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The Effect of Methanol Foliar Application on the Tolerance of Sugar Beet Cultivars to Drought Stress

Soltanmorad Khazaei*, Dariush Fathollah Taleghani** and Hossein Hassanpour Darvishi*
**Department of Agronomy, Shahr-e-Qods Branch, Islamic Azad University, Tehran, IRAN*
***Sugar Beet Seed Institute, Karaj, IRAN*

(Corresponding author: Dariush Fathollah Taleghani)
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ABSTRACT: This experiment was conducted in 2013 at fields of the Research Institute of Sugar Beet, Karaj, Iran, to study the effect of drought stress and methanol foliar application on sugar beet cultivars. The experiment was conducted in split plot factorial in the form of a randomized complete block design with four replications and three factors: (1) irrigation in two levels (irrigation after 90 mm and 200 mm evaporation from the A class pan), (2) foliar application of methanol in two levels (0 and 20% of volume percentage), and (3) sugar beet cultivars in six levels (Gadouk, 5RR-87HSF.33, Fd 415, Pars, Sbsi 016 and Brigita). The measured traits included: root yield, sugar content, white sugar yield, Na, K and N concentration. Analysis of variance indicated the significant effect of drought stress on root yield, sugar content and N content. Foliar application of methanol had no significant effect on any of the measured traits. Moreover, all measured traits were significantly different among different cultivars. The only interaction with significant effect was cultivar × irrigation. Mean comparison showed that the highest root yield (101.72 t/ha) and white sugar yield (11.75 t/ha) were related to Brigita. This cultivar had also the lowest content of K (4.66 eq/100 g sugar beet) and N (1.10 eq/100 g sugar beet). In Brigita cultivar, drought stress reduced root yield from 101.72 t/ha to 74.4 t/ha. However, drought stress increased sugar content of all cultivars.

Keywords: α-amino N, potassium, sodium, white sugar yield.

INTRODUCTION

Sugar beet production is an essential component of agricultural economics in many countries. Sugar beet cultivation was first developed in Europe about 200 years ago, in order to reduce the import of sugar to Europe from other parts of the world. Sugar beet contributes to about 40% of world sugar production and is the main source of sugar production in many countries. Sugar beet is not only important for sugar production, but it is a beneficial crop in agronomic rotation and is used in industries and human and livestock food production. Each tone of sugar beet yield has 9-14 kg molasses which contains 20% water, 60% carbohydrates, 10% ash and 10% protein (Cooke and Scott, 1993; Pakniat, 1899).

Water plays important role in plants and in some plant organs it contributes to 90% of the weight. It is required for all plant processes; reduction of available water affects plant physiological and biochemical processes (Koochaki *et al., 1996*). The primary effect of drought stress on plants is the reduction of cell turgor pressure. Then, drought stress affects stomata closure, structure of proteins and enzymes, activity of hormones, metabolism of carbohydrates, photosynthesis, respiration, and finally plant yield (Black and Ong, 2000; Cooke and Scott, 1993; Koochaki *et al.*, 1996; Levitt, 1980). Barlow *et al.* (1977) reported that drought stress reduced ATP content in plant cell by 40% and increased free amino acids by 20%. Aspinall and Paleg (1981) also reported that drought stress may increase plan proline content by 40-100%. Firoozabadi *et al.* (2003) tested the effect of drought stress on sugar beet and found that root yield in normal irrigation, moderate drought stress and extreme drought stress was 58.6, 45.8 and 34.7 t/ha, respectively. Choluj *et al.* (2004) found that drought stress reduced both root yield and sugar yield; depending on the stage it was occurred. Although sugar beet is tolerant to drought stress to some extent; however, drought stress induces morphological and physiological responses in sugar beet (Schittenhelm, 1999). So, development of sugar beet cultivars tolerant to drought stress is an important method to prevent yield loss in dry conditions. In arid or semi-arid regions which plants faces water shortage, sugar beet yield loss may reach to more than 20% (Ober, 2001).

Plants use different mechanisms to cope with the drought stress. Foliar application of methanol or other substances may help drought stressed plants. After spray of methanol to plant shoot, plant shows a systemic response; the response is observed even in leaves which have not received methanol directly. This indicated that methanol is transferred to other plant parts after it is sprayed to plant leaves (Ramberg *et al.*, 2002).

