Bangladesh J. Bot. **45**(5): 1069-1074, 2016 (December)

EFFECTS OF METHANOL FOLIAR APPLICATION ON NUTRIENT CONTENT AND RWC OF SUGAR BEET UNDER WATER DEFICIT STRESS

EBRAHIM KHALILVAND BEHROUZYAR* AND MEHRDAD YARNIA

Department of Agronomy and Plant Breeding, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Key words: Methanol foliar application, Sugar beet, Water deficit stress

Abstract

To study the effect of methanol foliar application on sugar beet under water deficit stress an experiment was conducted. Treatments were water deficit stress in three levels, a_1: mild stress (50% FC irrigation), a_2 : fair stress (75% FC irrigation) and a_3: normal irrigation (100% FC irrigation) and the foliar application of methanol in seven levels [b_1 : 0, b_2 : 5, b_3 : 10, b_4 : 15, b_5 : 20, b_6 : 25 and b_7 : 30 (v/v)]. The analysis of variance showed significant effect of water deficit stress and methanol foliar application on concentration of α-amino N, Na, K, RWC and sugar content ($p < 0.01$). Results showed that α-amino nitrogen and K contents were the highest in mild stress and the lowest in 20% (v/v) methanol foliar application. However, Na content was the highest in normal irrigation and 30% (v/v) methanol foliar application. Sugar content was the highest in mild stress (16.40%) and the lowest in the normal irrigation (15.86%). Besides, the results also proved that 20% (v/v) and 30% (v/v) methanol foliar application had the highest (17.16%) and lowest (15/31%) sugar content, respectively.

Introduction

Water deficit is a major abiotic stress that adversely affects the crop growth and yield (Jaleel *et al.* 2008). It reduces plant growth and development by affecting various physiological and biochemical processes (Jaleel *et al.* 2008, Farooq *et al.* 2008). Plants use different mechanisms to cope with the drought stress. The first step to achieving high yield per unit area is to increase production of dry matter, because almost 90% of plant dry weight is resulted from CO_2 assimilation during photosynthesis (Khalilvand and Yarnia 2013). Today in order to achieve this goal compounds such as methanol, ethanol and amino acids like glycine, glutamats and aspartate are used as C source for the most production. Recent investigation showed that C_3 crops yield and growth increased via methanol spray and methanol may act as C source for these crops (Makhdum *et al.* 2002). Methanol (CH_3OH) is the second most abundant organic gas in the atmosphere after methane (Hanson *et al.* 2000). Methanol metabolism in plants is poorly understood. Methanol is a naturally occurring, volatile organic compound emitted from the leaves of many plant species (MacDonald and Fall 1993, Nemecek-Marshall *et al.* 1995), but the origin of emission is uncertain. The diurnal pattern for volatile organic compounds (VOCs) and methanol suggests that accumulation of these substances is related to photosynthetic processes (Fall and Benson 1996). Interestingly, leaves typically harbor methylotrophic bacteria capable of metabolizing methanol (Corpe and Rheem 1989, Holland and Polacco 1994), and these bacteria may be associated with leaf surface methanol emission. Methylotrophic yeast is also capable of metabolizing methanol. Foliar applications of aqueous methanol have been reported to increase yield, accelerate maturity, and reduce drought stress and irrigation requirements in C_3 crops grown in arid environments, under elevated temperatures, and in direct sunlight (Ramirez *et al.* 2006). Metabolism of methanol to sugars would change leaf osmotic potential, resulting in increased turgor and stomatal conductance. Keeping the stomata open would increase the assimilation rate and, subsequently,

*Author for correspondence: < e.khalilvand@iaut.ac.ir>.

plant growth. The accelerated growth rate resulted in earlier maturation and thus less need for irrigation (Milton and McGiffen 1996). In calculating the carbon input from foliar applied methanol, Sadeghi-Shoae *et al.* (2014) reported that methanol foliar application enhanced total dry matter (TDM), root yield (RY), sugar yield (SY) and white yield sugar. Nadali *et al.* (2010) stated that 21% (v/v) methanol spray poses the greatest impact on yield, and other physiological traits. Bagheri *et al.* (2014) stated that spraying with 20% (v/v) methanol in lavender greatly increased leaf area, leaf fresh and dry mass. Khazaei *et al.* (2015) showed that Na content was the highest in normal irrigation (4.21 eq/100 g sugar beet) and the lowest in drought stress (2.82 eq/100 g); however, K and N contents were the highest in drought stress (5.11 and 2/82 eq/100 g, respectively) and the lowest in normal irrigation (4/99 and 1.38 eq/100 g). Positive effects of methanol foliar application on growth of plant have been confirmed in previous studies. Thus, the objectives of this study were to improve the nutrient and RWC of sugar beet under different regimes of irrigation.

