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Foliar application of methanol influences on growth and yield of rice (*Oryza sativa* L.) under different barnyard grass (*Echinochloa crus-galli*) densities

Efecto de la aplicación foliar con metanol y presencia de distintas densidades de pasto dentado (*Echinochloa crus-galli*) sobre el desarrollo y rendimiento de arroz (*Oryza sativa* L.)

Abouzar Abbasian[1], *Bahram Mirshekari*[*1], *Mohammad Naghi Safarzade Vishekaei*[2], *Varahram Rashidi*[1] *and Hashem Aminpanah*[2]

ABSTRACT

Recent reports indicate that vegetative growth and yield of C_3 crops are enhanced by foliar methanol application and that overall crop water use is reduced by methanol sprays. In order to evaluate the effects of methanol and barnyard grass density on rice (*Oryza sativa* cv. Shiroudi) yield and its components, a field experiment was conducted at the Rice Research Station of Tonekabon, Iran in 2012. The experiment was carried out as a randomized complete block design with a factorial arrangement in three replicates. Studied factors were aqueous methanol solutions (0, 6, 12, 18 and 24% (v/v)) and barnyard grass (*Echinochloa crus-galli*) densities (0, 16, 24 and 32 plants m^{-2}). Methanol was sprayed on the foliage of rice three times during its growth period with two-week intervals. Results indicated that effect of methanol was significant for tiller number and grain yield ($P < 0.05$), while the effect of barnyard grass density was significant for grain and biological yields ($P < 0.01$). Moreover, the interaction between methanol and barnyard grass density was significant for grain yield ($P < 0.05$) and 1000-grain weight ($P < 0.01$). The greatest mean values for grain yield and 1000-grain weights obtained at 18% (v/v) with weed-free condition, with 7440.8 kg ha^{-1} and 28.07 g, respectively. Under weed-free conditions grain yield increased as methanol dose increased from 0 to 18% (v/v) and then reduced significantly. At the highest weed density, grain yield decreased significantly as the methanol dose increased. It appears that foliar sprays of aqueous methanol may be recommended for rice under weed-free conditions.

Key words: rice, barnyard grass, grain yield, methanol, weed density.

RESUMEN

El crecimiento vegetativo y la producción de cultivos C3 se estimulan por la aplicación foliar de metanol y mejoran la eficiencia del uso total de agua. El objetivo de este trabajo fue evaluar el efecto de la aplicación foliar con metanol y la densidad de las pasto dentado en el rendimiento de arroz (Oryza sativa cv. Shiroudi). El experimento se realizó en la Estación Experimental del Arroz de Tonekabon, Irán en 2012. El diseño experimental fue de bloques completos al azar con un arreglo factorial 5X4, con tres repeticiones. Los factores estudiados fueron soluciones acuosas de metanol en concentraciones de 0, 6, 12, 18 y 24% (v/v) y densidades del pasto dentado (Echinochloa crus-galli) de 0, 16, 24 y 32 plantas m^{-2}*. El metanol se aplicó sobre el follaje de arroz cada dos semanas durante su período de crecimiento. Los resultados indican que el rendimiento del grano fue afectado por la aplicación de metanol (P <0,05) y la densidad de pasto dentado (P <0,01). La interacción entre concentración de metanol y densidad de maleza fue significativa para rendimiento (P <0,05) y peso de 1000 granos (P <0,01). Los mejores valores medios de rendimiento y peso de 1000 granos se obtuvieron con 18% (v/v) y libre de pasto dentado, alcanzando 7.440,8 kg* ha^{-1} *y 28,07 g, respectivamente. En ausencia de malezas el rendimiento de grano mejoró al aumentar la aplicación de metanol de 0 a 18% (v / v) y después se redujo significativamente. A mayor densidad de las malezas, el rendimiento de grano disminuyó significativamente a medida que la dosis de metanol se incrementó. Los resultados sugieren que las aplicaciones foliares de metanol pueden ser recomendados para el arroz en condiciones libres de malezas.*

