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The effect of foliar application of methanol on sugar beet (*Beta vulgaris* L.) plants grown in drought conditions

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Abstract: *The effect of foliar application of methanol on sugar beet (*Beta vulgaris *L.) plants grown in drought conditions.* To study the effect of water deficit stress and methanol foliar application on sugar beet, an experiment was conducted in split plot form based on a randomized complete block design (RCBD) with three replications, at the Research Station of the Islamic Azad University, Tabriz Branch, north-western Iran, during the 2013–2014 growing seasons. The treatments were three levels of water deficit stress: a_1 – mild stress (50% of field irrigation capacity); a_2 – fair stress (75% of field irrigation capacity); and a_3 – normal irrigation (100% of field irrigation capacity); and seven levels of foliar application of methanol (b_1:0, b_2:5, b_3:10, b_4:15, b_5:20, b_6:25 and b_7:30 percent by volume). The analysis of variance showed the significant effect of water deficit stress on root yield, sugar yield, chlorophyll content ($p < 0.01$), harvest index and leaf area index ($p < 0.05$). The results showed that 100% of field irrigation capacity had the greatest effect, and mild stress (50% of capacity) the least effect, on root yield, sugar yield, harvest index, leaf area index and chlorophyll content. Also, the effect of methanol foliar application on root yield, sugar yield, harvest index, leaf area index and chlorophyll content was significant ($p < 0.01$). It was found that 20% (v/v) methanol foliar application led to increased chlorophyll content (332) and harvest index (66.37%) compared with the control. The plants treated with 20% (v/v) methanol also had the highest root yield (7.014 kg·m^{-2}) and sugar yield (1,204 g·m^{-2}).

Key words: chlorophyll content, harvest index, irrigation, leaf area index, yield

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INTRODUCTION

Sugar beet accounts for about 40% of world sugar production, and is the main source of sugar in many countries. Sugar beet is a widely grown crop in Iran, with 4.73 million t cultivated on a total area of 97,101 ha, giving a yield of 48.7 t·ha^{-1} [Iranian Sugar Factories Syndicate 2014]. When plants do not receive sufficient water they are subject to a stress called water deficit. Growth is accomplished through cell division, cell enlargement and differentiation, and involves genetic, physiological, ecological and morphological events and their complex interactions. The quality and quantity of plant growth depend on these events, which are affected by water deficit [Farooq et al. 2009]. One of the most common stress tolerance strategies in plants is the overproduction of various types of compatible organic solutes [Serraj and Sinclair 2002]. Osmotic adjustment is a mechanism used to maintain water relations under osmotic stress. It involves the accumulation of a range of osmotically active molecules or ions including soluble sugars, sugar alcohols, proline, glycine betaine, organic acids, calcium, potassium, chloride ions, etc. Under water deficit and as a result of solute accumulation, the osmotic potential of the cell is lowered, which attracts water into the cell and helps maintain turgor [Subbarao et al. 2000]. The metabolism of methanol and its conversion to sugars change the osmotic potential of the leaves. Foliar applications of aqueous methanol have been reported to increase yield, accelerate maturity, and reduce drought stress and irrigation requirements in C3 crops grown in arid environments, under elevated temperatures, and in direct sunlight [Ramirez et al. 2006]. Sadeghi-Shoae et al. [2014] reported that methanol foliar application increased total dry matter (TDM), root yield (RY), sugar yield (SY) and white yield sugar. In addition, methanol application had alleviating impacts on chickpea exposed to drought stress conditions [Hossinzadeh et al. 2012]. Bagheri et al. [2014] reported that spraying with 20% (v/v) methanol in lavender greatly increased leaf area, leaf fresh and dry mass. This study investigates the effect of foliar application of methanol on some of the physiological traits of sugar beet under different irrigation regimes.

MATERIAL AND METHODS

The field experiment was carried out in split plot form on a randomized complete block design with three replicates, at the Research Station of the Islamic Azad University, Tabriz Branch, northwestern Iran, during 2013–2014. The *Beta vulgaris* variety used was SBSI-007, a diploid, monogerm spring variety that is widely cultivated in Iran. The first factor was water deficit stress in three levels: mild stress (a_1; 50% of field irrigation capacity), fair stress (a_2; 75% of field irrigation capacity) and normal irrigation (a_3; 100% of field irrigation capacity). 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 percent by volume), where to prevent methanol poisoning in the presence of light, 1 mg·dm^{-3} glycine and 1 mg·dm^{-3} tetrahydrofolate (THF) were added to the prepared solution [Khalilvand Behrouzyar and Yarnia 2013]. Each plot consists of five rows, with 60 cm row spacing and 20 cm plant intervals. Pre-planting fertilization was carried out using urea fertilizer at a rate of 150 kg·ha^{-1}. A physical and chemical analysis of the soil is given in Table 1. The pH of the soil was low-average alkaline (7.8 to 8.9), which makes it difficult for a plant to absorb micronutrients such as Fe, Mn, Cu, B and Zn.

