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# Effect of methanol foliar application on rice (*Oryza sativa* L.) growth and grain yield

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**Abstract**

The aim of this experiment was to evaluate the effect of methanol foliar application on growth and grain yield of two rice cultivars. The experiment was carried out in Bandar Anzali, north of Iran, during rice growing season in 2011 and 2012. The experiment was conducted as a randomized complete block design with a factorial treatment arrangement and three replicates. Factors were two rice cultivar ('Shiroudi' and 'Hashemi') and five aqueous methanol foliar applications (0, 7, 14, 21, and 28% v/v). ANOVA indicated that the effects of methanol and cultivar and cultivar × methanol interaction were significant for grain yield and yield components, aboveground dry weight, and root dry weight. These indicate that the cultivars had different response to methanol foliar application. For 'Shiroudi' cultivar, the highest grain yields were obtained when methanol was sprayed at 14% and 7% concentration, while the lowest one was observed in non-treated plants. For 'Hashemi' cultivar, the greatest grain yield was obtained when methanol was sprayed at 28% v/v concentration, while the lowest ones were observed in non-treated plants and plants treated with 7% v/v concentration. This experiment illustrated that methanol foliar application increased rice growth and grain yield.

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## Introduction

Rice is an important staple food for over half the world's people grows well in hot and humid regions. More than 90% of the world's rice is grown and consumed in Asia. Rice is a valuable carbohydrate energy source, and also provides a number of trace minerals, proteins, and vitamins. In 2012, it was the second most produced cereal after wheat with a world production of over 719 million tonnes (Fao, 2014). The total rice production in Iran was 2.4 million tonnes of paddy rice in 2012, which was harvested from 48000 ha (Fao, 2014). Rice is extensively grown in north provinces of Iran, i.e. Mazandaran, Guilan, and Golestan, in irrigated lowland fields.

Methanol is one of the simplest plant products (Fall and Benson 1996). Most plants produce and emit methanol especially during early stages of development because of pectin demithylation, and this volatile organic compound exits leaves via stomata (Nemecek-Marshall *et al.*, 1995). The main sources of methanol production in plants are: 1) pectin de-methylation in cell walls (Obendorf *et al.*, 1990), 2) protein repair pathways (Mudgett and Clarke, 1993), and 3) lignin degradation (Lewis and Yamamoto, 1990).

The role of methanol as a plant growth regulator (Dwivedi *et al.* 2001) and to accelerate plant maturity would need to be studied more in detail. Methanol induced growth stimulation has been reported in some $C_3$ crops, probably due to higher turgor and growth rate (Nonomura and Benson, 1992a, b). Hemming *et al.* (1995) declared that brief foliar application of aqueous methanol solutions increased the metabolic heat rate resulting in increased carbon conversion efficiency. Ramadan and Omran (2005) found that increasing the chlorophyll content, the leaf area and the number of stomata per unit leaf area by methanol application increased net productivity of vines. Nadali *et al.* (2010) and Abido (2012) reported that methanol foliar application increased significantly root and leaf fresh weights and sugar yield in sugar beet (*Beta vulgaris*). Zbieć *et al.* (1999) found that foliar application of methanol increased the activity of nitrate reductase and alkaline phosphatase in some crop leaves. However, others failed to see any positive impact of methanol on plant growth and the results of the field studies have proved to be largely non-reproducible (Hartz *et al.*, 1993; Mitchell *et al.*, 1994; Hemming *et al.*, 1995). It has been suggested that methanol acts as a photorespiration inhibitor in plants with $C_3$ metabolism. Therefore, this study was conducted to determine the effect of methanol foliar application on rice growth and grain yield.