Palabras clave: *arroz, pasto dentado, rendimiento de grano, metanol, densidad de malezas.*

1 Department of Agronomy and Plant Breeding, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
2 Department of Agronomy and Plant Breeding, Rasht Branch, Islamic Azad University, Rasht, Iran.
* Corresponding author: Mirshekari@iaut.ac.ir

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Introduction

Weeds are the greatest constraint to yield in upland or aerobic rice systems, resulting in yield losses between 30 and 98% (Aminpanah, 2012). Barnyard grass (*Echinochloa crus-galli*) is the principal weed in rice production, and it is a problem weed in 42 countries (Holm *et al*., 1979). Season-long competition from *E. crus-galli* reduced rice yields by 38% to 64% depending on the rice cultivar (Stauber *et al*., 1991).

Most plants produce and emit methanol, especially during the early stages of leaf expansion, because of pectin demethylation (Fall and Benson, 1996), and this volatile organic compound exits leaves via stomata (Nemecek-Marshall *et al.*, 1995). Methanol has been proved to show great positive effects on photosynthesis (Nonomura and Benson 1992; Li and Yi, 2004). However, understanding the effects of methanol on plants is still highly controversial because opposite conclusions were reached concerning the effects on photosynthetic activity and biomass increase. It has been suggested that methanol may act as a C source for the plant and a photorespiration inhibitor (Albrecht *et al*., 1995). A wide range of C_3 crops and ornamental plants increase their growth and yield of fruit or seed after being sprayed with 10–50% methanol. Treatment of cabbage (*Brassica oleracea capitata*) with methanol resulted in an increase of approximately 50% in vegetative fresh weight. Comparable enhancements of growth of wheat, radish, pea, and tomato have been reported (Devlin *et al.*, 1994; Rowe *et al.*, 1994). Methanol also stimulated growth of wheat *(Triticum aestivum* L.) and pea (*Pisum sativum* L.) (Devlin *et al.*, 1994); geranium (*Pelargonium* sp.) and bachelor's button (*Centaurea cyanus* L.) (Devlin *et al.*, 1995).

Some research has shown that application of methanol was not effective. No influence on yield was reported for spring barley *(Hordeum vulgare* L.), winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) (Albrecht *et al.*, 1995) or peppermint *(Mentha* x *piperita* L.) (Mitchell *et al*., 1994).

Nonomura and Benson (1992) reported that foliar methanol application dramatically increased the growth, yield and water use efficiency of many C_3 crop plants. The increased growth and yield has been attributed to the action of methanol as an inhibitor of photorespiration (Nonomura and Benson, 1992; Fall and Benson, 1996). The use of foliar applications of methanol to increase biomass production and water use efficiency of agricultural crops has received considerable attention. Such studies were stimulated by the initial report of Nonomura and Benson (1992) that even a single foliar application of 10 to 50% methanol increased growth and development of a number of crops grown in an arid environment under high sunlight intensity. They suggested that the long-term stimulation of growth by methanol may be related to the inhibition of photorespiration of plants as they found that positive effects were observed in C_3 plants (with photorespiration) but not in C_4 plants (without photorespiration). Methanol spray is a method that increases crop CO_2 fixation per unit area. Recent investigation showed that C_3 crop yield and growth increased via methanol spray and that methanol may act as C source for these crops (Makhdum *et al.*, 2002).

Because foliar application of methanol has no apparent effect on the growth of C_4 plants (Nonomura and Benson, 1992; Devlin *et al.*, 1994), it is possible that an effect of methanol in C_3 plants is to reduce photorespiration rates, unless gross photosynthesis is also decreased by methanol. Also, the field conditions under which methanol has been found to enhance growth (i.e. high temperature and high light intensity) are consistent with high rates of photorespiration (Nonomura and Benson, 1992). Reduction of photorespiration would enhance net CO_2 assimilation. This was supported by failure of C_4 plants to respond to foliar-applied methanol, by the high light requirements for beneficial effects of methanol in C_3 plants, and by finding that the ratio of sucrose to glycolate metabolites was increased by methanol. The main objectives of our experiments were to: (1) evaluate whether methanol enhances growth and yield of rice; (2) determine the efficacious alcohol concentration for foliar application and (3) assess whether methanol has any apparent effect on the growth of C_4 plants.

