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RESEARCH PAPER

Effects of the foliar application of methanol on the yield and growth of rice (*Oryza sativa* cv. Shiroudi)

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Abstract

A. Abbasian, B. Mirshekari, M.N. Safarzade Vishekaei, V. Rashidi, and H. Aminpanah. 2016. Effects of the foliar application of methanol on the yield and growth of rice (*Oryza sativa* cv. Shiroudi). Cien. Inv. Agr. 43(1):17-24. Approximately two decades have passed since it was reported that the foliar application of methanol increased plant biomass and yield. Many subsequent reports have been published concerning the ability or inability to reproduce these initial observations. To evaluate the effect of methanol on rice (*Oryza sativa* cv. Shiroudi) yield and rice components, a field experiment was conducted at the Tonekabon Rice Research Station in Iran in 2012 and 2013. The experiment had a randomized complete block design (RCBD) with three replicates. The plants were treated with aqueous methanol solutions (0, 6, 12, 18, and 24% (v/v)). Methanol was sprayed on the rice foliage three times during the experimental period at two-week intervals. The results indicated that, in general, the methanol did not affect rice growth or yield and seemed to be ineffective as a growth enhancer, so foliar spraying of aqueous methanol cannot be recommended for rice. The negative results could be due to the absence of *Methylobacterium* sp. or that the effects are cultivar-specific because positive effects on the growth and yield of rice have been reported in other cultivars.

Key words: Aqueous methanol solution, correlation, glycine, grain yield, growth, rice, Tween 80 as a surfactant.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal food crops (Eckardt, 2000). Global population estimates have predicted the need for a 70% increase in rice production over the next 30 years (Lee *et al.*, 2006) to provide a staple food for more than half of the world's population (Sasaki and Burr, 2000). Due to the constant increase of the

world's population and adverse climatic conditions, the increase in the rate of rice production adequate to satisfy the increased demand for food should be approximately 1% per year (Sass *et al.*, 2002). Rice is a principal food in Iranian cuisine, and the quality of cooked rice outweighs all other considerations among Iranian consumers. The total area under rice cultivation in Iran is more than 600,000 hectares and rice is grown in 15 provinces. The consumption of rice in Iran has been estimated at 28 kg per capita per year. As the supply and demand for rice in Iran are not

yet evenly balanced, Iran imports approximately 400,000 to 500,000 tons of rice for domestic consumption (Pourimani and Anoosheh, 2015).

Numerous experiments have shown that an increase in the CO₂ content of the air led to an increase in crop yields (Devlin *et al.*, 1994), accelerated flowering (Fisher *et al.*, 1997), and a greater accumulation of carbohydrates by plants (Abdel-Latif *et al.*, 1996). Spraying methanol is a method which increases crop CO₂ fixation in a given area. Recent investigation has shown that the yield and growth of C₃ crops was increased via methanol spraying and that methanol may be a C source for these crops (Makhdum *et al.*, 2002). In most cases, 25% of the carbon in C₃ plants is used for photorespiration, and the amount of photorespiration can be minimized by the foliar application of methanol (Gout *et al.*, 2000) because methanol is rapidly metabolized to CO₂ and water in plant tissue (Safarzadeh Vishekai *et al.*, 2007). The foliar application of methanol indirectly stimulates the methyltrophic bacteria that live on most plant leaves. These bacteria consume some of the methanol on the leaves and induce plant growth via auxin and cytokine production (Ivanova *et al.*, 2001).

A wide range of C₃ crops and ornamental plants have shown an increase in growth and the yield of fruits or seeds after spraying with 10–50% methanol. Positive responses have been reported for wheat (*Triticum aestivum* L.), peas (*Pisum sativum* L.) (Devlin *et al.*, 1994), oilseed rape (*Brassica napus* var. *oleifera*) (Karczmarczyk *et al.*, 1995), geranium (*Pelargonium* sp.), and bachelor's button (*Centaurea cyanus* L.) (Devlin *et al.*, 1995). However, some publications report no gain of biomass of treated plants (Hartz *et al.*, 1994; Mauney and Gerik, 1994; McGiffen *et al.*, 1995; Mitchell *et al.*, 1994). Nonomura and Benson (1992) reported large increases in the growth and yield of a wide range of C₃ species: tomato (*Lycopersicon esculentum* Mill.), 50%; strawberry (*Fragaria ananassa* Duchesne), 60%; eggplant (*Solanum melongena* L.), 60%;

