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Effects of methanol on yield and some quality characteristic of sugar beet (*Beta vulgaris* L.) in drought stress condition

I. Nadali[1*], M. Yarnia1, F. Paknejad[2], F. Farahvash1, S. Vazan[2]

[1]*Department of Agronomy and Plant Breeding, Islamic Azad University Tabriz Branch, Iran*

[2]*Department of Agronomy and Plant Breeding, Islamic Azad University Karaj Branch, Iran*

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Abstract

A study was conducted to evaluate the effects of Methanol on yield and some Quality Characteristic of Sugar Beet (Beta vulgaris L.) in drought stress condition in Maahdasht (Karaj, Iran), in 2012. Aqueous solutions were 0(control), 7, 14, 21 and 28 (v/v) methanol. Second factor was irrigation regime 1.normal irrigation (irrigation after 40% depletion of available water), 2.mild drought stress (irrigation after 60% depletion of available water) and 3.severe drought stress (irrigation after 70% depletion of available water). Irrigation system was dripping irrigation system (Tape) in this study. These solutions were sprayed overhead three times in two-week intervals on foliage parts of sugar beet. Results of this experiment indicated that there was a significant difference between effects of solutions on root yield, leaf yield, white sugar yield, molasses, white sugar content, N and Na concentrations. The best of root yield, leaf yield and white sugar yield was gained in 7% (v/v) of methanol with 76.62, 61.72 and 9.91(ton/h), respectively. There was also significant difference among three levels of irrigations on root yield, leaf yield, sugar content and K and N concentrations of white sugar content.

***Corresponding Author:** I. Nadali ✉ imnadal@ymail.com

Introduction

Stress is an altered physiological condition by factors that tends to disrupt the equilibrium, such as drought stress. Drought is a meteorological term commonly defined as a period without significant rainfall (*Ober, 2001)*. Around one third of cultivable lands of the world encounter water shortage and recently it has been reported that drought stress is the main factor in reducing the quality of sugar beet (*Clover et al., 1998*). Although sugar beet is resistant against drought stress but to reach high productivity, researchers have considered some actions to reduce drought stress (*Hsiao, 2000*). Most of these actions are focused to find the ways to reduce transpiration in order to maintain stability of Co_2 and reduce photorespiration in drought stress condition. According to Zbiec et al. (2003) increasing density of Co_2 can neutralize the effects resulted by drought stress, so applying materials to increase Co_2's density in plants eventually stabilizes the yield in drought conditions. One of the solutions to increase Co_2's density in the plants is using the compounds such as methanol, ethanol, propanol, butanol and also using Amino acids as glycine, glutamate and aspartate (*Nonomura, 1992*). It should be noted that methanol is common for plants because it is the simplest plant products, which is produced inside the plant (*Fall et al., 1996*). This organic compound escapes from the leaves via stomata or it is metabolized by plant tissues and becomes Co_2 for plant's consumption (*Gout et al., 2000*). Methanol's application causes to produce Co_2 in the leaves and increases photosynthesis so it can be used as carbon's source (*Zbiec et al., 1999*). Foliar application of methanol can increase activity of nitrate reductase and alkaline phosphatase in leaves (Zbieć et al., 1999). Andres et al. (1990) studied the effects of alcohols (methanol, ethanol, propanol, butanol) on the association of the thylakoid membrane with fructose-1,6-bisphosphatase (FBPase), one of the principal enzymes controlling is the activity of the photosynthetic carbon reduction cycle. The most important advantage of methanol is preventing and reducing inducted stresses of plants during their photorespiration (*Nonomura et al.,* 1992).