Materials and Methods

The field experiment was carried out in split plot form by completely randomized block design with three replicates at the Research Station of the Islamic Azad University, Tabriz Branch, north-western Iran, during the 2013 - 2014. The *Beta vulgaris* variety SBSI-007 was used (a diploid, monogerm and spring variety that is widely planted in Iran). The first factor was water deficit stress in three levels: a_1: mild stress (50% FC irrigation), a_2: fair stress (75% FC irrigation) and a_3: normal irrigation (100% FC irrigation). The second factor was the foliar application of methanol in seven levels [b_1 : 0, b_2 : 5, b_3 : 10, b_4 : 15, b_5 : 20, b_6 : 25 and b_7 : 30 volumetric percentage (v/v)] that to prevent of methanol poisoning at light presence, 1 g/l glycine and 1 mg/l tetrahydrofolate (THF) were added to prepared solution (Bayat *et al.* 2013). In all treatments, methanol spray was applied three times during stages of sugar beet development. The first spray was conducted in about 16 leaves (70 day after planting) and the other two sprays were conducted with 14 days interval. In the control plots, plants were sprayed with water. Water deficit stress was imposed from 8 leaves stage to physiological maturity. Each plot consists of 5 rows, 60 cm row spacing and 20 cm plant interval. Sodium and potassium contents were measured by flame photometry method. Moreover, α-amino N was measured by betalizer device (Clover *et al.* 1998). Also, sugar content estimated by using polarimetric method (Babaee *et al.* 2011). Leaves relative water content (RWC) was determined with method described by Matin *et al.* (1989):

$$RWC = \frac{Fw - Dw}{Dw - Tw} \times 100$$

where, Fw, Dw and Tw are fresh, dry and turgor leaf weights, respectively.

In order to check the normality of data, analysis of variance, and mean comparison MSTAT-C software were used. The means of the treatments were compared using the least significant difference (LSD) test at $p < 0.05$.

Results and Discussion

The analysis of variance presented in Table 1 showed significant effect of water deficit stress and methanol foliar application on concentration of α-amino N, Na, K, RWC and sugar content at 1% probability level.

Table 1. Analysis of variance of measured traits.

S.O.V	df	α-amino N	K	Na	Sugar content	RWC
Rep	2	0.061*	0.024*	0.005 ns	0.299*	1.621 ns
WDS	2	0.285**	0.304**	0.301**	1.52**	82.8**
Error	4	0.004	0.003	0.002	0.027	1.712
MFA	6	2.249**	0.054**	0.182**	4.691**	140.51**
MFA×WDS	12	0.005 ns	0.006 ns	0.003 ns	0.05 ns	1.874 ns
Error	36	0.015	0.005	0.003	0.153	74.55
CV		3.56	1/08	1.40	2.43	1.83

* and **significant at 5 and 1%, respectively. WDS: Water deficit stress, MFA: Methanol foliar application, SOV: Source of variation.

Mean comparison of water deficit stress levels (Table 2) indicated that α-amino nitrogen and K contents were the highest in mild stress (50% FC irrigation), (3.526 and 6.709 eq 100 gr/sugar, respectively) and the lowest in normal irrigation (100% FC irrigation), (3.293 and 4.468 eq100 gr/sugar); however, Na content was the highest in normal irrigation (4.168 eq100 gr/sugar) and the lowest in mild stress (3.929 eq100 gr/sugar). This represents that water deficit stress increased K and N content in sugar (Table 2). Besides, sugar content was the highest in mild stress (16.40%) and the lowest in the normal irrigation (15.86%). Our results also showed that the highest RWC were related to 100% FC irrigation (80.76%). In addition, 50% FC irrigation had the lowest RWC (76.82%), (Table 2). Under water deficit stress, plants break polysaccharides to monosaccharides to cope with the water deficit stress, which results in the enhancement of sugar content in plant (Ober 2001). Results of our experiment also showed that water deficit stress produced 3.4% more

Table 2. Mean comparison of water deficit stress on traits.