cotton (*Gossypium hirsutum* L.), 50%; savoy cabbage (*Brassica oleracea* L. Capitata Group), 50%; wheat (*Triticum aestivum* L.), 100%; rose (*Rosa* spp.), 40%; palm (*Washingtonia robusta* H. Wendl.), 70%; and watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai], 36%. Li *et al.* (1995) reported that methanol had a positive effect of the seed yield, seed weight, and the number of pods per plant of soybeans. Safarzadeh Vishekai *et al.* (2007) found that the foliar application of 20% methanol increased the leaf area index, the crop growth rate, the pod growth rate, the radiation use efficiency, the pod and grain yield, the 1000-grain weight, the number of ripened pods, and the grain protein concentration in peanuts. Nadali *et al.* (2010) demonstrated that the application of 21% (v/v) methanol had the greatest impact on the root yield, leaf weight, and yield of sugar beets. In addition to increasing the yield, some crops showed reduced photorespiration along with an increased cell turgor in plant tissue and an enhanced capacity for photosynthesis during the reproductive stage due to an increase of CO₂ (Nonomura and Benson, 1992). Armand *et al.* (2016) reported that treatment with 20% methanol in beans (*Phaseolus vulgaris* L.) at the seedling stage resulted in increased net photosynthesis (P_N), intercellular CO₂ concentration (C_i), and a decreased transpiration rate under non-stress conditions.

Understanding the impact of methanol on plants is still controversial because different studies on its impact on photosynthetic activity and biomass production in plants have produced different results (Zheng *et al.*, 2008). Some studies have suggested that both biomass production and photosynthetic activity in algae were increased at low methanol concentrations (Theodoridou *et al.*, 2002). Changes in photosynthetic metabolism due to a change in environmental conditions or agricultural practices lead to a change in plant's growth and productivity (Pallardy, 2010), while foliar applications of methanol increased CO₂ assimilation in plants (Ganjeali, 2012). There are few studies on methanol in Iran. Rice is a very

valuable economic product and an important food source that is consumed daily worldwide, but a water deficit is a problem for rice cultivation in Iran. The main objectives of our experiments were to (1) assess whether methanol enhances the growth and yield of rice and (2) determine the efficacious methanol concentration for foliar application.

Materials and methods

Growing Conditions

These experiments were conducted at the experimental farm of the Tonekabon Rice Research Station (36°51' N, 50°46' E; -20 m above sea level) in the north of Iran during April – August of 2012 and 2013. The soil at the experimental site was a Silty clay loam, with 3.2% organic matter, 30% clay, 50% silt, 20% sand, a pH of 7.61, a cation exchange capacity (CEC) of 29.9 meg 100 g, 0.338% total soil nitrogen, 3.25% organic carbon, 13.6 mg kg⁻¹ of phosphorus, and 88 mg kg⁻¹ available potassium. To simplify the comparison of the growing season weather, we considered the monthly total precipitation and temperature from May through August at the Tonekabon Rice Research Institute (Table 1).

Rice seeds were disinfected with 70% thiophanate-methyl WP (Melli Agrochemical Company (PAC);

Qazvin, Iran) fungicide at 200 g 100 kg⁻¹ seed and were then sown in the nursery. The seedlings were manually transplanted in the experimental field at the 2-3 leaf stage at a spacing of 25×25 cm². Recommended rates of nitrogen (100 kg ha⁻¹), phosphorous (100 kg ha⁻¹), and potassium (150 kg ha⁻¹) were applied. One-third amount of the nitrogen and all of the phosphorous and potassium were applied as a basal dose at the transplant stage. The remaining two-thirds of the nitrogen were applied in two split doses 30 days after transplanting (at the tiller stage) and at the panicle initiation stage. Weeds were controlled by hand weeding during the growth season. The permanent flood water level was maintained at 10 cm during the rice growing period.