Under drought stress conditions due to closeness of stomata and transpiration rate is reduced and Co_2 cannot enter into the leaves. Zbiec et al (1999) indicated that the reason for reducing photosynthesis in plants treated with methanol is rapid oxidation methanol to Co_2 and its combination with ribulose 5-1- diphosphate and elimination of oxygen exchange. Methanol causes the delay of senescence in leaves and influences on ethylene production in plant, which causes the increase photosynthesis activity. Zbiec et al (2003) reported increasing of 10% root yield on sugar beet in 20 and 30% (v/v) of methanol, meanwhile Makhdum et al (2002) reported that methanol has caused the increase cotton leaf's surface. A bacterium exists named Methyltrophic bacteria. These bacteria are capable to grow on methanol and generate plant growth regulators such as auxin and cytokinin (*Lee et al., 2006*). Weather is the most important external factor influencing on yield and technologic quality of sugar beet. Humidity restriction in the soil causes the decrease of leaf's surface and eventually photosynthesis and finally reduces root yield (*Cooke at al., 1993*). According to some reports, drought stress caused root yield decrease and white sugar yield (*Bazza et al., 1993*). Water shortage and high temperature in addition to reducing growth cause the increase of sugar in the root (*Abdollahian-noghabi et al., 1998*). Imposing drought stress at the end of the growth season causes the density increase of root's impurities particularly potassium, nitrogen and sodium and finally causes the increase molasses (*Ober, 2001*). Clover et al (1998) reported that losing water through roots and smallness of roots caused to increasing sugar level in drought stress condition. Some other reasons include breaking down of polysaccharide and converting to monosaccharide and finally increasing density of sugars in the cell (*Cooke at al., 1993*).

The aim of this research includes effects assessing of foliar application in methanol and drought stress on root yield, leaf yield, sugar content, sodium, potassium, nitrogen concentration, molasses, white sugar content and white sugar yield. As far as methanol acts as a C source for C3 crops to enhance yield, the main objectives of our experiments 1.) To evaluate the effect of foliar application of methanol on the root yield, leaf yield, white sugar yield, sugar yield and some quality properties 2.) Determine the efficacious alcohol concentration for foliar application of methanol.

Materials and methods

Field conditions

This research was conducted in a research farm of Islamic Azad university of Karaj, Iran (35^{o} 45' N and 51^{o} *56' E, 1160 M) during 2012-2013 growth season. The planting of sugar* beet was carried out in early may on sandy loam soil with an electrical conductivity (EC) of 4.28 dSm^{-1} and pH of 7.91.

Experimental design and plant material

Treatments arranged as split-plot experiment based on a randomized completely block design (RCBD) with 3 replications. Studied factorials included 0(control), 7, 14, 21and 28 (v/v) methanol, Plots related to control treatments were sprayed with water at the time of foliar application. The second factor was normal irrigation (irrigation after 40% depletion of available water), mild drought stress (irrigation after 60% depletion of available water) and severe drought stress (irrigation after 70% depletion of available water). Irrigation system was dripping (Tape) in this study. Soil moisture content was determined using chalk blocks based on humidity drainage of the field. Blocks were studied by Paknejad et al (2007) in this farm. Since that sugar beet is sensitive to environmental stresses such as drought stress so Irrigation was done enough from germination stage to perfectly stabilization of plant and after 8 leaf stages due to depletion of moisture, drought stress treatment was imposed.

Methanol application

Methanol solutions were sprayed overhead three times in two-week intervals on foliage parts of sugar beet. The first foliar application was applied during 80 days after planting. These treatments were applied on july 19^{th}, August 2^{nd} and August 17^{th}, between 14:00 pm to 16:00 pm during bright sunny days at hot temperature. Spraying foliage was continued until flowing of solution drops.

Sowing operation until harvesting

The planting density was approximately 10 plant $^{m-2}$ with the rows 50 cm apart. Plots were 7.5 m in width and 5m in length in each replication. The experimental field received 150 kg P_2O_5 h^{-1} , two third of which was applied during deep plough in autumn and remainder in spring prior to disk harrowing. Nitrogen fertilizer was applied at a rate of 150 kg N h^{-1} in the form of urea, the first half of which during harrowing in spring and the remaining half before hoeing when the plants reached the six-leaf stage. Weeds were controlled by hand weeding when necessary. Final harvesting was conducted on 17 Nov 2012 with ignoring a meter from each planting line in 3.6 meter square.

