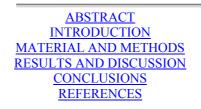
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RESPONSE OF SOME CULTIVATED PLANTS TO METHANOL AS COMPARED TO SUPPLEMENTAL IRRIGATION

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ABSTRACT

Field and laboratory experiments aimed at the assessment of the impact of diluted methyl alcohol applied overhead 4-5 times in one-week intervals on growth and yield of tomato, bean, sugar beet, oil seed rape, as compared to supplemental irrigation. The photosynthetic activity was measured in situ with an LC-4 gas analyser. The crops when treated with methanol solutions yielded 20-30% higher than the control. The yield increases were comparable to those caused by supplemental irrigation. The increased biomass synthesis caused either by irrigation or methanol application was due to enhanced carbon dioxide assimilation, transpiration, leaf conductivity, and higher activity of nitrate reductase and alkaline phosphatase.

Key words: methanol, irrigation, bean, sugar beet, tomato, oil seed rape

INTRODUCTION

Production of biomass by plants depends to a great extent on environmental factors, such as water supply, air temperature, insolation, carbon dioxide concentration in the canopy. Numerous experiments have shown that by increasing the CO_2 content in air, the crops yielded better [5], flowering was accelerated [6] and plants accumulated more carbohydrates [1]. Besford [2] who studied the effects of prolonged CO_2 enrichment on the photosynthetic performance and Calvin cycle enzymes of tomato plants found, that leaves reaching full expansion more than doubled their net rate of carbon dioxide fixation. Studies on the physiology of CO_2 effect aim at understanding mechanisms which control interactions of CO_2 with other environmental factors, such as water or sun deficit [3,4,5,9].

Methyl alcohol may be an alternate carbon source for plants. According to Nonomura and Benson [10] methanol treated plants showed increased turgor, higher growth rates and consequently gave higher yield than the control plants. Only C_3 plants, that is those which during photosynthetic carboxylation produce ribulose 1,5-diphosphate and then 3-phosphoglyceric acid respond to methanol by increased biomass production, since carbon dioxide resulting from rapid oxidation of methanol can successfully compete with oxygen for RuBisCO – [11]. Hemming et al. [7] who studied the rate of metabolism in pepper, petunia and tomato plant tissues found that brief exposure to aqueous methanol solutions increased the metabolic heat rate, which resulted in enhanced carbon conversion efficiency. Furthermore plants which grow in an CO_2 enriched atmosphere are less susceptible to drought, since their stomata are closed, transpiration decreases, and net photosynthesis is thus elevated [2,14].

Considering the possibility of using methanol as a measure for yield increases, and saving irrigation water, greenhouse and field experiments were carried out to assess the impact of methanol solution on selected crops cultivated in moderate climate zone.

MATERIAL AND METHODS

Three-year field experiments were carried out on 0.5 m^2 microplots in 4 replications. The first factor was overhead irrigation applied so as to maintain 70-80% field water capacity. The control plants grew under natural conditions. Methanol (second factor) was applied in aqueous solutions of 10, 20, 30 and 40%. Each solution, also the water applied to control plants contained 0.2% glycine and 0.4% Florovit (micro- and macrofertliser). Plants of high rate of photorespiration when treated with methanol yield two molecules of serine per entry of two molecules of glycine thus leading to twice the sucrose being produced. For this reason Nonomura and Benson [10] recommend addition of glycine to methanol spray. Methanol-treated plants show also increased demand for minerals, hence the use of Florovit (N - 3%, K - 2% and microelements - Cu, Fe, Mn, Mo, Zn, Ca, S).

Plants were treated with the methanol solutions 4-5 times over vegetation in one-week intervals, in June to July. The test crops were: 'Betalux' tomato, 'Cezar F-1' cucumber, 'Igołomska' bean, 'Colibri' sugar beet and 'Lirajet' winter rape. At the time of full vegetative development, photosynthetic activity - CO_2 assimilation, transpiration, leaf conductance and CO_2 concentration in substomatal cells were measured with a LC-4 gas analyser in situ. The activity of nitrate reductase was analysed in leaves, using reduced NADH as hydrogen donor, that of phosphatase by colorymetry.