WDS	α-amino N eq100 g/sugar	K eq100 g/sugar	Na eq100 g/sugar	sugar content (%)	RWC (%)
a_1 (100% FC)	3.293	4.468	4.168	15.86	80.76
a_2 (75% FC)	3.408	6.595	4.062	16.15	78.38
a_3 (50% FC)	3.526	6.709	3.929	16.40	76.82
LSD5%	0.054	0.046	0.038	0.140	1.12

WDS: Water deficit stress, FC: Field capacity.

sugar content than normal irrigation. Khazaei *et al.* (2015) tested the effect of drought stress on sugar beet and found that drought stress increased sugar content from 13.8 to 16.25%. Clover *et al.* (1998) reported that drought stress increase nitrogen content in sugar beet yield but had no significant effect on Na and K. The more suitable genotypes for arid areas are those which can maintain more water content and have a higher RWC without closing their stomata, due to positive effects of higher RWC on more stomata opening and CO_2 fixation and thereby increase photosynthetic capacity (Por-mousavi *et al.* 2007). On the other hand, however, reduction of RWC and stomata closing are among the first drought impacts on plants, which may reduce crop yield through disturbing photosynthesis processes (Paknejad *et al.* 2007). Under drought stress condition due to increasing ABA in mesophyll, stomata are closed and eventually stomata conduction reduced in the leaf and finally cell's turgid is decreased and decreasing turgid can confine growth (Hsiao 2000).

Mean comparison of methanol foliar application (Table 3) indicated that α-Amino nitrogen and K contents were the lowest in 20% (v/v), (2.931 eq100, 6.469 eq100 g/sugar, respectively) and the highest in 5% (v/v), (4.321 eq100, 6.694 eq100 g/sugar); however, Na content was the highest in 30% (v/v), (4.943 eq100 g/sugar) and the lowest in 5% (v/v) (3.863 eq100 g/sugar). Results of our experiment showed that 20% (v/v) methanol foliar application produced 32 and 3% less α-Amino nitrogen and K contents than 5% (v/v). Results presented in Table 3 showed that 20% (v/v) and 30% (v/v) methanol foliar application had the highest (17.16%) and lowest (15.31%) sugar content, respectively. Furthermore, the highest (82.39%) and the lowest (70.97) RWC were observed for 15% (v/v) methanol foliar application and control, respectively (Table 3). This study proved that methanol spraying decreased the levels of N and K and this reason is causes to absorbing Na to regulate osmotic pressure in sugar beet to increase turgidly and growth and accumulating root dry material. Interestingly, α-amino nitrogen and K

Table 3. Mean comparison of methanol foliar application on traits.

MFA	α-amino N eq100 g/sugar	K eq100 g/sugar	Na eq100 g/sugar	Sugar content (%)	RWC (%)
b_1 (control)	3.036	6.469	4.220	15.37	70.97
b_2 [5% (v/v)]	4.321	6.694	3.863	15.65	77.46
b_3 [10% (v/v)]	3.182	6.579	3.976	16.23	80.95
b_4 [15% (v/v)]	3.084	6.512	4.036	16.37	82.39
b_5 [20% (v/v)]	2.931	6.469	4.249	17.16	81.09
b_6 [25% (v/v)]	3.549	6.619	4.084	16.86	80.24
b_7 [30% (v/v)]	3.760	6.626	4.943	15.31	77.79
LSD5%	0.054	0.046	0.038	4.26	1.37

MFA: Methanol foliar application.

contents were expanded with increase in amount of methanol from 20 - 30 (v/v). Nadali *et al.* (2014) observed that Level 10% (v/v) and the control had the most amounts and the lowest amount of nitrogen concentration, respectively. Also, methanol caused significant difference on the concentration of sodium and the highest amount belongs to level control and the lowest rate belongs to level of 10% (v/v). Ramirez *et al.* (2006) also emphasized on increasing cell relative water content upon methanol application, which may be a possible reason for higher observed yields with methanol application. Makhdum *et al.* (2002) also reported higher leaf turgor when cotton plants were treated with 15% (v/v) of methanol, suggesting that methanol can improve water status of leaves thereby enabling them to maintain their chlorophyll. Other findings (Ramirez *et al.* (2006) and Zbiec *et al.* 2003) have also emphasized on increasing cell relative water content upon methanol application, which may be a possible reason for higher yields with methanol application.

It seems methanol can be used as rich source of carbon. Physiological characteristics of sugar beat are affected by methanol spray. As far as sugar beet spends it is the most sensitive growth stages periods in the hot weather of summer so using methanol as an anti stress material to reach higher yield is recommended. Based on the results, spraying of methanol up to 20% (v/v) had negative and poisonous effects on physiological characteristics.

Acknowledgement

The authors would like to thank Tabriz Branch, Islamic Azad University for the financial support on the basis of a research project contract.

References

Babaee B, Abdolahian Noghabi M, Jahad Akbar MR and Yousef Abadi M 2011. The appropriate method for determining of sugar content in sugar beet produced under drought, salinity and normal conditions. J. Sugar Beet. **29**(1): 53-59.

Bagheri HR, Ladan Moghadam AR and Afshari H 2014. The effects of foliar application of methanol on growth and secondary metabolites in lavender. Intl. Res. J. Appl. Basic. Sci. **8**(2): 150-152.