## Materials and methods

*Experimental design, plant culture and management*

Pot experiment was conducted in Bandar Anzali (37° 29' N, 49° 24' E), north of Iran, during rice growing season in 2011 and 2012. The experimental design was a randomized complete block with four replicates. Factorial combinations of two cultivars and five foliar applications of aqueous methanol solutions were the treatments of the experiment. Cultivars were 'Hashemi' and 'Shiroudi' (a traditional and high-yielding modern cultivar which extensively grown in north of Iran, respectively) and five foliar applications of aqueous methanol solutions were 0, 7 and 14, 21, and 28% v/v (M0%, M7%, M14%, M21%, and M28%, respectively). Monthly precipitation and temperature during rice season of 2011 and 2012 are shown in Table 1. The soil texture was clay (clay 50%, silt 37.5%, sand 12.5%), organic matter content 3.4%, pH 6.6, total N 0.13%, available N 115.3 mg $kg^{-1}$, available phosphorous 12.0 mg $kg^{-1}$, and available potassium 85.0 mg $kg^{-1}$, EC 0.3 ds $m^{-1}$. Twenty kilograms of the soil was weighed for each pot (35 cm in diameter and 45 cm in deep). Rice seeds were sown in the nursery on 30 and 28 April 2011 and 2012, respectively. Three healthy seedlings of 'Shiroudi' or 'Hashemi' cultivar were transplanted in hills on 29 and 27 May 2011 and 2012, respectively.
Aqueous methanol solution was sprayed on the plants with a manual sprayer at a rate of 50 ml per pot (plants were carefully wetted), with the nozzle approximately 20 cm above the leaf surface. Control plants were sprayed with water under the same conditions. Plants were sprayed three times during

growing period with 15 days intervals. The first methanol foliar application was applied at stem elongation stage. The recommended fertilizer dose of NPK (100 kg N $ha^{-1}$ as urea, 100 kg $P_2O_5$ $ha^{-1}$ as triple superphosphate, and 150 kg $K_2O$ $ha^{-1}$ as potassium sulfate) were applied, with half N and the entire P and K as a basal dose. The remaining N was applied at panicle initiation stage. Weeds were controlled by hand weeding when necessary. Consistent with the lowland paddy field practices in north of Iran, a 5- to 10-cm deep permanent flood was established during rice growing period.

*Sampling*

Mature plant height was measured from ground level to the tip of panicle excluding awns. At maturity stage, yield components of rice (Tiller number per $m^2$, grain number per panicle, and 1000-grain weight) were measured according to Gomez (1972). In each pot, all plants were harvested by hand-cutting from the above soil surface and subsequently leaf, stem, and grain were separated. Leaf areas for green leaves of each pot were measured using a leaf area meter (LI-3000A, LI-COR, Lincoln, Nebraska, USA). Rice grain yield was adjusted to 14% moisture content. Leaves and stems of each pot were placed in separate paper bags, dried at 75 °C for at least 96 h, and weighted. Harvest index was calculated as grain dry matter divided by aboveground dry matter. Rice roots from each pot were washed well with tap water to remove all traces of soil and placed in separate paper bags, dried at 75 °C for 96 h, and weighted.

*Statistical analyses*

All statistical analyses were conducted by using SAS (SAS Institute, 2004). Data were subjected to ANOVA and mean comparisons were made using Fisher's Protected LSD, when the F test for the cultivar or methanol was significant ($P < 0.05$). There were no year × treatment interactions for all traits, so data from two consecutive years were combined and years were considered random. When cultivar × methanol interaction was found to be significant, mean comparisons were made using SE among methanol concentrations for each cultivar.

**Results and discussion**

Methanol and cultivar main effects were significant for rice plant height, while the main effect of year and all 2- and 3-ways interactions were not significant (Table 2). Therefore, data were presented over two years. 'Hashemi' (131 cm) was significantly taller than 'Shiroudi' (96 cm) cultivar when average across methanol foliar applications and years. 'Hashemi' is a traditional cultivar with tall stature, while 'Shiroudi' is a semi-dwarf, high-yielding modern cultivar and, therefore, it is reasonable that 'Hashemi' be taller than "Shiroudi". Regardless of year and cultivar, the tallest plants were observed when methanol was sprayed at 28% v/v; however there were no significant differences in plant height among M28%, M21%, and M14% treatments. The shortest plants were observed in M7% and M0% treatments (Fig. 1). Similarly, significant increases in plant height due to methanol foliar application were reported by Makhdum *et al.* (2002) for cotton and Mirakhori *et al.* (2010) for soybean plants.

**Table 1.** Monthly precipitation and temperature from April to September in 2011 and 2012 for experimental site.

| Month | Precipitation (mm) | | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Maximum | | Minimum | | Average | |
| | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| April | 72.2 | 16.5 | 17.08 | 19.24 | 9.9 | 13 | 13.49 | 16.12 |
| May | 54.9 | 10.7 | 22.11 | 25.55 | 16.1 | 20.04 | 19.1 | 22.79 |
| June | 31.9 | 181.1 | 26.92 | 29.07 | 19.8 | 22.63 | 23.36 | 25.85 |
| July | 0.3 | 118.5 | 31.6 | 29.01 | 22.5 | 23.35 | 27.05 | 26.18 |
| August | 166.7 | 206.5 | 28.1 | 30.8 | 21.6 | 24.03 | 24.85 | 27.41 |
| September | 236.2 | 246.2 | 25 | 26.08 | 18.2 | 20.12 | 21.6 | 23.1 |