Nonomura and Beson (1992) reported that methanol application to plant leaves increased yield and alleviated the effect of drought stress on plant; these effects are more significant when plant is under stress. Ramberg *et al.* 2002 reported that spraying methanol to crop plants under drought stress results in the enhancement of biomass; however, it reduces the biomass of plants with sufficient available water. Mirakhori *et al.* (2009) tested the effect of methanol foliar application on soybean and observed that the treatment increased leaf area index, crop growth rate, relative water content, chlorophyll content, pod growth and yield, 1000 grain weight and grain yield. Paknejad *et al.* (2012) also observed that foliar application of methanol increased grain yield, biomass, oil content and protein content of soybean. Li *et al.* (1995) reported methanol application significantly increased grain yield, 1000-seed weight and the number of pods per plant of soybean.

Regarding the importance of sugar beet in world sugar production and the effect of drought stress on sugar beet yield loss, this experiment was conducted to evaluate the effect of methanol foliar application on the tolerance of various sugar beet cultivars to drought stress.

MATERIALS AND METHODS

This experiment was conducted in 2013 at fields of the Research Institute of Sugar Beet, Karaj, Iran (35° 45' N, 51° 6' E, 1313 m above the sea level). The annual precipitation in the area in 2013 was 256.3 mm. The average maximum and minimum daily air temperature at the area was 16.6°C and 2.6°C, respectively. Soil type at the test site was loamy with the pH of 7.86 and EC of 0.55 ds/m. Detailed soil properties are listed in Table 1.

Table 1: Physico-chemical properties of the test site soil.

Sand (%)	Silt (%)	Clay (%)	OC (%)	K (ppm)	No_3 (ppm)	NH_4 (ppm)	P (mg/kg)	Na (meq/l)
28.29	44.70	25.40	1.0	459.46	19.29	9.70	19.13	6.11

The experiment was conducted in split plot factorial in the form of a randomized complete block design with four replications and three factors:

Irrigation (main factor): in two levels including normal irrigation (irrigation conducted after 90 mm evaporation from the A class evaporation pan) and drought stress (irrigation was conducted after 200 mm evaporation from the pan). Drought stress was applied to the filed from the eight leaves stage. Totally, plots were irrigated 12 times in normal treatments and seven times in drought stress treatments.

Methanol: in two levels including 0 and 20% of volume percentage. Methanol was sprayed to plants in three times. The first spray was conducted 70 day after planting; other two sprays were conducted with 20 days interval. Spraying was conducted between 5-7 pm. In the control plots, plants were sprayed with water.

Sugar beet cultivars: in six levels including Gadouk, 5RR-87HSF.33, Fd 415, Pars, Sbsi 016 and Brigita.

The field was under fallow prior to sugar beet cultivation. Field preparation was conducted using moldboard plow, disk and leveler. Nitrogen fertilizer was split in two parts: the first part was added at the time of cultivation and the second part was added after thinning and weeding, when plants were fully established and they were in the six leaves stage. The total amount of nitrogen given to the field was 150 kg N/ha in the form of urea. Moreover, 100 kg P/ha in the form of triple-superphosphate was added to the field prior to cultivation, based on the results of soil sample analysis.

Each plot contained three planting rows with the length of 8 m. There was 50 cm space between the rows. The interval of plants on the planting rows was 20 cm and the planting density was 100,000 plants/ha. Seeds were planted on May 15th, 2-3 cm below the soil surface. The normal field operations were conducted during the growth period, and harvest was done on Nov 16th. The measured traits included: root yield, sugar content, white sugar yield, Na, K and N concentration.

To measure root yield, all plants were harvested from 4.8 m^2 area and shoot and root were divided. Roots were counted and transferred to the Sugar Technology Laboratory. In the laboratory, roots were weighted before providing pulp from them. White sugar yield was obtained by multiplying root yield × sugar percentage.

White sugar yield is the most important parameter in sugar beet production which is the amount of sugar that can be extracted from roots. It is always lower than the total sugar yield (Cooke and Scott , 1993). To obtain sugar content, the Polarimetry method by Saccharomat instrument was used which is the most common method (Clover et al., 1998). Sodium and potassium contents were measured by flame photometry method. Moreover, α-amino N was measured by betalizer device (Clover *et al.*, 1998).

The analysis of variance was conducted by SAS software and means were compared according to Duncan's multiple range test and also LSD.

