Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [11] October 2014: 57-61
©2014 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Effect of Methanol in promoting sugar beet (*Beta vulgaris* L.) yield and some Quality Characteristic in Drought stress Condition

Iman Nadali, Mehrdad Yarnia, Farzad Paknejad, Farhad Farahvash, Saeid Vazan
Department of Agronomy and Plant Breeding, Islamic Azad University Karaj Branch, Iran.

ABSTRACT

In order to evaluate the effects of Methanol on yield and some Quality Characteristic of Sugar Beet (Beta vulgaris L.) in drought stress condition a study was conducted in 2012 in maahdasht (Karaj, Iran). Aqueous solutions 0(control), 7, 14, 21 and 28 (v/v) methanol. Second factor were irrigation regime1.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 in this study was dripping irrigation system (Tape). These solutions were sprayed overhead 3 times in two week intervals on foliage parts of sugar beet. Results of this experiment indicated that there was 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, 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 between three levels of irrigations on root yield, leaf yield, sugar content and K and N concentrations also white sugar content.

Key word: *Sugar beet, Methanol, Root yield, White sugar yield and Drought stress*

Received 01.05.2014 Revised 19.06.2014 Accepted 10.09. 2014

INTRODUCTION

Fertilizers for higher plants generally include nitrogen phosphorus and potassium which are referred to as primary nutrients or macronutrients and as well as various minerals such as iron sulfur calcium and magnesium as micronutrients [1]. therefore little attention has been paid to providing fertilizers which act directly to enhance carbon fixation in higher plants. Today in order to achieve this goal compounds such as methanol, ethanol, propanol, butanol and amino acids like glycine, glutamats and aspartate are used as C source for the most production [2]. Recently methanol spry is a method, which increases crop CO_2 fixation in unit area. The main reason for using of Methanol due to generate by plant through demethylation of pectin-by-pectin methylesterase for tightening of the cell especially throughout the early stage of leaf expansion [3]. Some methanol emission has also been observed during changes in cell wall construction during the development of roots and fruits [4]. 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 [3]. Also these bacteria are associated with nitrogen metabolism in plants through production of bacterial urea [5]. Methanol accumulates in the intercellular air space or in the liquid pool at night when the stomata close and is rapidly converted to formaldehyde formic acid and CO2 to prevent damage by alcohol oxidase [6]. Radio 14C and 13C NMR studies revealed that methanol is metabolized by alcohol oxidase to formaldehyde and formic acid, which are further converted to serine methionine, purine and thymidylate [5]. The CO2 produced from the oxidization of methanol is utilized within the calvin cycle for glucose metabolism. The clear distinctions between C3 and C4 plants in the effect of methanol were attributed to the inhibition of photorespiration during methanol assimilation [2]. Plants not undergoing photorespiration could not assimilate methanol and showed toxicity symptoms following methanol applications.

The aim of this research includes assessing effect of foliar application of 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 act 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) to determine the efficacious alcohol concentration for foliar application of methanol.

MATERIALS AND METHOD

This research was conducted a research farm of Islamic Azad university of Karaj, Iran (35° 45' N and 51° 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 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 time of foliar application. These solutions were sprayed overhead three times in two-week intervals on foliage parts of sugar beet. The first foliar application was applied in 80 days after planting. These treatments were applied on July 19, August 2thand August 17, between 14:00 pm to 16:00 pm during bright sunny days with hot temperature. Spraying foliage was continued until flowing solution drops. 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 in this study was dripping (Tape).Soil moisture content was determined using chalking blocks based on humidity drainage of the field. Paknejad et al [7] in this farm studied the blocks.

The planting density was approximately 10 $plant^{m-2}$ 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^{-1} , two third of which was applied during deep plough in autumn and reminder in spring prior to disk harrowing. Nitrogen fertilizer at a rate of 150 kg N h^{-1} 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. Weeds were controlled by hand weeding when necessary. Since that sugar beet is sensitive to environmental stresses such as drought stress, so from germination stage to perfectly stabilization of plant Irrigation was done enough and after 8 leaf stage due to depletion of moisture, drought stress treatment was imposed. Final harvesting was conducted on 17 Nov 2012 with ignoring a meter from each planting line in 4.8 meter square. Obtained roots of each plot was washed and after weighing, they were placed in special dishes randomly after covering trays by nylon cover they were transferred to freezer immediately and were kept in -20°c until time of qualitative analysis. 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, alimpid syrup was obtained. In the obtained syrup, sugar content was measured by polarymetery method by sodium and potassium saccharide meter device by liquid digit betalizer device [8]. 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:

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

Sugar wastage of sugar factory was estimated as 0.6.

