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Effects of methanol on sugar beet (*Beta vulgaris*)

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Abstract

In order to evaluate the effects of methanol on sugar beet quality and yield, a field study was conducted at Research Station of Islamic Azad University of Karaj, Iran, during 2008-2009. Aqueous methanol solutions with 0 (control), 7, 14, 21, 28 and 35% (v/v) concentrations were sprayed on foliage parts of sugar beet three times during growth season with two week intervals. The first foliar application was done at 80 days after planting. After 190 days, plants were harvested, and the fresh weight of root and leaf, sugar, and white sugar yields, the relative content of molasses, sugar and white sugar, and the content of Na, K, and N in roots were measured. Results indicated that there was a significant difference ($p<0.01$) between control plants and plants with methanol treatment in the fresh weight of root, leaf, sugar, and white sugar. Foliar application of 21% methanol solution increased root and leaf fresh weights and sugar yield. The plants with 14% (v/v) methanol application had the maximum white sugar yield (9.28 ton/h). The other parameters were not affected by methanol application. This study indicates that foliar application of 14-21% (v/v) methanol increase sugar yield of sugar beets.

Keywords: sugar yield, white sugar yield, methanol, molasses, potassium, sodium, sugar beet.

Introduction

Production of biomass by plants depends to great extent on environmental factors such as water supply, air temperature and carbon dioxide concentration in the canopy (Zbieć et al., 2003). Numerous experiment have shown that by increasing the dioxide carbon content in air, the crops yield increased and plants accumulated more carbohydrates because almost 90% of plant dry weight is resulted from CO_2 assimilation during photosynthesis (Abdel-Latif et al., 1996). Methanol spry is a method which increases crop CO_2 fixation in unit area. 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). Abundant dioxide carbon supply from methanol causes the photorespiration to be shifted from catabolism to anabolism (Zbieć et al., 1999). Photo respiration can be minimized with methanol spray, since 25% of carbon wastes during photorespiration (Desclaux et al., 2000). That is because methanol is absorbed in plant and rapidly metabolized to CO_2 in plant tissue due to smaller size of methanol rather than CO_2 (Gout et al., 2000). The major source of methanol production in plant is cellular pectin demethylation . Such volatile organic compound i.e., methanol exist leaves via stomata and it is obvious that plant tissues metabolize methanol (Galbally et al., 2002). A small proportion of this endogenous methanol reaches leaf surfaces, where it is volatilized or consumed by methylotrophic bacteria. These bacteria are capable to grow on methanol and generate plant growth regulators such as auxin and cytokinin (Lee et al., 2006). Also these bacteria are associated with nitrogen metabolism in plants through production of bacterial urea (Fall et al., 1996). Glycine has effective roll in drought stress and other stress induced physiological response (Zbieć et al., 2003). Only C_3 plants which produce ribolose 1,5-diphosphate and then 3phosphoglyceric acid during their photosynthetic carboxylation respond to methanol by increased biomass production, since carbon dioxide resulting from rapid oxidation of methanol can successfully compete with oxygen for RuBisco (Ramirez et al., 2006). Foliar application of methanol can increase the 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 the activity of the photosynthetic carbon reduction cycle. They found that moderately concentrated (2-20%) alcohols stabilized the hydrophobic binding between FBPase and other membrane bound proteins, probably due to the hydrophobic character of the alcohols, and increased FBPase activity. Alcohols have been shown to delay senescence of oat (*Avena fatua*) via inhibition of the ethylene production (Satler et al., 1980). Hemming et al. (1995) measured metabolic heat rate, carbon dioxide production and oxygen uptake rates of bell pepper (*Capsicum annuum* L.) after exposing leaf tissues to methanol. They reported a strict increase in carbon conversion efficiency which lasted several weeks. Frequent methanol applications reduce the require-

Table 1. Results of analysis of variance for qualitative and quantitative traits in sugar beet

(S.O.V)	df	Root yield	Leaf yield	Sugar content	White sugar content	Sugar yield	White Sugar yield	Molasses	K	Na	N
Block	2	830.7^{**}	106.5^{**}	0.25^{ns}	0.812^{ns}	16.2^{**}	6.95^{**}	0.161^{ns}	0.06^{ns}	3.01^{ns}	1.20^{ns}
Methanol	5	187.4^{**}	54.18^{**}	0.31^{ns}	0.534^{ns}	4.68^{**}	2.12^{**}	0.16^{ns}	0.14^{ns}	0.45^{ns}	0.66^{ns}
Error	10	25.53	11.102	0.401	0.582	0.279	0.288	0.094	0.24	0.316	0.40
C.V(%)	-	6.11	7.2	4.2	7.2	4.26	6.22	7.82	7.16	12.45	19.23