RESULTS AND DISCUSSION

A. The effect of drought stress

Analysis of variance indicated the significant effect of drought stress on root yield at P≤0.05, sugar content at P≤0.01 and N content at P≤0.01; the effect was not significant on white sugar yield, Na and K content (Table 2). Mean comparison of drought stress levels (Table 3) indicated that root yield was the highest in normal irrigation (88.60 t/ha) and the lowest in drought stress (75.83 t/ha). However, sugar content and white sugar yield were the highest in stressed plants (16.25% and 9.74 t/ha, respectively) and the lowest in the normal irrigation (13.80% and 9.02 t/ha, respectively). In fact, drought stress reduced root yield by about 85% but increased white sugar yield by about 8%. Mean comparison also 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). This represents that drought stress increased K and N content in sugar (Table 2).

Table 2: Analysis of variance of the effect of treatments on the measured traits.

SOV	df	Mean Squares (MS)					
		Root yield	Sugar content	White sugar yield	Na	K	N
Rep	3	ns	ns	ns	ns	ns	ns
Irrigation (A)	1	*	**	ns	ns	ns	**
Error A	3	173.32	2.11	3.73	6.31	2.81	1.17
Methanol (B)	1	ns	ns	ns	ns	ns	ns
Cultivar (C)	5	**	**	**	**	**	**
A × B	1	ns	ns	ns	ns	ns	ns
A × C	5	**	**	**	**	ns	ns
B × C	5	ns	ns	ns	ns	ns	ns
A × B × C	5	ns	ns	ns	ns	ns	ns
Error	66	75.5	1.26	1.04	0.81	0.18	0.19
CV (%)	-	10.57	7.47	10.87	25.60	8.38	20.76

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 3: The effect of drought stress on the measured traits.

Treatments	Root yield (t/ha)	Sugar content (%)	White sugar yield (t/ha)	Na (eq/100 g sugar beet)	K(eq/100 g sugar beet)	N(eq/100 g sugar beet)
Normal	88.60a	13.80b	9.02a	4.21a	4.99a	1.38b
Stress	75.83b	16.25a	9.74a	2.82a	5.11a	2.84a

Means in a column followed by the same letter are not significantly different at P≤0.05.

Drought stress reduces sugar beet yield because it reduces cell turgor pressure and increases soil water potential. However, under drought stress plants break polysaccharides to monosaccharides to cope with the drought stress, which results in the enhancement of sugar content in plant. Results of our experiment also showed that drought stress increased sugar content from 13.8% to 16.25% (Black and Ong, 2000; Cooke and Scott, 1993).

Ober (2001) also found that drought stress increased dark respiration in sugar beet and broke starch to sucrose, resulting in the enhancement of sugar content. Firoozabadi *et al.* (2003) tested the effect of drought stress on sugar beet and found that root yield in normal irrigation, moderate drought stress and extreme drought stress was 58.6, 45.8 and 34.7 t/ha, respectively. Choluj *et al.* (2004) found that drought stress reduced both root yield and sugar yield; depending on the stage it was occurred.

Hammer *et al.* (1994) tested the effect of nine different irrigation regimes on sugar beet and observed that white sugar yield was 9.82 t/ha in plots without irrigation and 10.78 t/ah in plots with the highest amount of irrigation. They reported that sugar yield is directly correlated to the water supply. They also reported that drought stress inhibits plant growth, but growth continues after the drought period. The damages depend on the physiological age of plant, the severity of stress and plant species (Hammer *et al.*, 1994).

Ghosh *et al.* (1994) conducted an experiment to test the effect of drought stress on oilseed rape and found that drought stress extremely reduced plant yield. Korte *et al.* (1983) observed that irrigating plants at the vegetative growth stage had significant effect on their growth and height; however, irrigation and the grain filling stage increased grain weight and grain yield.

Sodium, potassium and nitrogen reduce the quality of sugar in sugar beet. Clover *et al.* (1998) reported that drought stress increase nitrogen content in sugar beet yield but had no significant effect on Na and K.

B. The effect of methanol

Results of our experiment indicated that foliar application of methanol had no significant effect on any of the measured traits (Table 2, Table 4). However, there are various reports about the effect of methanol foliar application on growth, yield and quality of different crop plants. For example, Nadali (2009) conducted experiments to test the effect of drought stress and foliar application of methanol on sugar beet and reported that methanol application significantly affected leaf weight, root yield and sugar yield. MacDonald and Fall (1993) also reported that foliar application of methanol increased yield and reduced plant need for water under warm and dry environmental conditions. Mirakhori *et al.* (2009) tested the effect of methanol foliar application on soybean and observed that the treatment increased leaf area index, crop growth rate, relative water content, chlorophyll content, pod growth and yield, 1000 grain weight and grain yield. The non-significant effect of methanol on sugar beet in our experiment may be related to the environmental conditions and experimental conditions and methods.

