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Effect of methanol spraying and seed inoculation with *Azospirillum lipoferum* on sugar beet performance under different regimes of irrigation

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Abstract

The field study was carried out to evaluate the effects methanol concentrations and seed inoculation with *Azospirillum* in water stress conditions. Experiment was conducted using a randomized complete block design with a split-plot arrangement of treatments and four replications. The first treatment factor was two levels of water stress (based on 40 and 70% depletion of available soil moisture) as the main plots. The second treatment factor was methanol concentrations at three levels (0, 14 and 28 % (v/v) methanol) and the third treatment factor was the absence and presence of seed inoculation with *Azospirillum lipoferum* Stain 21 bacteria as subplots. RY (Root Yield), SC (Sugar content), SY (Sugar Yield), WSY (White Sugar Yield), Na, K and N contents, WSC, molasses sugar, alkalite were verified. Results showed a significant difference between different concentrations of methanol on RY, SC, SY, WSY, and N contents. The greatest RY (60.01 ton ha⁻¹) was observed in plots with 28 % (v/v) methanol spraying. The maximum WSY was found in 28 % (v/v) methanol treatment, followed by 14 % (v/v) methanol spraying (6.97 and 6.46 ton ha⁻¹, respectively). Results also indicated that RY, SC, SY, WSY, Na, N contents, molasses sugar and alkalite were influenced by different levels of irrigation. Normal irrigation had a higher RY than deficit irrigation, 60.63 vs. 47.64 ton ha⁻¹. Furthermore, WSY was decreased under water stress. *Azospirillum lipoferum* had no effects on the evaluated traits.

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Introduction

Water stress is a critical factor limiting photosynthesis. Net assimilation is the first process affected when plants were exposed to water stress. The rate of photosynthesis remarkably decreases with increasing in water stress and closest to zero at higher level of stress. Water stress reduces photosynthesis via reduction in leaf area, closing stomata and reduction of carbon fixation efficiency. Reduction in photosynthesis rate induced by water stress is attributed to the closed stomata preventing CO_2 fixation (Gzik, 1996). Nonomura and Benson (1992) found that methanol spraying on plants shoot considerably increased plant growth in arid and warm areas. They also reported that foliar application of methanol can increase plant turgor pressure and reduce damage due to direct sunlight particularly in warm areas. These authors stated that the beneficial effects of foliar-applied methanol on plant growth could be due to methanol consumption by plants as a major source of carbon in photosynthesis. Other studies also showed that application of methanol on crops exposed to water stress led to increasing the biomass (Ramberg *et al.*, 2002; Ramirez *et al.*, 2006; Gout *et al.*, 2000; Zbiec *et al.*, 2003). Methanol molecules, which are smaller than CO_2 , can easily pass through the stomata and use for increasing the photosynthesis by C_3 plants (Li *et al.*, 1995; Kotzabasis *et al.*, 1999).

In sugarbeet, Sadeghishoae *et al.* (2012) found that foliar application of methanol increased root yield, sugar yield and white sugar yield. They also reported significant difference between different levels of irrigation on root yield, leaf yield, sugar content %, sugar yield and white sugar yield, Na content, extraction coefficient and alkalinity. Sadeghishoae *et al.* (2012) showed that spraying the aerial parts of vetch with different concentrations of methanol increased seed yield, protein yield, seed number per pod, pod number, seed number per plant and plant height in mung bean. C_3 plants show toxicity signs when treated by methanol, to reduce methanol toxicity in the presence of direct sunlight; 2 g L^{-1} glycine was added to all solutions including zero

methanol (Nonomura and Benson 1992; Ramberg *et al.*, 2002). Li *et al.* (1995) found that grain yield, seed weight, and pod number can be increased by spraying the leaves with 25 % (v/v) methanol.

Madhaiyan *et al.* (2006) studied the effects of three kinds of *Azotobacter* bacteria (84-86-201) and two kinds of *Azospirillum lipoferum* (4-5) on roots of two cultivar of sugarbeet (Dana and Alina) under farm conditions, who found that the performance of bacteria varied based on bacteria strain and sugarbeet cultivar. The authors also reported that combined effects of *Azospirillum* and *Azotobacter* on yield increase were more when compared with the inoculation with *Azospirillum*/*Azotobacter* alone.