**Table 2.** Mean squares for the combined analysis of variance for rice plant height (H), panicle number per plant (PN), grain number per panicle (GN), 1000-grain weight (ThGW), grain yield (Y) as affected by methanol foliar application (M) and cultivar (C).

| Source | df | H | PN | GN | ThGW | Yield |
|---|---|---|---|---|---|---|
| Year (Y) | 1 | 60 ns | 0.4 ns | 69.5ns | 0.01 ns | 79.5 ns |
| R (Y) | 4 | 8 | 0.5 | 22.1 | 0.33 | 198.0 |
| Methanol (M) | 4 | 233 *** | 12.1*** | 212.1*** | 3.10** | 4312.4*** |
| M * Y | 4 | 3 ns | 0.1 ns | 12 ns | 0.01 ns | 10.9 ns |
| Cultivar (C) | 1 | 18077*** | 0.1ns | 2793.4*** | 81.66 *** | 11002.6*** |
| Y * C | 1 | 2 ns | 0.2 ns | 0.1 ns | 0.06 ns | 2.7 ns |
| M * C | 4 | 64 ns | 2.7 *** | 110.9 * | 1.29 ** | 879.4 ** |
| M * C * Y | 4 | 1 ns | 0.1 ns | 2.3 ns | 0.06 ns | 9.2 ns |
| Error | 36 | 1068 | 0.3 | 35.3 | 0.28 | 220.3 |

*, **, ***: significant at the 0.05, 0.01, and 0.001 probability levels, respectively

ns, not significant at the 0.05 probability level.

Grain yield and yield components (panicle number per plant, grain number per panicle and 1000-grain weight) were significantly influenced by methanol foliar application (M), cultivar (C). Moreover, the interaction between methanol and cultivar was significant for yield and yield components (Table 2). This indicates that rice cultivars ('Shiroudi' and 'Hashemi') showed different response to methanol foliar application in regard to grain yield and yield components. ANOVA also indicated that the main effect of year and other 2- and 3-way interactions were not significant (Table 2). For 'Shiroudi' cultivar, the highest grain yields were obtained when methanol was sprayed at 14% and 7% concentrations, while the lowest one was observed in non-treated plants (Table 3). For 'Hashemi' cultivar, the greatest grain yield was obtained when methanol was sprayed at 28% v/v concentration, while the lowest ones were observed in non-treated plants and plants treated with 7% v/v concentration (Table 3). For 'Shiroudi' cultivar, panicle number per plant was significantly increased from 7.0 ± 0.2 to 10.0 ± 0.2, when methanol concentration increased from 0 to 7% v/v, but thereafter remained constant (Table 3). For 'Hashemi' cultivar, panicle number per plant was significantly increased from 8.8 ± 0.2 to 10.4 ± 0.2, when methanol concentration increased from 0 to 14% v/v, but it was significantly reduced at higher methanol concentrations (Table 3). For 'Shiroudi' cultivar, grain number per panicle was significantly increased from 77.9 ± 3.5 to 85.9 ± 1.8 as methanol concentration increased from 0 to 14%, but it was significantly reduced at higher methanol concentrations (Table 3). For 'Hashemi' cultivar, an inconsistent trend in grain number per panicle was observed for different methanol concentration. For this cultivar the highest grain numbers per panicle were observed for M0, M14%, and M28% treatments, while the lowest one was observed for M7% treatment (Table 3). For 'Shiroudi' cultivar, methanol foliar application increased significantly 1000-grain weight; however, there were no significant differences in 1000-grain weight among M7%, M14%, M21%, and M28% concentrations (Table 3). For 'Hashemi' cultivar, the highest and the lowest 1000-grain weights were observed for M28% and M0%, respectively (Table 3). Main effect of methanol was significant for aboveground biomass (Table 4). Moreover, the interaction between cultivar and methanol was significant; indicating that rice cultivars had different response to methanol foliar application in aboveground biomass production (Table 4). ANOVA also indicated that the main effects of year and cultivar and the interactions of M × Y, C × Y, and M × C × Y were not significant (Table 4). For 'Shiroudi' cultivar, aboveground dry weight was significantly increased from 162.2 ± 3.5g to 254.1 ± 3.4g when methanol concentration increased from 0 to 14% v/v, but thereafter was significantly reduced (Table 3). For 'Hashemi' cultivar, aboveground dry weight was significantly increased from 188.2 ± 0.2g to 251.4 ± 3.5g when methanol concentration increased from 0 to 28% v/v (Table 3). Harvest index was significantly affected only by cultivar (Table 4). Harvest index for