Lab analysis

The obtained roots of each plot were washed and after weighing they were placed in special dishes randomly after covering trays by nylon cover they were transferred to freezer immediately and kept in -20°C until time of qualitative analysis. Each paste sample was placed in 20^{o} c to perform qualitative analysis and after thawing, 26 g paste from each sample with 177 m.lit so stat lead were mixed for three minutes. Limpid syrup was obtained After transferring mixture to funnel. In the obtained syrup, sugar content was measured by polarymetery method by sodium and potassium saccharide meter device by liquid digit betalizer device (*Clover et al., 1998*). As for density of impurities in white sugar content per gram sugar in 100 gram sugar beet and percentage of Molasses sugar per gram sugar in 100 gram sugar beet were estimated by following equation:

White sugar content(%) = sugar content(%) – (Molasses+ 0.6)

Sugar wastage was estimated as 0.6 in sugar factory. Also, the white sugar yield was measured by these equations:

White sugar yield (t/ha) = root yield (ton [fresh weight]/ha) ×white sugar content (%).

Molasses Amount is estimated based on potassium sodium and Nitrogen by one of the most common experimental formulas gathered.

Statistical analysis

The SAS was used to analyze all the data and means were compared by the least significant differences (LSD) test at 0.05 probability level.

Results and Discussion

Studying methanol's effect on qualitative and quantitative properties of sugar beet

The results of variance analysis showed that there were significant differences ($p<0.01$) between levels of methanol solutions and control on concentration of nitrogen and also methanol affected significantly on root yield, leaf yield, white sugar yield, molasses, sodium and white sugar content ($p<0.05$) (Table 1). Among different levels of methanol on root yield there was a significant difference ($p<0.05$) and the most yield of roots was obtained in 7, 21 and 14 (v/v) of methanol respectively. The optimum foliar applied for root yield is 7% (v/v) of methanol with 76.62t ha^{-1} (Table 2). The minimum root yield was observed at control with 61.33t ha^{-1} (Table 2). Results showed that methanol has increased roots yield by 23% compared to 0 (control). It was reported that methanol increases root yield of sugar beet by 10 % in 20- 30% (v/v) of methanol (*Zbiec et al., 2003*). The leaves of many plants were covered by methyl-bacterium. These bacteria are capable to grow on C1 compounds such as methanol and generate plant growth regulators such as Auxin and Cytokinin (*Satler et al., 1980*). It has been observed that applying methanol by solution spraying method increases fresh weight of tobacco (*Ramirez et al., 2006*). According to Nonomura et al (1992), the Plant treated by methanol can increase their net photosynthesis and improve their yield. They said that methanol improves carbon converting process. Methanol is smaller than CO_2 molecules which it can be used by C_3 plants to increase yield (*Ramirez et al., 2006*). Methanol increases activity of photosynthesis in the leaves by delaying senescence in the leaves and finally increases yield. There was a significant difference ($p<0.05$) between levels of methanol solutions and control on leaf yield (Table 1). The maximum leaf yield was observed at 7% (v/v) methanol with 61.72 t.ha^{-1} and the lowest amount belonged to control with 49.2 t ha^{-1} (Table 2).

