS treatments, the methanol-sprayed and CK plants were not significantly different (Table 2). The S3 application showed that for the NS treatment methanol concentrations increased P_N significantly compared with CK. Under the MS treatment, M10 and M20 resulted in a significant increase compared with CK, but under SS, the methanol-treated and CK plants showed no significant difference (Table 3).

Low P_N is one of the most important effects how water stress affects plants. Researchers have attributed low P_N under mild stress conditions to stomata closure and under severe stress conditions to impairment of biochemical reactions (Rahbarian *et al*. 2011, Karimi *et al*. 2015). When a plant experiences water deficiency, stomata closure is a natural phenomenon that occurs in order to reduce water loss. However, stomata closure blocks an access to CO_2 from air to cells in leaves, so that photosynthesis is reduced to a point lower than that of the compensatory point (Pagter *et al*. 2005). The most important advantage of treating plants with methanol is that methanol sprayed on leaves is easily absorbed and plants can use this compound as a carbon source in addition to atmospheric carbon (Gout *et al*. 2000). Various studies have reported that plants treated with methanol spray showed a significant increase in P_N in comparison with controls (Farzad *et al*. 2007, Hosseinzadeh *et al*. 2014). Therefore, it was generally observed that spraying methanol solution under mild stress conditions, which decreases P_N due to stomata closure, was very effective and can nullify the negative impact of mild stress. However, under conditions of severe stress, when destructive biochemical reactions occur in plants, spraying methanol solution cannot reduce negative effects.

***C*$_i$**: The results of the S1 application demonstrated that under NS and SS conditions, methanol concentrations significantly increased C_i over the CK. After the MS treatment, only the M20 concentration significantly increased C_i over the CK group (Table 1). During the S2 stage, the results showed that all methanol treatments (M10, M20, and M30) significantly increased C_i over the CK in all water-stressed plants (Table 2). After the S3 spraying, methanol concentrations significantly increased C_i over the CK level under the NS treatment. For the MS treatment, M10 and M20 significantly increased C_i

compared with CK, while after the SS treatment, only M20 significantly enhanced C_i (Table 3).

A reduction of C_i under conditions of water deficiency has been reported in many legumes (Zlatev and Yordanov 2004, Ganjeali *et al*. 2011). In this study, MS and SS also resulted in a significant decrease in C_i in comparison with the NS conditions. Downie *et al*. (2004) reported that after spraying, methanol is converted to formaldehyde and then to formate (methanoic acid) by the methanol oxidase enzyme. This formate is then converted to CO_2 by the enzyme formate dehydrogenase; it leads to an increase of C_i in a plant. The Rubisco enzyme has an important role in the oxidation process of this compound (Rahbarian *et al*. 2011) in addition to its important role in carboxylation of ribulose-1,5-bisphosphate (RuBP). Oxidation of RuBP follows photorespiration (Makhdum *et al*. 2002). Methanol is rapidly metabolized to carbon dioxide and the increase in CO_2 brings about further carboxylation and reduces oxygenation in a plant (Hosseinzadeh *et al*. 2014). Leaf pores play a key role in a plant's response to water shortage stress, thus understanding water stress is associated with stomata behaviour (Johnson *et al*. 2002).

TChl: Results after the S1 stage showed that after the NS treatment, the application of M10 and M20 significantly increased TChl compared with CK. For the MS and SS treatments, no significant difference was observed between the methanol-sprayed and CK plants (Table 1). After spraying at the S2 stage, we showed that for the NS and MS treatments, the methanol concentrations significantly increased TChl compared with CK (Table 2). In the S3 stage, the results showed that M20 significantly increased TChl over CK during the NS treatment. For the MS treatment, M10 and M20 significantly increased TChl in comparison with CK. Under the SS treatment, there was no significant difference in TChl of the plants treated with various methanol concentrations (Table 3).

The Chl content is known as an index to evaluate tolerance to water stress in plants (Anyia and Herzog 2004). As water availability diminishes in plants, so does TChl in the green tissues (Flexas and Medrano 2008). We showed the significant reduction of TChl under MS and SS in comparison with NS conditions. Under conditions of water deficiency, TChl constant values imply that plants are unaffected by stress (Flexas and Medrano 2008). The results of our study indicated that under SS conditions, methanol had no effect on TChl. However, under the NS and MS conditions, methanol treatments resulted in the significant increase of TChl in comparison with CK. The studies of Ramadant and Omran (2005) revealed that spraying methanol solution elevated Chl contents in leaves.