The present study was also carried out with the purpose of studying the effects of methanol spraying and seed inoculation with *Azospirillum lipoferum* on sugarbeet performance under different regimes of irrigation.

Materials and methods

Field conditions and treatments

Field experiments were carried out in 2011 at research farm of Islamic Azad University Karaj, Iran (35°45' N, 51°6' E, and 1113 m above mean sea level). The soil was clay loam with pH of 7.6 and 2.7 ds.m^{-1} soil water conductivity at the depth of 0-30 cm. The first treatment factor was two levels of water stress (based on 40 and 70% depletion of available soil moisture). The second treatment factor was methanol concentrations at three levels (0, 14 and 28 % (v/v) methanol). The third treatment factor was the absence and presence of seed inoculation with *Azospirillum Lipoferum* Stain 21 bacteria. The experiment was a split-plot factorial design with water stress as the main plots and methanol concentrations and *Azospirillum Lipoferum* subplots with four replications. At the time of seed treatment, *Azospirillum Lipoferum* Stain 21 was used at a rate of 15 gr per 1 kg of seed. For better adherence of the product, 10-12 milliliter (mL) of sugar solution was used (10-20% sugar concentration) was used and mixed well. Seeds inoculated with liquid inoculants placed to dry in the shade. The sugarbeet

seeds were planted with density of 10000 plants ha⁻¹ on 10 May in 2011. The first spraying was done on July 20 and 70 d after sowing. Methanol spraying was administered in the evening (1900-2100 h). Plants were sprayed by methanol to run-off. Soil water depletion and hence irrigation time was established using gypsum blocks previously calibrated according to moisture depletion curves provided by Paknejadet *al.* (2007) (Figure 1).

Data collection

At the crop maturity, plants were harvested from two central rows in an area measuring 5 m². Plants were transferred to the laboratory for qualitative analysis after separating shoots of roots. The harvested roots were washed with gently running water and dough samples was prepared randomly from the total roots by automatic machine after weighting, then **it was placed in special trays and the samples were covered with nylon cover.** The trays were transferred to a refrigerator with -20 °C. For qualitative analysis, from each sample 26 g pulp was mixed with 177 ml of Phacetate after placing them at 20° C. Then, the mixture was stirred and clear extracts were prepared from the following passage of special filters. The extract was poured at the special glasses and was sucked by suction of betalizer device. Sugar content (SC) was determined by sacchary meter according to polarimetry method and Na, K and N contents were calculated by betalizer (Payne, 1968).

Molasses sugar content was estimated according to the following equation:

$$MS = 0.343(K + Na) + 0.094(X - \text{amino-N}) - 0.31 \text{ (equation I)}$$

Extractible sugar percentage was found as below:

$$\text{Extractible sugar (\%)} = (\text{sugar \%} - \text{molasses sugar}) + 0.6 \text{ (equation II)}$$

White sugar was calculated using the following relationship:

$$\text{White sugar yield} = \text{sugar (\%)} + \text{root yield} \text{ (equation III)}$$

Total dry matter (TDM), root yield (RY), sugar content % (CS), sugar yield (SY), white yield sugar

(WYS), Na, K, harmful N, recoverable sugar content (CCS), extract efficiency (EE), molasses content sugar (MCS) and alkalite were calculated.

Statistical analysis

All data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 2002). Treatment means were compared using Duncan's Multiple Range Test at $P < 0.05$. The graphs were fitted using Excel 2003 statistical software.

Results and discussion

Root yield

A significant difference between different levels of methanol spraying on root yield was found ($p > 0.01$) (Table 1). The highest (60.01 ton ha⁻¹) and lowest (47.51 ton ha⁻¹) root yield were detected in plots with 28 % (v/v) methanol spraying and control, respectively. Result also indicated that with 14 % (v/v) methanol spraying treatment was intermediate (47.51 ton ha⁻¹) (Table 2). Foliar application with 28 % (v/v) methanol increased sugarbeet root yield by 26 %. Sadeghishoae *et al.* (2012) also found that application of 14 % (v/v) methanol produced the maximum sugarbeet root yield. Zbiec *et al.* (1999) found that that foliar application of 20-30% methanol to sugarbeet increased root yield by 10%. Foliar application of methanol increased fresh weight of tobacco (Ramirez *et al.*, 2006) and soybean (Li *et al.*, 1995).