Materials and Methods

In order to evaluate effects of methanol spraying on rice yield, a field experiment was conducted at the Rice Research Station in Tonekabon, Iran (36°51' N, 50°46' E with altitude of -22 m above sea level) during the 2012 growing season. Soil properties were 2.2% organic matter content, 37% clay, 44% silt, 19% sand, pH=6.8 and CEC= 29.9

(meg 100 g). Rice seeds were sown in the nursery on 8 April, 2012 and transplanted on 15 May, 2012.

The experiment was conducted as a randomized complete block design with a factorial treatment arrangement and three replications. Studied factors were 0 (as control), 6, 12, 18 and 24% (v/v) methanol and four barnyard grass (*Echinochloa crus-galli*) densities; 0, 16, 24 and 32 plants m^{-2}.

These solutions were sprayed three times on foliage parts of rice at two week intervals. The first foliar application was applied in 45 days after transplanting. These treatments were applied on 30 June, 10 July and 31 July, between 16:00 pm to 19:00 pm during bright sunny days with hot temperature. Cossins (1964) reported that during evening hours air temperature is relatively low, which reduces evaporation of methanol from the leaf surface, and thus increases the possibility for methanol to penetrate into the plant. Methanol spraying was carried out in a way that covered all aboveground parts of rice plants. A back engine sprayer with a capacity of 20 L was used to spray; the sprinkler was held 40 cm above the plants. To reduce the probability of methanol toxicity, Nonomura and Benson (1992) recommended adding glycine to the methanol spray. They reported that application of glycine in methanol treatments caused increased turgidity and stimulated plant growth without injury under indirect sunlight. Exogenous application of glycine-betaine has been shown to increase drought stress tolerance and increase biomass production and yield in drought-stressed maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) (Agboma *et al*., 1997).

The total fertilizer applied was 200 kg N ha^{-1}, 100 kg P ha^{-1} and 150 kg K ha^{-1} with split application broadcast at the transplanting stage (30% N and 100% P.K.), panicle initiation (35% N), and 5 days before flowering (35% N). Irrigation was performed twice a week until 100 days after transplanting. Moreover, during the growing season all weeds except the planted barnyard grass were hand weeded.

At the maturity stage, plant height (from the soil surface to the top of the plant canopy) was measured. Plants were harvested by hand cutting at the soil surface and subsequently aboveground biomass of rice was measured and tillers of each plot were counted. Rice aboveground biomass from each plot was placed in a separate paper bag, dried at 72 °C for 48 h, and weighed.

The agronomic traits included tiller number and 1000-grain weight, measured according to the standard evaluation system. Plants were harvested 107 days after transplanting. Plots were hand harvested for rough rice yields at 2.5 m^2 and adjusted to 14% moisture.

The SAS software package was used to analyze all the data (SAS Institute, 2001) and means were compared by the least significant difference (LSD) tests at 0.05 probability level. Data obtained were subjected to correlation and path coefficient analysis using the statistical software PASW Ver. 18.0. Path coefficient analysis was performed using simple Pearson correlation coefficients using grain yield as dependent variable and the other characters as influential variables. The direct and indirect effects of influential variables on grain yield were calculated according to the method of Dewey and Lu (1959).

Results and Discussion

Analysis of variance showed that methanol had significant effects on tiller number and grain yield (Table 1). There were significant ($P< 0.01$) effects of weed densities on rice seed and biological yields. Moreover, the interactions between methanol and barnyard grass densities were significant for grain yield and 1000-grain weight. Harvest index was not influenced by the presence of weeds and methanol treatments (Table 1); this indicates that percentage reductions of grain yield and biomass due to weeds and methanol were similar. Treatments showed significantly different responses to different weed densities and methanol application.