Also white sugar yield was measured by these equations:

2) 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. 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 analysis of variance showed that there was a significant differences ($p<0.01$) between levels of methanol solutions and control on concentration of nitrogen and also methanol effected 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 different ($p<0.05$) 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 zero (control). It was reported that methanol increases root yield of sugar beet by 10 % in 20- 30% (v/v) of methanol [9]. The leaves of many plants have covered by methylobacterium.These bacteria are capable to grow on C1 compounds such as methanol and generate plant growth regulators such as Auxin and Cytokinin [10].It has been observed that applying methanol by

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solution spraying method increases fresh weight of tobacco [11]. According to Nonomura et al [2], 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 [11]. Methanol increases activity of photosynthesis in the leaves with 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 belongs to control with 49.2t ha^{-1} (Table 2) .

Results showed that methanol caused increase leaf yield by 31%comparison to control. Methanol increases turgidy in the cells of the leaves which this contributes growth of the leaf [9,12]. It seems that methanol with increasing leaf area duration caused increasing photosynthesis period in the plants and protects leaves and also increases leaf yield and root yield. This organic material can delay senescence of the leaves by effecting on rate of producing ethylene [10]. Nadali et al [13] 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 [14], 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. Methanol had not significant effect on potassium content (Table, 1).Level 7% (v/v) and the control had the most amounts and the lowest amount of nitrogen concentration respectively (Table, 2). This reason likely is due to absorbing this element to regulate osmotic pressure in sugar beet to increase turgidly and growth and accumulating dry material [15]. Increasing root yield and leaf yield in 7% (v/v) of methanol shows that in this level, nitrogen absorption is high which causes 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 application of methanol tend to absorbing of elements such as N, K, NA [9], as far as nitrogen has the best effects on plants growth, it seems 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 waste of sugar through the molasses in comparison with nitrogen [16]. According to this research the control had the maximum sodium absorption 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 white sugar content by 11% compared to control. As mentioned before level 7 % (v/v) of methanol has the lowest amount of molasses and control has the most amounts of molasses (Table, 2) so according to equation of 2, it is likely reason 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 compared to 0(control) had increase as 47 percentages 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 , such as results of this research. Therefore, it is possible to improve white sugar yield by increasing root yield through foliar application of methanol.

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

The result of analysis of variance 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 it can reduce root yield basically especially due to decreasing turgid pressure [15].

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 turgid is decreased and decreasing turgid can confine root's growth [17].

The main factor in root's growth is supplying 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 [18] reported that growth reduction of leaf and root under drought stress conditions. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and decrease in cell enlargement and growth. The results of analysis of variance showed significant differences ($p<0.01$) between 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 depletion water through roots and small size of roots under this condition [16]. One of the mechanisms of the plant under drought stress conditions is, breaking polysaccharide and converting to monosaccharide and eventually increasing sugar materials density in the cell in order to maintain of osmotic adjustment [15]. 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 amount was observed at the severe drought stress (Table, 2). Usually in the drought stress conditions, impurities of root will increase in order to maintaining turgor by osmotic adjustment [19]. Rates of sodium for irrigation levels did not show significant difference (Table, 1). According to table 2 With lowering water content and under severe drought stress conditions white sugar content was increased and this property between normal, mild drought stress and severe drought stress levels was significant ($p<0.05$) (Table, 1). Probably increasing white sugar content under drought stress conditions is due to increasing percentage of sugar content in root [20]. Between of irrigation regimes in amount of molasses, there was no significant difference (Table, 1) and irrigation regime was placed in same group in comparing means (Table2). White sugar yield was not significantly affected by irrigation treatments. This reason likely is due to increasing the white sugar content significantly under mild drought stress and severe stress comparison to normal irrigation. Its indicated that white sugar content was more effective than root yield on white sugar yield.

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.

CONCLUSION

In general, it can be concluded that methanol can be used as rich source of carbon. 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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CITATION OF THIS ARTICLE

Iman N, Mehrdad Y, Farzad P, Farhad F, Saeid V .Effect of Methanol in promoting sugar beet (*Beta vulgaris* L.) yield and some Quality Characteristic in Drought stress Condition.Bull. Env. Pharmacol. Life Sci., Vol 3 [11] October 2014: 57-61