The growth of winter rape seedlings was tested in a growth chamber. 15 seeds were planted in 8-cm styrofoam cups containing vermiculite, kept under constant conditions of light (126 μ E · m⁻² · s⁻¹, 16h light, 8h dark), and temperature 22 ± 1°C). After emergence 30 ml of ½ strength Hoagland medium were applied to each cup. 20-old-day plants were treated with 0, 10, 20, 30, 40% methanol solution fortified with 0.2% glycine. Two days after treatment, 10 largest seedlings were selected from each cup for shoot length, fresh as well as dry matter determination. The plant yield was analysed with Tukey test, at p = 95%.

RESULTS AND DISCUSSION

Data presented in <u>Tables 1-3</u> showed that supplemental irrigation and methanol treatment positively affected the plant photosynthesis. As a result of overhead irrigation, an increased CO₂ assimilation and transpiration rates were statistically confirmed in leaves of all three tested plants, whereas such effect of methanol was found in tomato and sugar beet leaves, to a lesser extent in bean. As for sugar beet, the impact of methanol on carbon dioxide assimilation surpassed that of supplemental irrigation. According to Nonomura and Benson [10] methanol reduces the plant photorespiration, and the rapidly oxidized methanol leads to formaldehyde incursion with tetrahydrofolate. As a result, the doubling of serine content could lead to twice the sucrose being produced through the serine intermediate [13]. Abundant CO₂ supply from methanol causes the redirection of photorespiration from catabolism to anabolism [8,12]. Data of <u>Tab. 5</u> showed that young plants rapidly reacted to diluted methanol application. The leaves activity of nitrate reductase was by over 50% higher, and of alkaline phosphatase by 32%. These findings support the view of Nonomura and Benson [10] who reported that methanol was rapidly oxidized to CO₂ and then incorporated into structural compounds. Furthermore, the increased sucrose production improved the plant turgidity, hence a lesser susceptibility of methanol – treated plants to water deficit.

Table 1. Photosynthetic activity of bean leaves

Trea	Treatment		т	Ci	Gs
Water	Methanol	A	I	Ui	Gs
Control	Control	10.51	2.14	305	0.15
Control	30%	11.22	2.98	290	0.13
Irrigation	Control	11.23	3.12	270	0.13
Irrigation	30%	12.34	3.46	274	0.14
	LSD p=0.05 for: irrigation		0.57	ns	0.02
methanol		1.96	ns	ns	ns
inter	action	ns	ns	ns	ns

*A – μ M CO₂·m⁻² · s⁻¹, T – transpiration M · m⁻² · s⁻¹, C_i – CO₂ concentration in stomatal chamber, G_s – stomatal conductance, ns – non-significant difference

Table 2. Photosynthetic activity of sugar beet leaves

Trea	Treatment		т	Ci	G
Water	Methanol	A	I	Ui	Gs
Control	Control	8.3	3.20	364	0.66
Control	30%	12.3	4.13	362	0.06
Irrigation	Control	10.7	5.25	380	0.10
Irrigation	30%	15.4	5.74	377	0.12
LSD p=0.05	LSD p=0.05 for: irrigation		0.56	Ns	0.02
methanol		1.66	0.61	ns	ns
inte	raction	1.95	ns	ns	ns

* for explanations, see Table 1

Table 3. Photosynthetic activity of tomato leaves

Tre	Treatment			<u> </u>	
Water	Methanol	A	I	C _i	Gs
Control	Control	2.56	0.75	147	0.03
Control	30%	3.88	1.17	136	0.04
Irrigation	Control	6.86	2.16	188	0.10
Irrigation	30%	8.78	2.51	189	0.13
	LSD p=0.05 for: irrigation		0.082	13.3	0.006
methanol		0.83	0.30	ns	ns
inte	eraction	ns	ns	ns	ns

* for explanations, see Table 1

Table 4. Growth of winter rape seedlings

Methanol	Shoot length		Fresh	weight	Dry weight	
treatment %	mm	%	g·plant ⁻¹	%	g·plant ⁻¹	%
Control	298	100	13.9	100	1.90	100
10	379	127	23.1	166	2.98	157
20	401	134	26.1	187	3.46	182
30	392	131	25.4	182	3.48	183
40	328	110	17.5	125	2.55	134
LSD p=0.05	21.0		2.0	07	0.33	