In each column, ns and ** means non-significant and significant at 0.01 probability level, respectively.

ment for fungicide application to mildew (*Sphacrotheca panosa*) (Rajala et al., 1998). Methanol enhanced the growth of oilseed rape, soybeans, small beans, cabbage and sugar beet (Zbieć et al., 2003). It has been reported that foliar application of methanol caused increase in seed cotton yield and it had positive effect on physiological processes, water relation and plant structure (Makhdum et al., 2002). Also in another experiment on sunflower (*Helianthus annuus* L.) methanol increased stem length, leaf area index, stem dry weight, number of floret primordial and accelerated completion of floral development by 5 day (Hernandez et al., 2000). As far as methanol act as a C source for C_3 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) to determine the efficacious alcohol concentration for foliar application of methanol.

Materials and methods

In order to evaluate effects of methanol on sugar beet quality and yield, a field experiment was conducted at Research Farm of Islamic Azad University of Karaj, Iran (35° 45′ N, 50° 56′ E, 1160 M) during 2008-2009 growth season. Sugar beets were planted in early may on sandy loam soil with an electrical conductivity (EC) of 5.55 dS/m and a pH of 7.6. The planting density was approximately 10 pl/m with rows 60 cm apart. Plots in each replication were 7.5 m in width and 5m in length. The experimental field received 150 kg P_2O_5/h , two third of which was applied during deep plough in autumn, while the rest was applied in spring prior to disk harrowing. Nitrogen fertilizer at a rate of 150 kg N/h was applied 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. The sugar beet was established with furrow irrigation system. Weeds were controlled by hand weeding when necessary. The experiment was a completely randomized block with three replications. The treatment were 0 (control), 7, 14, 21, 28, and 35% (v/v) methanol and each solution contained 0.2% glycine. To reduce probability of methanol toxicity, Nonomura and Benson (1992) recommended adding glycine to methanol spray. These solutions were sprayed on foliage parts of sugar beet three times with two week intervals. The first foliar application was applied in 80 days after planting (10-12 leaf stage). These treatments were applied on July 28th, August 10th and August 23th, between 14:00 pm to 16:00 pm during bright sunny days with hot temperature. Methanol spray was carried out in a way that all above ground parts of sugar beet plant were covered. Back engine sprayer with a capacity of 12 L was used for spray and sprinkler was held 40 cm above the plants. Plant were harvested on 11 November by pulling the beet manually, and topped by cutting the crown at the base of the leaves. To qualitative analysis each paste sample was placed in 20 °C and after thawing, 26 g paste from each sample with 177 m/lit so stat lead were mixed for three minutes. After transferring mixture to funnel, a limpid syrup was obtained. In the obtained syrup, sugar content was measured by polarymetery method by sacchary meter device and sodium, potassium and nitrogen was measured by betalizer device (Payne, 1968). As for concentration of impurities in white sugar content (mg/100g sugar) and percentage of Molasses sugar (mg/100g sugar) were estimated by following equation:

White sugar content (%) = sugar content (%) – (Molasses (%) – 0.6) [1]

Sugar wastage of sugar factory was estimated as 0.6. Molasses Amount is estimated based on potassium, sodium and nitrogen by one of the most common experimental formulas gathered . Also white sugar yield and sugar yield was measured by these equations:

White sugar yield (t/ha) = root yield (ton [fresh weight]/ha) × white sugar content (%) [2]

Sugar yield (t/ha) = root yield (ton [fresh weight]/ha) × sugar content (%) [3]

data were collected at harvest on root yield (ton [fresh weight]/ha), leaf yield (t/ha), sugar content (t/ha), sugar content (%), molasses (%), white sugar content (%), white sugar yield (t/ha),white sugar (t/ha), nitrogen (mg/100g sugar), sodium (mg/100g sugar) and potassium (mg/100g sugar). As far as these parameters are the most significant as for this reason these parameters was measured. Data given in percentages were subjected to arcsine transformation before statistical analysis. The SAS software package was used to analyze all the data (SAS Institute, 2001) and means were compared by the least significant differences (LSD) test at 0.01 probability level.