The bacteria levels of *Azospirillum* didn't have a significant effect on sugarbeet root yield (RY) at the probability level of 5% (Table 1). RY in normal conditions (60.63 ton ha⁻¹) was considerably more than that of under drought conditions (47.63 ton ha⁻¹) (Table 2). Water shortage reduces sugarbeet growth, especially reduce the cell inflammation and increase the soil potential (Cooke and Scott, 1993). The decreased root yield and growth was also found by Abdollahian Noghabi and Williams (1998) and Sadeghishoae *et al.* (2012).

Plant growth due to water shortages may be influenced by gene expression changes stimulating synthesis

raction of newprotein. Under drought stress, because of increased abscisicacidin themesophylicpaths, stomata closes, leaf stomata conductance decreases, and co2 penetration into the

plants reduces for assimilation (Clover *et al.*, 1998). Thesupplyofcarbohydratesfrom leavestoroots is the main factor determining root growth (Cooke and Scott, 1993).

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

Mean Square												
S.O.V.	d.f.	Root yield	Sugar content	Sugar yield	White sugar yield	Na	K	N	White sugar content	ESC	Molasses	Alcalite
Replication	3	738.54*	14.32ns	20.51**	14.78**	3.87ns	3.65ns	1.63ns	15.36*	30.2*	0.05ns	3.98ns
Irrigation(A)	1	2022.80*	19.91**	19.63**	19.60*	4.61**	2.47ns	0.66*	2.12**	14ns	0.06**	4.94*
rep×A	3	163.34ns	2.14ns	3.92ns	2.26ns	1.58ns	1.48ns	0.14ns	1.11ns	2.8ns	0.01ns	4.90ns
Methanol(B)	2	630.88**	17.67**	3.15*	7.76*	0.81ns	0.76ns	0.25*	0.55*	2.8ns	0.08ns	0.34ns
Bacteria(C)	1	1.15ns	4.99ns	0.39ns	1.06ns	1.94ns	1.13ns	1.38*	8.19ns	9.6ns	0.05ns	3.38ns
A×B	2	23.65ns	8.10ns	4.44*	3.95ns	1.41ns	3.03ns	0.79ns	8.26ns	12ns	0.06ns	1.56ns
A×C	1	11.48ns	1.49ns	0.09ns	0.17ns	0.23ns	0.54ns	0.31ns	1.79ns	5.0ns	0.02ns	1.18ns
B×C	2	67.81ns	6.06ns	5.3ns	3.24ns	5.01ns	1.07ns	1.17ns	5.96ns	16ns	0.01ns	6.66*
A×B×C	2	11.81ns	4.24ns	2.13ns	1.80ns	0.14ns	0.76ns	1.57ns	3.95ns	1.6ns	0.13ns	1.71ns
Error	30	24.29	4.02	1.24	1.35	2.48	1.26	0.52	4.65	9.5	0.05	1.98
C.V.(%)	-	9.17	13.12	13.62	18.62	24.75	16.65	21.47	18.66	13.97	9.21	18.20

In each column, ns, * and ** means non-significant and significant at 0.05 and 0.01 probability level, respectively.

Sugar content

A significant different between different levels of methanol concentrations on sugar content (SC) was detected (at 0.01 level) (Table 1). The maximum SC (16.45 %) was found in control treatments, followed by 14 % and 28 % methanol spraying (14.94 % and 14.43 %, respectively). This can be due to the inverse relationship between root yield and sugar content. Given that RY increase under methanol concentrations than control, lower SC in treatments containing methanol than control was expected. Similar observations were also detected by Sadeghshojae *et al.* (2012), who found methanol spraying had no effect on SC.