We recorded yield changes following methanol applications to plant foliage. Considering the high yields from the field trials (Table 2), it seems likely that rice suffers significantly from any methanol treatment. The highest grain yield was at 18% (v/v) and there were significant differences between 18% methanol and other treatments (Figure 1).

Grain yield and yield components were influenced significantly when rice was exposed either to weed densities or methanol application. The greatest mean values for grain yield and 1000-grain weight were obtained at 18% (v/v) with weed free condition, 7440.8 kg ha^{-1} and 28.07 g, respectively (Figure 1 and 2).

As the barnyard grass population increased, grain yield decreased constantly. The results indicated that barnyard grass at 16, 24 and 32

Table 1: Analysis of variance for determined characteristics in rice as affected by methanol and barnyard grass density treatments.

SOV	df	Grain yield	Biological yield	HI	1000 - grain weight	Tiller number	Plant height
Rep (Y)	2	1145347.58	3624071.1	17.69	.47	10.85	249.66
Methanol (M)	4	611227.19*	1368956.87ns	1.959ns	.097ns	9.86*	9ns
Weed (W)	3	1506776.87**	8644177.24**	4.29ns	.38ns	7.24ns	20.85ns
M×W	12	363583.69*	1775912.93ns	2.06ns	.92**	3.89ns	7.91ns
Error	38	183857.03	1322789.2	3.77	.26	2.84	10.78
CV (%)		6.61	10.93	3.59	1.92	8.34	3.31

ns= not significant; * , ** significant at the 5% and 1% levels of probability, respectively.

Table 2. Mean comparison of simple effects for determined characteristics in rice as affected by methanol and barnyard grass density treatments

Treatment	Grain yield (Kg ha^{-1})	Biological yield (Kg ha^{-1})	1000 - grain weight (g)	Tiller number
M1	6706.2±177.77	10456.5±401.82	26.78±0.15	20.77±0.68
M2	6572.2±165.45	10635.3±335.14	26.9±0.22	20.78±0.55
M3	6485.1±195.96	11040.2±453.67	26.85±0.15	20.63±0.37
M4	6575.1±166.04	10237.3±437.12	26.67±0.27	20.17±0.52
M5	6111.8±112.42	10222.5±350.63	26.75±0.09	18.64±0.58
W1	6936±108.63	11652.2±400.31	27.01±0.2	20.7±0.53
W2	6496±144.45	10154.1±268.41	26.69±0.2	20.78±0.4
W3	6279.7±91.31	10048.5±283.19	26.65±0.11	20.03±0.54
W4	6248.8±121.52	10218.7±298.23	26.81±0.12	19.29±0.54

M1, M2, M3, M4 and M5: 0 (control), 6, 12, 18, and 24% (v/v) methanol, respectively.
W1, W2, W3 and W4: 0 (control), 16, 24 and 32 plants/m^2, respectively.
Each value represents mean ± S.E. of three replicates per treatment.