Methanol application

Methanol was sprayed on the rice foliage three times at two week intervals. The first foliar application was 45 days after transplanting on June 30th, and the second and third applications were on the 13th and the 27th of July, between 1600 to 1900 hours on bright, hot sunny days. Cossins (1964) reported that methanol was utilized and converted to sugars and amino acids when applied to plant tissue during darkness. The methanol was sprayed so all of the above ground parts of the rice plants were covered. A back engine sprayer with a 20 L capacity was used to spray, and the sprinkler was positioned

Table 1. Monthly precipitation and temperature from May to September for the growing season (2012-2013) at the Tonekabon Rice Research Institute, Iran.

Month	Year	Precipitation (mm)	Temperature (°C)		
			Maximum	Minimum	Average
May	2012	28.3	25.29	18.45	21.87
June	2012	117.9	28.38	21.23	24.81
July	2012	125	29.12	22.81	25.96
August	2012	86.5	30.9	24.22	27.56
May	2013	26.3	23.3	16.1	19.7
June	2013	14.3	27.7	20.7	24.2
July	2013	70.1	29.5	23.1	26.3
August	2013	27.1	28.4	22.2	25.3

at 40 cm above the plants. The methanol (Merck Company, Darmstadt, Germany) solutions also included 0.1% Tween 80 (v/v) (J.T. Baker Chemical Company, Phillipsburg, New Jersey) as a surfactant. Glycine apparently increased the rate of methanol metabolism. Glycine was added to the sprayed solution to prevent injury from high methanol concentrations or when the light intensity was low (Nonomura and Benson, 1992). The addition of up to 2 g L⁻¹ glycine to the methanol solution enabled the use of higher methanol concentrations without visible injury.

At maturity, the plant height (from the soil surface to the top of the plant canopy) and the tiller number were measured. The plants were harvested by hand-cutting at the soil surface and subsequently, the aboveground biomass of the rice was determined. The rice aboveground biomass from each plot was placed in a separate paper bag, dried at 72°C for 48 h, and weighed. The yield components included the tiller number, and the 1000-grain weight was measured according to the standard evaluation system. Plants were harvested at 107 days after transplanting. The plots were hand harvested for rough rice yields at 2.5 m² and adjusted to 14% moisture.

Statistical analysis

The experimental design was a randomized complete block with three replicates and five treatments: 0, 6, 12, 18, and 24% (v/v) methanol. The statistical analysis was conducted using the SAS software (SAS Institute Inc. Cary, USA) (SAS Inst., 1990) to determine whether the effects of the application of methanol was significantly different between treatments. Means were compared using Fisher's protected LSD test at $\alpha=0.05$. A correlation analysis was used to draw inferences about the relationship between the agronomic traits under consideration using the PASW Ver. 18.0 statistical software (SPSS Inc., Hong Kong).

Results and discussion

An analysis of variance showed that methanol treatments had no significant effect on any trait at the 5% probability level (Table 2). For both years (2012 and 2013), the rice grain yield was not significantly increased following methanol foliar application. On the contrary, Nonomura and Benson (1992) reported that the plant growth rate was significantly increased in response to the foliar application of methanol. Furthermore in our study, no differences in the biological yield, harvest index (HI), plant height, tiller number, or unfilled grain number (Table 3) were evident. The highest 1000-grain weight was observed in the methanol treatments in comparison with control treatment. Additionally, the highest filled grain number occurred in the control treatment. A correlation analysis provides a good indication of the association between growth parameters and helps to identify the most important growth parameter(s) to be considered for the effective selection for a higher yield. The correlation analysis (Table 4) showed a significant positive correlation between the rice yield and the biological yield ($P\leq 0.01$, 0.75), plant height ($P\leq 0.01$, 0.64), and the tiller number ($P\leq 0.05$, 0.36).

In our study, the quantities of methanol applied to the plants were so small compared to carbon fixation in the plant canopy during the growing season that changes in growth could not be expected to result from the alcohol application. Moreover, the extent of the methanol absorption and utilization in the plant it is not clear. Cossins (1964) observed a large variation in the utilization of methanol when it was fed to different cell tissues of various crop species. The methanol may not have affected the rice because of the rainy weather during the rice growth period (Table 1) because the effect of methanol is greater during a water shortage and stress conditions (Safarzadeh Vishekaei *et al.*, 2007).