In all treatments, methanol spray was applied three times during the stages of sugar beet development. The first spraying was performed at around the 16-leaf stage (BBCH 19.6), and the subsequent two sprayings were performed at 14-day intervals (BBCH 31 and 39). In the control plots, plants were sprayed with water. Water deficit stress was imposed from the eight-leaf stage to physiological maturity. Root yield, sugar yield, chlorophyll content (estimated by a SPAD-502 device, a portable, non-destructive device for measuring the chlorophyll

TABLE 1. Physical and chemical analysis of the soil

pH	Ec × 103	C (%)	N (%)	P (ava) (ppm)	K (ava) (ppm)	Sand (%)	Silt (%)	Clay (%)
7.8–8.9	20	0.57	0.051	56	323	71	17	12

content of leaves; 90 days after planting, BBCH 31–33), harvest index and leaf area index were obtained from an area of 1 m^2. To measure root yield, all plants were harvested from the 1 m^2 area and the shoots and roots were divided. Roots were counted and transferred to the Sugar Technology Laboratory. In the laboratory, the roots were weighed before being converted into pulp. Sugar yield was calculated by the following formula:

sugar yield ($kg \cdot m^{-2}$) = root yield ($kg \cdot m^{-2}$ [fresh weight]) × white sugar content (%)

To check the normality of data and to perform analysis of variance and mean comparison, MSTAT-C software was 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 showed the significant effect of water deficit stress on root yield (RY), sugar yield (SY), chlorophyll content ($p < 0.01$), harvest index (LAI) and leaf area index ($p < 0.05$) – Table 2.

Effect of irrigation levels on qualitative and quantitative properties of sugar beet

The results for different water deficit stress levels (Table 3) indicated that root yield and sugar yield were highest under normal irrigation (100% of field irrigation capacity), at 6.18 $kg \cdot m^{-2}$ and 983 $g \cdot m^{-2}$ respectively, and lowest under mild stress (50% of field irrigation capacity), at 5.63 $kg \cdot m^{-2}$ and 927 g m^{-2} respectively. Chlorophyll content was highest under mild stress (324.7) and lowest under normal irrigation (309.4). It was also found that water deficit stress produced 8.8 and 44% lower root yield and sugar yield than normal irrigation. Abdollahian-Noghabi and Froud-Williams [1998] reported reduced leaf and root growth under drought stress conditions. Firoozabadi et al. [2003] tested the effect of drought stress on sugar beet and found that root yield under normal irrigation, moderate drought stress and extreme drought stress was 58.6, 45.8 and 34.7 $t \cdot ha^{-1}$, respectively. Under drought stress conditions, due to increasing ABA in mesophyll, the stomata are closed, and eventually stomata conduction is

TABLE 2. Analysis of variance of measured traits

SOV	df	Root yield	Sugar yield	Chlorophyll content	Harvest index	Leaf area index
Rep	2	0.269*	36.80*	120.846 ^{ns}	8.29 ^{ns}	0.035 ^{ns}
WDS	2	1.591**	169.73**	1276**	199*	3.016**
Error	4	0.033	7.203	59.042	11.38	0.022
MFA	6	4.293**	2 091**	2 040**	526**	6.795**
MFA × WDS	12	0.057 ^{ns}	19.118 ^{ns}	27.181 ^{ns}	8.68 ^{ns}	0.044 ^{ns}
Error	36	0.104	34.657	19.853	23.88	0.087
CV		5.46	6.15	1.41	8.82	6.71

*, ** – significance at 5 and 1%, respectively.
WDS – water deficit stress, MFA – methanol foliar application, SOV – source of variation.

TABLE 3. Effect of water deficit stress on selected traits of sugar beet

WDS	Root yield ($kg \cdot m^{-2}$)	Sugar yield ($g \cdot m^{-2}$)	Chlorophyll content	Harvest index (%)	Leaf area index
100% FC	6.18	983	309.1	15.86	4.74
75% FC	5.93	967	315.7	16.15	4.72
50% FC	5.63	927	324.7	16.40	3.99
LSD5%	0.054	2.3	0.038	0.140	0.12

WDS – water deficit stress, FC – field capacity.

reduced in the leaf and carbon dioxide penetration for assimilation in the plant is reduced; finally the cell's turgidity is lowered, which can limit root growth [Hsiao 2000]. Photosynthesis, a process occurring in chlorophyll-containing tissues, provides the main materials for other metabolically vital processes [Bagheri et al. 2014]. It has been documented that alterations in photosynthetic metabolism due to environmental changes or agricultural practices will eventually modify plant growth and productivity [Bagheri et al. 2014]. In the present study, water deficit stress increased chlorophyll content in the leaves. Drought may initially inhibit leaf growth and development [Gazanchian et al. 2007], and although chlorophyll content is closely associated with leaf development, response patterns of cell number and size [Lecoeur et al. 1995] and chlorophyll content to water stress depend on the period of leaf development. At the cellular level, moderate water deficits (mild stress) had opposite effects on cell number and cell size, but more severe deficits reduced both variables [Aguirrezabal et al. 2006]. Thus, compared with severe water deficit stress, under moderate drought the youngest leaves might increase their chlorophyll content, demonstrating adaptation to environmental stress (Table 2). Harvest index was highest under normal irrigation (58.54%) and lowest under mild stress (52.38%). Leaf area index (LAI) is a dimensionless quantity that characterizes plant canopies; the highest LAI value (4.74) was obtained under 100% FC irrigation, while 50% FC irrigation produced the lowest LAI (3.99) – Table 2. Leaf area index is one of the important growth indicators which have been used as measures of the photosynthetic system. This trait is related to biological and economic yields: an increase in LAI causes higher yields [Singh et al. 2009].