Methanol treatment %	Alkaline phosphatase µ·100 g⁻¹	Nitrate reductase µMNO₂·g⁻¹·h⁻¹	Fresh matter mg·m ⁻² ·h ⁻¹	Dry matter mg·m ⁻² ·h ⁻¹
0	1.23	31.6	1.62	0.13
10	1.58	36.7	2.49	0.19
20	1.62	47.9	3.11	0.24
40	1.46	42.0	1.66	0.13
LSD p=0.05	0.079	4.75	0.629	0.049

Table 5. Enzyme activities and biomass synthesis by spring rape seedling leaves, 20-day old plants

Data presented in <u>Tables 6</u>, <u>7</u> and <u>8</u> have shown that yield increases caused by methanol solutions ranged from 12% for sugar beet, 20% for bean to 30% for tomato. Comparison of methanol effects on seedlings and mature plants indicates that the young seedlings reaction to this treatment was more pronounced than that of the resulting crop (<u>Tab. 4</u> and <u>9</u>). The yield of seeds given by rape plants treated with 30 or 40% methanol exceeded that of the control plants by 30%, whereas the dry matter of seedlings sprayed with 30% methanol was higher by over 80%. This discrepancy may indicate that maturing plants had a greater need for biomass supply. The plant products of photosynthesis are used for the development of seeds, but first of all for building of leaves, branches and fruit. This is also in concord with the elevated photosynthesis of methanol – treated leaves. Similar results were reported by Zbieć et al. [14].

Table 6. Bean seed yield, $g \cdot 0.5 \text{ m}^{-2}$

Treatment									
Not irrigated					Irrigated				
Methanol treatment, %									
0	10	20	30	40	0 10 20 30				
209	216	223	251	245	250 248 248 261 26				263
	LSD _{p=0.05} for: irrigation 7.03 methanol 6.14 interaction ns								

Table 7. Sugar beet yield, kg \cdot 0.5 m⁻²

Methanol treatment, %	Not irr	Not irrigated		ated	
	roots	leaves	roots	leaves	
0	5.92	5.39	7.04	6.27	
10	6.06	5.48	7.08	6.40	
20	6.64	5.46	7.49	6.76	
30	6.65	5.43	7.63	6.93	
40	6.48	5.22	7.22	6.72	
LSD _{p=0.05} for: irrigation methanol interaction	0.45 0.56 ns		0.61 ns ns		

Table 8. Tomato yield, kg \cdot 0.5 m⁻²

Treatment									
Not irrigated					Irrigated				
Methanol treatment, %									
0	10	20	30	40	0	10	20	30	40
2.49	2.71	3.25	3.26	2.80	3.25	3.48	3.61	3.58	3.68
	LSD _{p=0.05} for: irrigation 0.18 methanol 0.45 interaction 0.60								

Table 9. Winter rape yield and yield components

Methanol treatment	Number of	branches	Number of	Number of siliques		yield
%	per plant	%	per plant	%	g·plant ⁻¹	%
0	5.1	100	115	100	25.9	100
20	6.8	133	150	130	32.6	126
30	6.8	133	151	130	33.5	129
40	7.0	137	151	130	34.2	132
LSD p=0.05	0.39		6.54		3.47	

Since the effects of supplemental irrigation were better than those of methanol application, it can be assumed that the beneficial impact of methanol is expressed to a greater extent in plants which are more susceptible to water deficit and grow better under hot weather conditions and well supplied with water. Information provided by some Israeli scientists who did experiments with methanol in greenhouses (personal information) supports the view that methanol application for yield increase can be successful in controlled environment conditions, since under hot climate and in the field, methanol rapidly evaporates, thus is ineffective.

CONCLUSIONS

- 1. Water solutions of methanol alcohol applied to plants of the C-3 carbon conversion cycle caused increase in carbon dioxide assimilation and biomass synthesis. Plants which had been treated with methanol solutions, particularly tomato and sugar beet showed an 50% increase in nitrate reductase activity, and significantly enhanced CO₂ assimilation.
- 2. Bean, sugar beet, tomato, oil seed rape treated with 30% methanol solution yielded by 12 to 30% higher than the control plants.
- 3. The methanol treated plants were less susceptible to water deficit, in some cases their yield equalled that of the irrigated plants.

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