Bayat V, Paknejad F, Ardakani M and Vazan S 2013. Can spraying with methanol improve the reproductive performance of soybean under deficit irrigation? International Journal of Agronomy and Plant Production **4**(12): 3164-3170.

Clover G, Smith H and Jaggard K 1998. The crop under stress. British Sugar Beet Review. **66**(3): 17-19.

Corpe WA and Rheem S 1989. Ecology of the methylotrophic bacteria on living leaf surfaces. FEMS Microbiol. Ecol. **62**: 243-250.

Fall R and Benson AA.1996. Leaf methanol the simplest natural product from plants. Trends in Plant Science **1**: 296-301.

Farooq M, Basra SMA, Wahid A, Cheema ZA, Cheema MA and Khaliq A 2008. Physiological role of exogenously applied glycinebetaine in improving drought tolerance of fine grain aromatic rice (*Oryza sativa* L.). Agronomy and Crop Science **194**: 325-333.

Hsiao TC 2000. Leaf and root growth in relation to water status. Hort. Sci. **35**: 1051-1058.

Hanson AD, Gage DA and Shachar-Hill Y 2000. Plant one-carbon metabolism and its engineering. Trends in Plant Science **5**(5): 206-213.

Holland MA and Polacco JC 1994. PPFMs and other covert contaminants: Is there more to plant physiology than just plant? Annu. Rev. Plant Physiol. Mol. Biol. **45**: 197-209.

Khalilvand Behrouzyar E and Yarnia M 2013. Physiological response of sunflower (*Helianthus annuus* L.) to foliar application of methanol under water deficit stress. Res. on Crops **14**(3): 839-844.

Khazaei S, Fathollah Taleghani D and Hassanpour Darvishi H 2015. The Effect of methanol foliar application on the tolerance of sugar beet cultivars to drought stress. Biological Forum - An International Journal. **7**(1): 1502-1507.

Jaleel CA, Gopi R, Sankar B, Gomathinayagam M and Panneerselvam R 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. Comptes Rendus Biologies **331**(1): 42-47.

MacDonald RC and Fall R 1993. Detection of substantial emissions of methanol from plants to the atmosphere. Atmos. Environ. **27A**: 1709-1713.

Makhdum MI, Malik MNA, Din SU, Ahmad F and Chaudhry FI 2002. Physiological response of cotton to methanol foliar application. J. Res. Sci. **13**: 37-43.

Matin MA, Brown JH and Ferguson H 1989. Leaf water potential, relative water content and diffusive resistance as screening techniques for drought resistance in barley. Agron. J. **81**: 100-05.

Milton E and McGiffen JR 1996. The role of methanol in promoting plant growth: A current evaluation. HortScience 31(7): 1092-1096

Nadali I, Yarnia M, Paknejad F, Farahvash F and Vazan S 2014. Effect of methanol in promoting sugar beet (*Beta vulgaris* L.) yield and some quality characteristic in drought stress condition. Bull. Env. Pharmacol. Life Sci. **3**(11): 57-61

Nemecek-Marshall M, MacDonald RC, Franzen JJ, Wojciechowski CL and Fall R 1995. Methanol emission from leaves: Enzymatic detection of gas-phase methanol and relation of methanol fluxes to stomatal conductance and leaf development. Plant Physiol. **108**: 1359-1368.

Ober E 2001. The search for drought tolerance in sugar beet. British Sugar Beet Review **69**(1): 40-43.

Paknejad F, Nasri M, Tohidi-Moghadam HR, Zahedi H and Jami Al-Ahmad M 2007. Effects of drought stress on chlorophyll fluorescence parameters, chlorophyll content and grain yield of wheat cultivars. J. Biol. Sci. **7**: 841-47.

Por-Mousavi M, Galavi M, Danshiyan J, Ghanbari A and Basirani N 2007. Effects of drought stress and manure on leaf relative water content, cell membrane stability and leaf chlorophyll content in soybean (*Glycine max*). J. Agric. Sci. Nat. Resour. **60**: 125-34.

Ramirez I, Dorta F, Espinoza V, Jimenez E, Mercado A and Pen a-Cortes H 2006. Effects of foliar and root applications of methanol on the growth of arabidopsis, tobacco and tomato plants. J. Plant Growth Regul. **25**: 30-44.

Sadeghi-Shoae M, Kashani A, Paknejad F, Fathollah Taleghani D and Tookallo MR 2014. Response of sugar beet to concentrations and intervals of methanol spraying under deficit irrigation. International J. Biosci. **4**(5): 128-134.

Zbiec I, Karczmarczyk S and Podsiado C 2003. Response of some cultivated plants to methanol as compared to supplemental irrigation. Elec. J. Polish Agric. Univ. Agron. **6**: 1-7

(Manuscript received on 22 April, 2016; revised on 22 May, 2016)