The effect of bacteria levels on SC was significant (Table 2). Results indicated that SC rates in drought stress were higher than that of under normal conditions (15.92 % vs. 14.33 %). Increasing SC of sugar beet under drought stress may be due to small roots, reduction in root water and increase in soluble. However, because the roots weight decrease and given that there is a negative correlation between increased root weight and sugar content, increasing the percentage of sugar is justified in treatments under drought stress. Increased sugar percentage

under deficit irrigation was reported by Cooke and Scott, (1993).

Sugar yield

Result of ANNOVA showed that sugar yield (SY) was affected by foliar application of methanol ($P \leq 0.05$). The highest (8.64 ton ha⁻¹) and lowest (7.76 ton ha⁻¹) SY was observed in plots with 28 % methanol and control, respectively (Table 2). As regards the SY depends on root yield and sugar content, increasing either of these parameters lead to increase SY (Firoozabadi *et al.*, 2003). In this study, although SC was decreased, methanol spraying increased significantly RY. The increased RY have had a more effect on SY, thus the increased SY is justified. Similar observations were reported by Sadeghshojae *et al.* (2012).

Results also indicated no statistically significant difference between bacteria levels on SY (Table 1). Results also indicated a statistically significant difference between normal and deficit irrigation on SY at the probability level of 1% (Table 1), so that the mean SY was remarkably decreased under deficit irrigation (Table 2).

White sugar yield

The Effect of methanol spraying on white sugar yield (WSY) was significant at the probability level of 5% (Table 1). Methanol spraying increased WSY by 24 % compared to control. Moreover, the greatest WSY was

reported in plots with 28 % and 14 % methanol spraying, 6.97 and 6.46 ton ha⁻¹, respectively (Table 2). The lowest WSY was related to control treatments, 5.58 ton ha⁻¹. This finding is in general agreement with that of sadeghishoe *et al.* (2012).

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

Treatment	Root yield (ton.h ⁻¹)	Sugar content (%)	Sugar yield (ton.h ⁻¹)	White sugar yield (ton.h ⁻¹)	Na	K Mg/100g root	N	White sugar content (%)	ESC	Molasses (%)	Alcalite
Irrigation											
Normal	60.63a	14.33b	9.27a	6.88a	4.84a	6.98a	3.94a	10.35b	77.10a	2.97a	4.21a
Stress	47.64b	15.92a	7.13b	5.06b	4.23b	6.53a	3.48b	11.77a	78.22a	2.11b	3.36b
Methanol											
0(control)	47.51c	16.45a	7.76b	5.58b	4.33a	6.64a	3.03b	11.91a	78.04a	2.61a	3.52a
14%	54.90b	14.94b	8.31a	6.46a	4.49a	6.61a	3.86a	10.86b	77.73a	2.58a	3.71a
28%	60.01a	14.43b	8.64a	6.97a	4.78a	7.01a	3.94a	10.93b	77.21a	2.72a	3.81a
Bacteria											
Control	53.98a	15.61a	8.26a	6.39a	4.33a	6.91a	3.12b	11.97a	78.11a	2.67a	3.41a
Application	54.29a	14.95a	8.08a	6.09a	4.73a	6.61a	3.96a	11.35a	77.21a	2.61a	3.94a

Mean with the same letters in each column have not significant differences at 0.05 and 0.01 probability level.

Based on the WSY was not affected by the bacteria levels (Table 1). Results also indicated significant effects of the irrigation levels on WSY at the probability level of 5% (Table 1), so that plots with normal irrigation produced a higher WSY than plots under drought stress, 6.88 and 5.06 ton ha⁻¹, respectively (Table 2). Although sugar content was greater under water, this rate was not so higher to naturalize the exceed difference of root yield in white sugar yield. Cooke and scott (1993) and Vazanet *al.* (2002) found WSY reduction in drought stress.