F_v/F_m: Under the NS conditions, methanol significantly increased F_v/F_m compared with CK. Under the MS treatment, M10 significantly increased F_v/F_m over CK values. Under the SS conditions, M10 and M20 significantly increased F_v/F_m (Table 1). During the S2 stage, M10 and M30 significantly enhanced F_v/F_m during the NS treatment. Under MS, methanol significantly increased F_v/F_m, while only M30 significantly increased F_v/F_m under SS (Table 2). Results at the S3 stage revealed that under NS conditions, methanol treatment led to a significant increase in F_v/F_m in comparison with CK. Under conditions of MS and SS, methanol treatments did not show any significant effect (Table 3). The F_v/F_m ratio is generally accepted as an efficiency indicator of PSII in plants exposed to environmental stress, including drought and heat (Giorio 2011). Reduction of the F_v/F_m ratio in plants under conditions of water stress indicates diminished efficiency of PSII and lowered electron transfer from PSII to PSI (Liu *et al*. 2015). Numerous studies have reported that, under conditions of severe drought, the F_v/F_m ratio is reduced significantly. The reason for this reduction seems to be destruction of the centers in PSII (Farzad *et al*. 2007). In general, reduction of F_v/F_m represents lowered photoprotection and can be considered as a reason that drought stress significantly affects the efficiency of photosynthesis (Zlatev and Yordanov 2004). Our study demonstrated that MS and SS resulted in a significant decrease of the F_v/F_m ratio in comparison with the NS conditions. However, under conditions of MS and SS, methanol treatments stimulated F_v/F_m only at the seedling and flowering stages. Increased F_v/F_m ratio was also observed after applying methanol to leaves in studies of Hosseinzadeh *et al*. (2014) and Ramadan and Omran (2005).

Content of elements in leaf tissue: A comparison of the average data on the interaction of methanol and leaf K concentration showed that MS and SS significantly decreased the K concentration in leaves. There was no significant difference for water stress *vs*. methanol concentrations (Fig. 1). The Ca concentrations showed that MS and SS significantly decreased Ca concentration in comparison with the NS treatment. There were no significant differences between methanol and CK at all levels of water stress (Fig. 2). Methanol treatments and water stress had no significant effect on the Na concentration in leaf tissue.

Absorption of nutrients from the soil is directly associated with the status of available water in soil. This decrease is caused by a lower E that affects active transfer and reduces membrane permeability, resulting in less nutrients being transferred to aerial parts of a plant (Khoshbakht *et al*. 2014). Another important effect of water stress on absorption of elements, such as K and Ca, is reduced mobility of these elements in the soil (Hu and Schmidhalter 2005, Khoshbakht *et al*. 2014). Osuagwu *et al*. (2010) reported that drought stress reduced

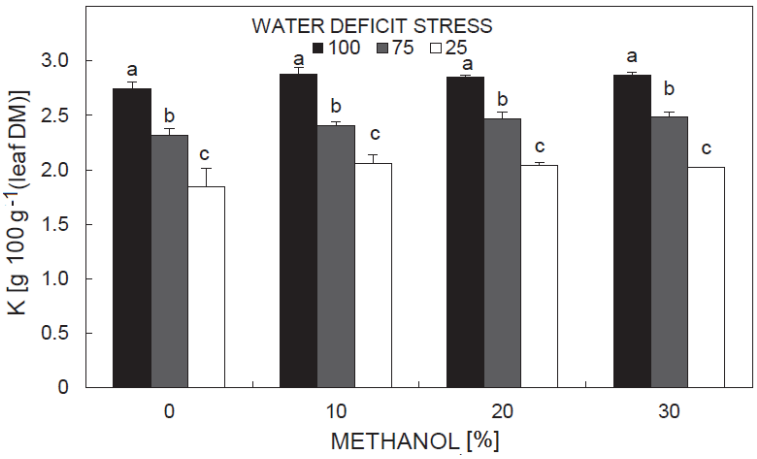

Fig. 1. Effect of methanol and water stress interaction on the K concentration. Columns with *the same letter(s)* are not significantly different at $p \leq 0.05$ probability.

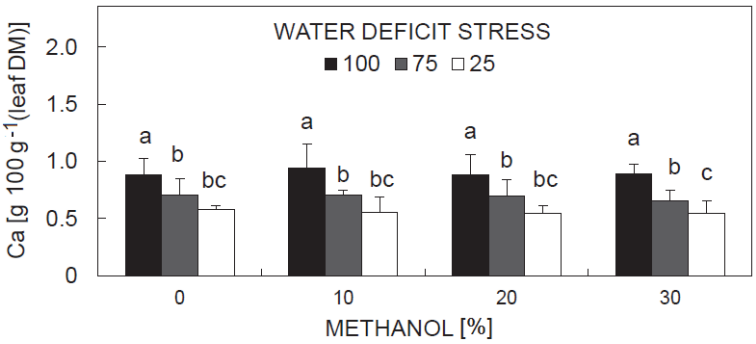

Fig. 2. Effect of methanol and water stress interaction on the Ca concentration. Columns with *the same letter(s)* are not significantly different at $p \leq 0.05$ probability.

contents of K and Ca in leaves of *Ocimum gratissimum* possibly due to movement of these elements from leaves to roots, because under these circumstances they act as osmotic protectors. In this study, concentrations of K and Ca were also significantly lowered under conditions of SS and MS compared with the NS conditions.

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