Effect of methanol on qualitative and quantitative properties of sugar beet

Results for the effect of methanol foliar application (Table 4) indicate that root yield and sugar yield were highest under 20% (v/v) application (7.014 $kg \cdot m^{-2}$ and 1,204 $g \cdot m^{-2}$ respectively) and lowest under 30% application (4.981, 762 $g \cdot m^{-2}$), while chlorophyll content was highest under 20% application (332) and lowest in the control (286). Khalilvand Behrouzyar and Yarnia [2013] showed that foliar application of 21% (v/v) methanol under 50% FC irrigation caused increases of 9, 20 and 16% in chlorophyll a and chlorophyll b content and total chlorophyll a + chlorophyll b compared

TABLE 4. Effect of methanol foliar application on selected traits of sugar beet

MFA	Root yield ($kg \cdot m^{-2}$)	Sugar yield ($g \cdot m^{-2}$)	Chlorophyll content	Harvest index (%)	Leaf area index
Control	5.399	829	286	48.93	5.77
5% (v/v)	5.633	881	312	52.55	4.92
10% (v/v)	5.718	962	321	51.13	4.78
15% (v/v)	6.498	1 063	327	59.92	4.36
20% (v/v)	7.014	1 204	332	62.80	4.08
25% (v/v)	6.153	1 036	321	66.37	3.48
30% (v/v)	4.981	762	314	46.06	3.27
LSD5%	0.308	5.62	4.26	4.67	0.31

MFA – methanol foliar application.

with plants under mild stress treatment without methanol spraying. Ramirez et al. [2006] found that spraying methanol on water-deficit plants can increase their leaf chlorophyll content. Zheng et al. [2006] found that foliar application of methanol on wheat plants increased leaf chlorophyll content and the photochemical efficiency of photosystem II, which led to increased photosynthesis and stomata conductance in a flag leaf plant and had a significant effect on grain yield. Makhdum et al. [2002] also reported higher leaf turgor when cotton plants were treated with 15% (v/v) methanol, suggesting that methanol can improve the water status of leaves, thereby enabling them to maintain their chlorophyll level. Treatment with 25% (v/v) methanol led to the highest HI (66.37%), while 30% (v/v) methanol produced the lowest value (Table 2). Furthermore, 20% (v/v) methanol as compared with 30% gave a 24% increase in LAI (from 3.272 to 4.084) – Table 2. Mirakhori et al. (2009) tested the effect of methanol foliar application on soybean, and observed that the treatment increased leaf area index.

CONCLUSIONS

In this study, foliar spraying with methanol was found to have a significant effect on all traits. Moreover, use of methanol in a concentration of 5–10% had the greatest stimulating effect. Increasing the concentration to 20% led to negative and poisonous effects on physiological characteristics.

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Streszczenie: *Wpływ dolistnego traktowania metanolem na wzrost buraka cukrowego (*Beta vulgaris *L.) w warunkach stresu suszy*. Badania nad wpływem dolistnej aplikacji metanolu w warunkach deficytu wody na wzrost buraka cukrowego przeprowadzono w latach 2013–2014 w północno-zachodnim Iranie – Research Station of the Islamic Azad University, Tabriz Branch. Doświadczenie założono w układzie split plot metodą losowanych bloków w trzech powtórzeniach. Testowano wzrost roślin, stosując trzy poziomy nawadniania: 50, 75 i 100% polowej pojemności wodnej; metanol zastosowano w siedmiu stężeniach (v/v): b_1:0, b_2:5, b_3:10, b_4:15, b_5:20, b_6:25 and b_7:30. Analiza statystyczna wykazała istotny wpływ deficytu wody na plon korzeni i cukru, zawartość chlorofilu ($p < 0{,}01$), wskaźnik witalności i wskaźnik powierzchni liścia ($p < 0{,}05$). Największe różnice w wymienionych parametrach wystąpiły między kombinacjami o skrajnych poziomach nawadniania (50 i 100% polowej pojemności wodnej). Dolistna aplikacja metanolu miała także istotny wpływ na plon korzeni i cukru, zawartość chlorofilu, wskaźnik witalności i wskaźnik powierzchni liścia ($p < 0{,}01$). Wzrost zawartości chlorofilu w liściach (332%) i wskaźnika witalności (o 66,37%) w porównaniu z kontrolą wykazano u rośliny opryskiwanych 20-procentowym (v/v) metanolem. W tej kombinacji odnotowano także największy plon korzeni (7,014 $kg \cdot m^{-2}$) i cukru (1204 $g \cdot m^{-2}$).