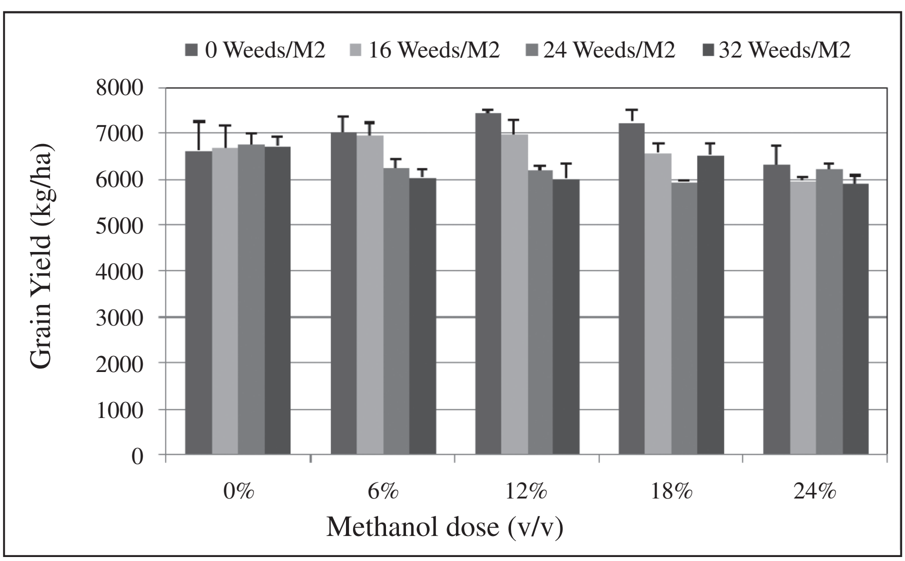

Figure 1. Grain yield after foliar spray of methanol at different weed densities. Error bars are ±S.E.

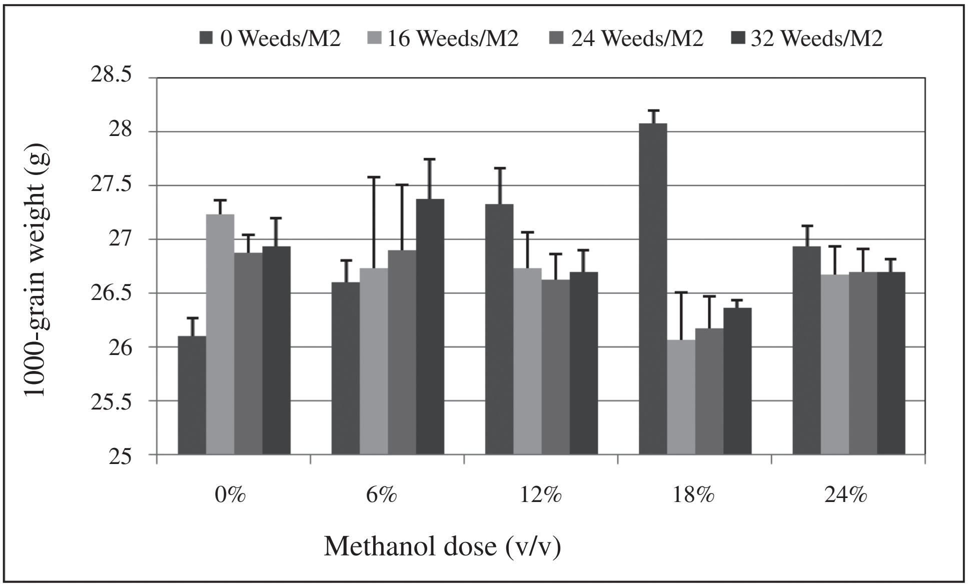

Figure 2. 1000-grain weight after foliar spray of methanol at different weed densities. Error bars are ±S.E.

plants m^{-2} reduced grain yields of rice by 6.34, 9.46 and 9.9%, respectively. Grain yield reductions were due to decreases in plant height, tiller number and biological yield per plant (Table 2). Rice grain yields were reduced when rice plants grew between barnyard grass clumps spaced 20 or 40 cm apart, but yields were not affected when rice was grown between weed clumps separated by 80 or 100 cm (Stauber *et al*., 1991). It appears that the main reason for significant differences between treatments in plant height, seed and biological yield is the result of weed effects rather than methanol application, because there were no significant differences between treatments of methanol application (M_3) and the control treatments (M_1).

In this study the quantities of methanol applied to the rice presumably were so small that changes in growth cannot be expected such as those resulting from methanol application in other plants. Moreover, it is not clear to what extent the methanol is absorbed and utilized in the plant. Probably a large proportion of the methanol is lost via evaporation, especially when applied on a sunny, warm day. Under water stress conditions, methanol might function as an osmoprotectant, protecting plant vital processes and enabling quick recovery when the stress is removed. In the present experiment, however, the rice was not exposed to any drought stress or extreme temperatures, which may explain why different effects were found. Foliar sprays of 10-50% aqueous methanol increase the growth and development of cut roses and tomatoes (Nonomura and Benson, 1992; Rowe *et al*., 1994) as well as different field crops (Nonomura and Benson, 1992; Devlin *et al*., 1994). Mitchell *et al*. (1994), however, found no effect of methanol on the growth of peppermint. Nonomura and Benson (1992) concluded that foliar sprays of methanol strongly enhanced plant growth in arid and warm (up to about 40-45 °C) environments. 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.