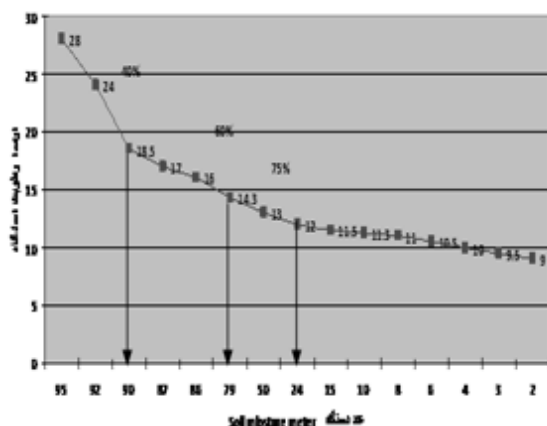


Fig. 1. Calibration and changes of electrical conductivity of gypsum blocks (Paknejad *et al.*, 2007).

Na, K and N

The result of ANOVA revealed no significant effects of bacteria levels, and methanol concentrations on Na rates (Table 1). According to the results, Na rates were affected by the levels of irrigation (Table 1). Similar observations were also found by Sohrabiet *al.* (2006) and sadeghishoe *et al.* (2012).

The levels of Methanol spraying, Azospirillum bacteria and irrigation had no effect on K rates (Table 1). Clover *et al.* (1998) also found that drought increased the rate of amino nitrogen in the roots and had little effects on Na and K. Reduction in Na and K rates under drought was due to the lack of minerals in tissues (Sohrabiet *al.*, 2006).

The result of ANOVA demonstrated significant effects ($p > 0.05$) of methanol spraying on harmful N (Table 1), so that the highest N was related to in treatments of 28 % and 14 % (v/v) methanol, 3.94 and 3.86 meq/100 g of root sugar beet, respectively. Increasing harmful N in treatments of 28 % and 14 % (v/v) methanol can be attributed to increasing root yield and thus improving uptake of nutrients under these treatments. These findings were consistent with those

of sadeghishoaeet *al.* (2012), who found that methanol had no effects on harmful N.

Molasses sugar content

Methanol concentration and *Azospirillum* bacteria didn't have a significant effect on the molasses sugar content of sugarbeet (Table 1). Result also indicated that the effect of the different levels of irrigation on the molasses sugar content of sugarbeet was significant at the probability level of 1% (Table 1), so that plots with normal irrigation showed better performance than plots under drought stress (2.97 % and 2.11 %, respectively) (Table 2). Molasses sugar percentage is calculated directly from the root impurities, so that no significant difference was found between the levels of irrigation on K content and Na and harmful N rates was greater under normal and stress conditions, respectively. Water shortage increased the molasses sugar content and root impurities of sugarbeet (Ranji *et al.*, 2000; Cooke and Scott, 1993). So given that the elements are absorbed through the water and due to soil surface dries quickly in dry conditions, then nutrient uptake can be reduced and dramatically decrease the amount of these elements at the root (Sohrabiet *al.*, 2006).

Alkalinity

Result showed that alkalinity was affected by the various concentrations of methanol (Table 1). The maximum alkalinity was detected in plots control (3.94), followed by in plots with 28 % and 14 % (v/v) methanol spraying (3.19 and 3.07, respectively) (Table 2). The alkalite rate was affected by the different levels of *Azospirillum* (at 0.05 level) (Table 1), so that the rate of alkalinity factor in the absence of bacteria (3.89) was higher compared to application of *Azospirillum* bacteria (3.01). These results were expected according to comparison of their harmful N rates.

The result of ANOVA demonstrated significant effects of irrigation levels (at $P \leq 0.01$) on alkalinity factor (Table 1). Mean comparison indicated that alkalinity factor under normal irrigation was greater

than that of under drought stress (4.21 vs. 3.36). This is due to the high rates of harmful N under drought stress having an inverse relationship with alkalinity factor. Similar observations were also reported by sohrabiet *al.* (2006) and sadeghishoaeet *al.* (2011).

Conclusion

Methanol spraying increased RY, SC and WSY, so that application of 28 % (v/v) methanol had the best performance on the above traits. Application the different levels of *Azospirillum* bacteria had no statistically significant effect on any of the traits but harmful N and alkalite. Sugarbeet RY, SC and WSY were decreased under deficit irrigation.

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