Results showed that methanol caused increase leaf yield by 31% in comparison to control. Methanol increases turgidy in the leaves cells which contributes in the growth of leaf (*Zbiec et al, 1999 and Hemming et al., 1995*). It seems that methanol with increasing leaf area duration caused increasing photosynthesis period in the plants and protects leaves and increases leaf yield and root yield. This organic material can delay senescence of the leaves by effecting on ethylene production rate (*Satler et al., 1980*). Nadali et al (2010) indicated that methanol caused increase leaf yield by 31%. There was no significant difference between concentrations of methanol in sugar content (Table 1). According to Demeres and Derks (1996), increasing dioxide carbon content will not essentially result in increased sugar content in plants, because there is a negative correlation between sugar content and root yield (*Demeres and Derks, 1996*). Methanol had no significant effect on potassium content (Table, 1). 7% level (v/v) and the control had the most amounts and the lowest amount of nitrogen concentration respectively (Table, 2). This reason likely due to the absorbing of this element to regulate osmotic pressure in sugar beet to increase turgidity and growth and accumulating of dry material (*Cooke et al., 1993*). Increasing root and leaf yields in 7% (v/v) of methanol shows that, nitrogen absorption is high in this level, which causes the growth. Methanol caused significant difference ($p<0.05$) on the concentration of sodium (Table, 1) and the highest amount belongs to level control and the lowest rate belongs to level of 7% (v/v) (Table, 2). Plants after methanol application tend to absorbing the elements such as N, K, NA (*Zbiec et al., 2003*), as far as nitrogen has the best effects on plants growth, it seems that nitrogen absorption is preferred to sodium absorption for growth and level of 7% (v/v) had the maximum nitrogen and the minimum sodium. As shown in table 1 there was a significant difference ($p<0.05$) between of methanol levels on molasses and control had the most molasses comparison to other levels of methanol solutions. Sodium has an important role in sugar westage through the molasses in comparison with nitrogen (*Ober, 2001*). According to this research the control had the maximum sodium absorption and then increasing of molasses in the control was logical. Methanol had a significant difference ($p<0.05$) between of methanol levels and control on white sugar content and 7%(v/v) methanol caused increase of white sugar content by 11% compared to control. As mentioned before, the 7 % level (v/v) of methanol has the lowest amount of molasses and control has the maximum molasses amount (Table, 2) so according to equation 2, it is likely the reason of obtaining this result on white sugar content. Methanol caused a significant increase ($p<0.05$) in white sugar yield among different levels of methanol and control (Table, 1). Levels 7, 21, 14 and 28% (v/v) of methanol have the most amount of white sugar yield, respectively and have not significant differences with each other. Level 7% (v/v) of methanol with 9.91 $t.ha^{-1}$ had the most amounts of white sugar yield and control level with 6.74 $t.ha^{-1}$ had the lowest amount (Table, 2). Level 21% (v/v) of methanol as compared to 0 (control) had increase of 47 percent in white sugar yield. In sugar beet, white sugar yield is a component of accumulated dry weight of the roots, and the maximum white sugar yield is obtained when dry weight of the roots is in its highest amount (*Ranji et al., 2000*), such as results of this research. Therefore it is possible to improve white sugar yield by increasing root yield through foliar application of methanol.

Table 1. Analysis of variance quantitative and quality traits in sugar beet.

		MS								
S.O.V	DF	WHITE SUGAR YIELD	MOLASSES	WHITE SUGAR CONTENT	N	K	Na	SUGAR CONTENT	SHOOT YIELD	ROOT YIELD
Block	2	6.231^{ns}	1.559^{**}	2.583^{ns}	4.96^{**}	0.67^{ns}	10.84^{**}	0.139^{ns}	117.8^{ns}	1880^{ns}
Irrigation	2	5.22^{ns}	0.367^{ns}	11.337^{*}	4.46^{*}	1.392^{*}	1.66^{ns}	10.01^{*}	1076.1^{**}	1085.3^{**}
Error a	4	5.99	0.47	12.109	2.477	0.237	4.872	9.74	39.38	47.79
Methanol	4	10.61^{*}	0.631^{*}	6.27^{*}	1.86^{**}	0.07^{ns}	5.193^{*}	3.49^{ns}	163.05^{*}	272.06^{*}
M×I	8	1.617^{ns}	0.454^{ns}	1.652^{ns}	0.58^{ns}	0.37^{ns}	2.43^{ns}	0.807^{ns}	136.1^{ns}	95.31^{ns}
Error b	24	2.83	0.246	3.853	0.353	0.353	1.619	3.063	50.02	76.15
CV (%)	-	18.58	13.9	15.8	20.12	12.33	19.12	10.55	12.82	12.57

ns: Non-significant

* and **: significant at the 5% and 1% probability levels, respectively.

Table 2. Comparison means for quantitative and quality traits in sugar beet.