Results and discussion

Foliar application of methanol had a significant effect on root yield (Table 1). The highest root yields were obtained at 21%, 14% and 7% (v/v), respectively. The optimum applied solution concentration for root yield was 21% (v/v) of methanol with 92.3 ton/h (Table 2). The minimum root yield was observed at control with 70.54 ton/h. Applying 21% (v/v) methanol caused root yield to be increased by 30%, compared to 0 (control). Table 1 According to these results, treating plants with methanol can enhance their net photosynthesis, thus improving the yield. Nonomura and Benson (1992) reported that foliar-applied methanol reduces the plants photorespiration, and the rapidly oxidized methanol leads to formaldehyde incursion with tetrahydrofolate. As a result, the doubling of serine content could lead to twofold the sucrose to be produced through the serine intermediate (Rowe et al., 1994). Also Methanol causes to delay senescence in leaves and influences on ethylene production in plant which this causes to increase photo synthesis activity (Zbieć et al. 1999). Zbieć et al. (2003) observed the yield of

Table 2. Comparisons of means for qualitative and quantitative traits in sugar beet

Methanol levels	Root yield	Leaf yield	sugar yield	white sugar Yield	sugar content	white sugar content	molasses	K	Na	N
	-----(t ha^{-1})-----		(%)	(t/ha)	----------(%)----------			--(mg/100g sugar)--		
control	70.54c	40.2b	10.37a	7.19b	14.76a	10.26a	3.92a	6.66a	4.74a	3.14a
7%	83.8ab	45.03ab	12.74ab	9.07a	15.28a	10.94a	3.75a	7.06a	3.9a	3.06a
14%	89.3ab	47.37ab	13.3a	9.28a	14.91a	10.41a	3.9a	6.71a	3.75a	3.57a
21%	92.3a	53.03a	13.72ab	9.20a	14.85a	9.95a	4.3a	7.2a	5.06a	4.15a
28%	81.89abc	44.05b	12.7ab	9.02a	15.53a	11.04a	3.88a	6.94a	4.47a	2.97a
35%	77.73bc	46.82ab	11.44bc	8.02ab	14.71a	10.32a	3.79a	6.82a	4.33a	2.94a
Lsd (0.01)	13.077	8.62	1.36	1.39	1.63	1.97	0.79	1.28	1.45	1.64

Mean with the same letters in each column have not significant differences at 0.01 probability level.

roots increased by 10% using 20 or 30 % methanol solutions. Data presented in table 1 showed that methanol treatments positively affected the leaf yield. The maximum leaf yield was observed at 21% (v/v) (Table 2). Results showed that methanol caused increase leaf yield by 31% in comparison with 0 (control). It seems that methanol with increasing leaf yield caused increasing photosynthesis in the plants and protects leaves and probability it was due to increases root yield. There are some reasons for increase leaf yield. The leaves of many plants have covered by methylobacterium. These bacteria are capable to grow on C_1 compounds such as methanol and generate plant growth regulators such as auxin and cytokinin (Omer et al., 2004; Ivanova et al., 2001). Also according to view of Makhdum et al. (2002), methanol treated cotton showed increased leaf area index and turgidy. Table 2 There was no significant difference between concentration of methanol in respect to gross and white 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 not significant effect on molasses, and potassium, sodium and nitrogen contents (Table 1). The concentration of K, Na and N present as impurities in extracted root sap have been shown to be inversely related to the amount of extractable sugar (Jaggard et al. 1998). There was a significant difference ($p<0.01$) between levels of methanol solutions control on sugar yield (Table 1). As shown in table 2, sugar yield increased by 32% as an influence of applying 21% methanol solution, compared to control. Sugar yield is a function of root yield and sugar content (Jaggard et al. 1998). As mentioned before, there was no any significant difference between different levels of methanol solutions in respect to sugar content, so it seems foliar applied methanol has caused a parallel increase in sugar yield by increasing the root yield. Methanol caused a significant increase ($p<0.01$) in white sugar yield. The maximum and the minimum white sugar yield were observed at 14% (v/v) solution and control respectively (Table 2). 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). Therefore it is possible to improve white sugar yield by increasing root yield through foliar application of methanol.

Conclusion

In general it can be concluded that methanol can be used as rich source of carbon to enhance root yield, leaf yield, sugar yield and white sugar yield. As far as sugar beet spends it the most sensitive growth stages periods in the hot weather of summer so using these materials as an anti stress material to reach higher yield is recommended.

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