Table 4: The effect of methanol on the measured traits.

Treatments	Root yield (t/ha)	Sugar content (%)	White sugar yield (t/ha)	Na (eq/100 g sugar beet)	K(eq/100 g sugar beet)	N(eq/100 g sugar beet)
0 volume percentage	82.30a	12.25a	9.42a	3.52a	5.06a	2.06a
16 volume percentage	82.14a	12.16a	9.33a	3.50a	5.04a	2.16a

Means in a column followed by the same letter are not significantly different at P≤0.05.

Table 5: The effect of interaction of cultivar × drought stress on the measured traits.

Treatments	Root yield (t/ha)	Sugar content (%)	White sugar yield (t/ha)	Na (eq/100 g sugar beet)	K(eq/100 g sugar beet)	N(eq/100 g sugar beet)
Gadouk × Normal	75.63c	14.09b	7.77d	5.02ab	4.91abc	1.30bcd
Pars × Normal	85.99b	13.49b	8.32c	4.42b	5.20ab	1.72a
Sbsi × Normal	92.69ab	11.34c	6.61c	5.87a	5.31a	1.22cd
5RR × Normal	90.99b	13.62b	9.20c	4.12bc	4.82bc	1.51ab
Fd 415 × Normal	84.60bc	15.63a	10.47b	2.60d	5.04abc	1.40bc
Brigita × Normal	101.72a	14.62ab	11.75a	3.21cd	4.66c	1.10d
LSD (5%)	10.23	1.253	1.075	1.006	0.4918	0.2870
Gadouk × Stress	63.52c	17.35a	8.82bc	2.40bc	2.79ab	2.80ab
Pars × Stress	78.53ab	15.97b	9.89ab	2.90b	2.90a	2.90a
Sbsi × Stress	81.10ab	15.28b	9.43bc	3.14ab	2.95a	2.95a
5RR × Stress	73.87b	15.44b	8.66c	4.02a	3.23a	3.23a
Fd 415 × Stress	83.55a	16.01b	10.64a	2.49bc	2.91a	2.91a
Brigita × Stress	74.4b	17.44a	10.98a	1.94c	2.27b	2.27b
LSD (5%)	7.874	1.132	1.096	0.9047	0.5919	0.5919

Means in a column followed by the same letter are not significantly different at P≤0.05.

C. Sugar beet cultivars

Analysis of variance indicated that all measured traits were significantly different among different cultivars (Table 2). Mean comparison showed that the highest root yield (101.72 t/ha) and white sugar yield (11.75 t/ha) were related to Brigita. This cultivar had also the lowest content of K (4.66 eq/100 g sugar beet) and N (1.10 eq/100 g sugar beet). Moreover, studying the interaction of cultivar × irrigation (Table 5) indicated that drought stress reduced root yield of all cultivars. In Brigita, drought stress reduced root yield from 101.72 t/ha in the normal irrigation to 74.4 t/ha. The reducing trend was observed in all other cultivars. However, drought stress increased sugar content of all cultivars. Again in Brigita, drought stress increased sugar content from 14.62% to 17.44%. Enhancement of sugar content in sugar beet root is a plant response to the stress. In another experiment, Ober (2001) also found that drought stress broke starch to sucrose, resulting in the enhancement of sugar content. Results of our experiment showed that the effect of drought stress on white sugar yield of different cultivars was not the same; drought stress reduced white sugar yield of 5RR-87HSF.33 and Brigita but increased white sugar yield of Gadouk, Pars, Sbsi 016 and Fd 415. In addition, drought stress reduced Na and K content of all cultivars but increased their N content (Table 5).

Breeding new cultivars which are tolerant to drought is a very important method to prevent yield loss in arid and semi-arid regions. In these regions, drought stress reduces sugar beet yield about 10-20% on average (Mohammadian *et al.,* 2003). Taiz and Zeiger (1998) reported that comparing the growth and yield of plants under normal irrigation condition and drought stress condition is a suitable method for the selection of cultivars tolerant to drought.

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