Treatment	WHITE SUGAR YIELD	MOLASSES (%)	WHITE SUGAR CONTENT (%)	N (meq. 100 g sugar^{-1})	K (meq. 100 g sugar^{-1})	Na (meq. 100 g sugar^{-1})	SUGAR CONTENT (%)	SHOOT YILED (T/ha)	ROOT YIELD (T/ha)
					METHANOL				
Control	6.74b	4.006a	10.75b	1.9c	5.4a	6.61a	15.48a	49.29c	61.33c
7%	9.91a	3.54ab	12.96a	2.83a	5.48a	4.37b	17.15a	61.72a	76.62a
21%	8.68a	3.26b	12.77a	2.81ab	5.26a	5.034b	16.62a	52.88bc	68.05bc
28%	8.66a	3.61ab	12.11ab	2.05bc	5.4a	5.47ab	16.37a	57.44ab	73.03ab
35%	8.64a	3.4b	12.43ab	2.12abc	5.3a	4.95b	16.49a	56.27ab	69.48ab
					IRRIGATION				
Normal	9.03a	3.73a	11.094b	2.039b	5.11b	5.58a	15.46b	65.95a	80.13a
Mild drought stress	8.07a	3.49a	12.25ab	2b	5.33ab	5.38a	16.33ab	49.2b	65.11b
Severe drought stress	8.47a	3.46a	13.2a	2.99a	5.66a	4.9a	17.5a	51.4b	63.8b

Means, in each column and for each factor, followed by at least one letter in common are not significantly different at the 5% probability level-using LSD test.

Studying effect of irrigation levels on qualitative and quantitative properties of sugar beet

The result of variance analysis showed that (table, 1) there was significant difference among normal levels, mild drought stress and severe drought stress on root yield ($p<0.01$). The reason for reducing root yield under drought stress conditions is water shortage, which can reduce root yield basically especially due to decreasing turgidity pressure (*Cook et al,* 1993).

Under drought stress condition due to increasing ABA in mesophyll, stomata are closed and eventually stomata conduction reduced in the leaf and dioxide carbon's penetration is reduced for assimilation in the plant and finally cell's turgidity is decreased and decreasing turgidity can confine root's growth (*Hsiao,* 2000).

The main factor in root's growth is supplying of carbohydrates from leaves to root. When stress reduces this supplying, root's growth deforms unavoidably. There was significant difference ($p<0.01$) between effects of normal, mild drought stress and severe drought stress on leaf yield (Table, 1) and the maximum leaf yield was observed by normal irrigation (Table 2).

Abdollahian Noghabi *et al.* (1998) reported that growth reduction of leaf and root under drought stress conditions. Severe water stress may result in the decreasing of photosynthesis, disturbance of metabolism and decrease in cell enlargement and growth. The results of analysis of variance showed significant differences ($p<0.01$) among levels of irrigation in amount of sugar content (Table 1). Amount of sugar content in severe drought stress level had high sugar content compared to normal irrigation regime (Table, 2). High sugar content in severe drought stress conditions is due to water depletion through roots and small size of roots under this condition (*Ober, 2000*). One of the plants mechanisms under drought stress conditions is, breaking polysaccharide and converting to monosaccharide and eventually increasing sugar materials density in the cell maintain the osmotic adjustment (*Cook et al, 1993*). Nitrogen and potassium amounts showed significant difference between severe drought stress, mild drought stress and normal levels (Table, 1). Results showed that the optimum Nitrogen and potassium amounts were observed at the severe drought stress (Table, 2). Usually in the drought stress conditions, the impurities of root will increase to maintaining turgor by osmotic adjustment (*Smith et al., 1977*). Rates of sodium for irrigation levels did not show significant difference (Table, 1). According to table 2 white sugar content was increased With lowering water content and under severe drought stress conditions and this property was significant among normal, mild and severe drought stress levels($p<0.05$) (Table, 1). Probably increasing white sugar content under drought stress conditions is due to increasing percentage of sugar content in root (*Firoozabadi et al., 2003*). There was no significant difference Between the irrigation regime of molasses amounts (Table, 1) while irrigation regimes were placed in same group in comparison with means (Table2). White sugar yield was not significantly affected by irrigation treatments. This reason is likely due to increasing the white sugar content significantly under mild.

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