Table 2. Analysis of variance of the effects of methanol application on the growth and yield of rice.

SOV*	df	Mean squares							
		Grain yield	Biological yield	HI	Plant height	Tiller number	1000 - grain weight	Number of filled grain	Number of unfilled grain
Year (Y)	1	8815.9ns	1306887.8ns	34.59*	54.41ns	5.57	4.33*	469.26*	963.9**
Rep.	2	427546.5	488038.5	2.40	5.9	7.49ns	0.096	217.21	13.84
Methanol (M)	4	507927.7ns	1439270.6ns	8.87ns	17.26ns	3.08ns	1.04ns	153.67ns	4.81ns
M×Y	4	725133.7ns	1875692.8ns	1.25ns	3.82ns	4.98ns	0.7ns	36.82ns	14.49ns
Error	18	851951.8	2638856.6	7.26	29.32	4.54	0.38	73.1	19.79
CV (%)		13.34	14.19	5.05	5.3	10.51	2.26	7.85	25.05

*Sources of variation.

ns= non-significant; *, **significant at the 5% and 1% probability levels, respectively.

Table 3. Comparison of the means of the growth parameters of rice as affected by methanol treatment.

Ingredients	Treatments (% alcohol (v/v))					LSD _{0.05}
	0	6	12	18	24	
Grain yield (kg ha ⁻¹)	7024.6 a	7168.3 a	6928.1 a	7051.8 a	6421.4 a	1119.6
Biological yield (kg ha ⁻¹)	7024.6 a	11341.6 a	11811.9 a	11861.4 a	10651.5 a	1970.4
HI (%)	51.67 a	54.65 a	54.32 a	53.55 a	52.65 a	3.27
Plant height (cm)	103.37 a	101.2 a	103.47 a	102.95 a	99.55 a	6.57
Tiller number	20.8 a	20.57 a	20.67 a	20.29 a	19.03 a	2.58
1000 - grain weight (g)	26.83 b	27.25 ab	27.63 a	27.93 a	27.28 ab	0.75
Number of filled grain	116.13 a	110.23 ab	107.3 ab	109.04 ab	102.13 b	10.37
Number of unfilled grain	17.93 a	16.2 a	17.97 a	18.3 a	18.4 a	5.39

Each value represents mean ± S.E. of three replicates per treatment.

Under water stress conditions, methanol might function as an osmoprotectant, protecting the plant's vital processes and enabling a quick recovery when the stress is removed. Another possible explanation for the positive response to water stress conditions could be that methanol blocks the senescent effect of ethylene in stressed plants and therefore improves recovery (Safarzadeh Vishekaei *et al.*, 2007). Further studies are being conducted to reveal more precisely the effect of methanol on stressed plants (Rajala *et al.*, 1998). Nonomura and Benson (1992) reported that the foliar application of aqueous methanol increased the yield, accelerated the maturity, and reduced the drought stress and irrigation requirements in crops grown in an arid environment at high temperature in direct sunlight.

Rajala *et al.* (1998) did research on some of the C₃ crops, including spring cereal (barley, wheat, and

oats), peas, and summer turnip rape, and observed that methanol did not affect the growth and the yield of any of the crop species investigated and was therefore ineffective as a growth enhancer. They stated that the effect of methanol depends on a relatively low air temperature during the evening, which reduces the evaporation of methanol from the leaf surface and increases the possibility that methanol will penetrate into the plant. This is especially important at a high methanol concentration. Accordingly, it is likely that the plants transformed the methanol into other compounds in the field experiments, even though the methanol was not applied during a period of high light intensity. It is also likely that the methanol penetration into the plant is greater when applied at lower temperatures during the night. Wilson *et al.* (1996) applied aqueous methanol (6 concentrations from 0 to 50%) on barley and found that none of the treatments significantly affected crop performance. Accord-

Table 4. Correlation coefficients for the growth parameters of rice.