Simple correlation coefficients between studied traits are illustrated in Table 3. All the characters except harvest index and 1000-grain weight were positively correlated with grain yield. The results showed that plant height had the most positive correlation (r=0.63**) with grain yield, followed by biological yield r=0.55**; plant height and biological yield were significantly correlated (r=0.48**). This indicates that grain yield can be increased whenever there is an increase in characters that show positive and significant association with grain yield. Hence these characters can be considered as criteria for selection for higher yield, as they were mutually and directly associated with yield. As in the present study, grain yield was reported to be positively

Table 3. Correlation coefficients for determined characteristics of rice as affected by methanol and barnyard grass density treatments.

Traits	Tiller number	Grain yield	Biological yield	HI	1000 - grain weight
Plant height	.08	.63**	.49**	-.27*	.26*
Tiller number	1	.34**	.32*	-.17	.06
Grain yield		1	.55**	-.13	.24
Biological yield			1	.01	.26*
HI				1	-.11

ns= not significant; * , ** significant at the 5% and 1% levels of probability, respectively.

Table. 4. Path coefficients for grain yield components as affected by methanol and barnyard grass density treatments. Underlined numbers on the diagonal are direct effects of traits on grain yield.

Traits	Plant height	Tiller number	Biological yield
Plant height	.434	.02	.098
Tiller number	.034	.255	.064
Biological yield	.213	.081	.2

Error= 0.662 (Residual effect)

correlated with plant height (Singh *et al*. (1979) and with number of grains per plant Sharma and Sharma (2007). But negative correlation of grain yield was reported with harvest index by Kishor *et al*. (2008).

Correlation analysis simply measures the correlation between two traits, and cannot elucidate the related mechanisms among them. Path analysis can dissect the correlation coefficient into direct and indirect effects, and quantify the relative contribution of each component to the overall correlation (Yao *et al*., 2011). Path coefficient analysis has been useful in determining selection criteria for rice (Samonte *et al*., 1998). In order to identify a trait as an indirect selection criterion for grain yield through path coefficients, the trait should have a positive direct effect on grain yield as well as significant positive correlation with grain yield (Das and Taliaferro 2009). Path coefficients of agronomic traits on grain yield of rice are shown in Table 4. The path coefficient analysis based on grain yield as a dependent variable revealed that all traits exhibited positive direct effects on grain yield (Table 4).

Similar to the correlation analysis, path analysis of grain yield and its components demonstrated that plant height wielded the highest positive direct influence (0.434) on grain yield, followed by tiller number and biological yield. Marjanovic-Jeromela (2009) also observed direct positive influences of these characters on the dependent variable. Hence these traits may be explored more confidently as selection criteria for yield improvement in rice.

All C_3 crop plants tested, including tomato and watermelon, responded positively to methanol treatment. According to Nonomura (personal communication), one application was sufficient to improve plant productivity, but multiple applications were required to achieve maximum benefits. Our data support these claims. Following Nonomura and Benson's treatment protocol, we found that foliar methanol application is effective overall in enhancing any measure of rice plant performance under irrigated field conditions.

Conclusion

The results from this experiment indicated that, in general, methanol affected growth and yield in the rice crop examined, and therefore appears to be effective as a growth enhancer. Foliar sprays of aqueous methanol, at least so far, can be recommended for rice. Barnyard grass reduced grain yield of the Shiroudi cultivar as weed density increased. Grain yield reductions were due to decreases in plant height, tiller number and biological yield per plant. All the characters except harvest index and 1000-grain weight were positively correlated with grain yield. Hence these characters can be considered as criteria for selection for higher yield, as they were mutually and directly associated with yield. The results of the path analysis suggested that plant height is a direct contributor to grain yield. These results concluded that improvement of grain yield in rice is linked with these traits, so these parameters should be an integral part of effective selection criteria leading to yield enhancement in rice.

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