Traits	Grain yield	Biological yield	HI	Plant height	Tiller number	1000 - grain weight	Number of filled grain
Biological yield	0.75**	1					
HI	-0.07ns	-0.12ns	1				
Plant height	0.64**	0.65**	-0.27ns	1			
Tiller number	0.36*	0.33ns	-0.06ns	0.17ns	1		
1000 - grain weight	0.02ns	-0.02ns	-0.13ns	0.13ns	-0.1ns	1	
Number of filled grain	0.17ns	0.23ns	-0.12ns	0.39*	-0.16ns	0.06ns	1
Number of unfilled grain	-0.12ns	-0.04ns	-0.45*	0.39*	-0.06ns	0.44*	0.22ns

ns= non-significant; *, **significant at the 5% and 1% levels of probability, respectively.

ing to Nonomura (personal communication), one application was sufficient to improve plant productivity, but multiple applications were required to achieve the maximum benefit. We followed Nonomura and Benson's treatment protocol, but we found that foliar methanol application did not effectively enhance any rice plant performance parameter under irrigated field conditions.

It could be useful to verify the presence of the methyltrophic bacteria in the plant tissues (leaves) because the absence of these bacteria could be another cause of the negative effect of methanol on the growth and yield of the rice. It has been shown that bacterial species of the genus *Methylobacterium* can help to consume some of the methanol on the leaves and induce plant growth via auxin and cytokine production for the best growth and yield of rice. We recommend a test to assess the presence or absence of these bacteria for a fuller explanation of our results.

In conclusion, this study indicated that methanol application did not seem to have a growth promoting effect on rice in the growing conditions in Iran. It seems that the rainy weather during the experimental period was responsible for the lack of an effect of the methanol on rice. It could be useful to verify the presence of methyltrophic bacteria in the plant tissues (leaves) because the absence of these bacteria could be another cause of the negative effect of the methanol on the growth and yield of the rice. It has been shown that bacterial species of the genus *Methylobacterium* can help to consume some of the methanol on the leaves and induce plant growth via auxin and cytokine production for the best growth and yield of the rice cultivar Co-47 (*Oryza sativa* L.) (Madhatyan *et al.*, 2004). We recommend a test to assess the presence or absence of these bacteria before and after of the application of methanol for a fuller explanation of our results.

Resumen

A. Abbasian, B. Mirshekari, M.N. Safarzade Vishekaei, V. Rashidi y H. Aminpanah. 2016. Efectos de la aplicación foliar de metanol en el rendimiento y el crecimiento de arroz (*Oryza sativa* cv. Shiroudi). Cien. Inv. Agr. 43(1):17-24. Hace aproximadamente dos décadas se informó que la aplicación foliar de metanol produce aumento de la biomasa y el rendimiento de las plantas. Desde entonces, muchos informes han sido publicados describiendo la capacidad o incapacidad de reproducir estas observaciones iniciales. Con el fin de evaluar el efecto de metanol en el arroz (*Oryza sativa* cv. Shiroudi) en el rendimiento y sus componentes, se desarrolló un experimento de campo en la Estación de Investigación del Arroz de Tonekabon, Irán, durante 2012 y 2013. El diseño estadístico usado fue de bloques completamente aleatorios (DBCA), con tres repeticiones. Los tratamientos estudiados fueron soluciones acuosas de metanol (0, 6, 12, 18 y 24% (v/v)). El metanol se pulverizó sobre partes de follaje de arroz tres

veces durante su período de crecimiento, con intervalos de dos semanas. Los resultados de estos experimentos indicaron que, en general, el metanol no afectó el crecimiento y el rendimiento del arroz y, por lo tanto, parecía ser ineficaz como un promotor de crecimiento, en cambio, no se pueden recomendar pulverizaciones foliares de metanol acuoso para el arroz. Esto podría ser debido a la ausencia del *Methylobacterium* sp. o que el cultivar no ha sido adecuada, ya que otros cultivares de arroz se produce un efecto positivo en el crecimiento y rendimiento de arroz. Se otorgan mayores comentarios en las conclusiones finales del estudio.

Palabras clave: Arroz, correlación, crecimiento, glicina, rendimiento de grano, soluciones acuosas de metanol, Tween 80 como agente tensioactivo.

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