'Shiroudi' (56.5) was significantly grater than that for 'Hashemi' (46.6) (Table 3). Methanol had no significant effect on harvest index, indicating that the effect of methanol on grain and aboveground biomass was the same. Our findings were in agreement with the results reported by Nishio *et al.* (1994), and Valenzuela *et al.* (1994). Moreover, foliar application of methanol solutions to Arabidopsis plants resulted in significant increases in fresh and dry weight. Li *et al.* (1995) reported that seed weight, seed yield, and pod number were significantly higher for soybean plants treated with methanol. In contrast, McGiffen *et al.* (1995) Mitchell *et al.* (1994) reported that methanol foliar application had no significant effect on growth and grain yield in $C_3$ plants. The stimulation effect of methanol on growth and yield of $C_3$ plants was attributed to the use of the methanol as a direct carbon source via serine biosynthesis, decreased carbon loss from photorespiration, increased water use efficiency, increased cell turgor and stomatal conductance, and increased assimilation rate in methanol treated plants (Nonomura and Benson,1992a, 1992b). Cossins (1964) showed that methanol is mainly converted to CO2, which probably can be used as a source of carbon in plants. The reduction in grain yield at concentration higher than 14% methanol for 'Shiroudi' cultivar may be due to the toxicity effect of methanol at high concentration level.

**Table 3.** mean comparison for methanol foliar application × cultivar interaction for panicle number per plant (PN), grain number per panicle (GN), 1000-grain weight (ThGW), grain yield (Y), aboveground dry weight (ADW), and root dry weight (RDW) over two years.

| | PN (No. pot$^{-1}$) | | GN (No. pot$^{-1}$) | | ThGW (g) | | Y (g pot$^{-1}$) | | ADW (g pot$^{-1}$) | | RDW (g pot$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Methanol (% v/v) | 'Shiroudi' | 'Hashemi' | 'Shiroudi' | 'Hashemi' | 'Shiroudi' | 'Hashemi' | 'Shiroudi' | 'Hashem' | 'Shiroudi' | 'Hashemi' | 'Shiroudi' | 'Hashemi' |
| 0 | 7.0±0.2 | 8.8±0.2 | 77.9±3.5 | 71.5±0.9 | 25.4±0.2 | 27.1±0.2 | 95.7±1.9 | 94.2±4.0 | 162.2±3.5 | 188.2±0.2 | 20.8±0.6 | 39.6±0.6 |
| 7 | 10.0±0.2 | 9.4±0.1 | 80.1±1.2 | 57.7±0.6 | 25.8±0.1 | 27.6±0.2 | 154.1±3.1 | 98.2±6.8 | 229.7±4.6 | 190.7±11.6 | 24. ±0.6 | 40.9±0.8 |
| 14 | 10.2±0.2 | 10.4±0.2 | 85.9±1.8 | 73.1±3.7 | 25.9±0.2 | 28.4±0.4 | 156.6±2.0 | 123.7±3.9 | 254.1±3.4 | 216.9±19.2 | 27.0±0.7 | 44.0±0.9 |
| 21 | 10.1±0.4 | 9.8±0.1 | 80.4±1.9 | 64.1±3.4 | 25.9±0.1 | 28.3±0.3 | 146.0±5.8 | 112.8±12.5 | 240.3 ± 1.1 | 233.8±4.2 | 25.6±1.4 | 43.7±0.8 |
| 28 | 10.1±0.1 | 9.9±0.3 | 82.7±1.3 | 72.4±0.9 | 25.9±0.2 | 29.3±0.2 | 151.8±1.3 | 131.3±3.0 | 236.9±10.1 | 251.9±3.5 | 24.9±0.7 | 46.3±0.7 |

Root dry weight was significantly influenced by year, methanol, cultivar, M × C interaction, while other 2- and 3-way interactions were not significant (Table 4). Rice plant produced greater root dry weight in 2011 (33.1 g pot$^{-1}$) compared to 2012 (34.3 g pot$^{-1}$). For 'Shiroudi' cultivar, the highest and the lowest root dry weights were observed in non-treated plants and plants treated with 14% methanol concentration, respectively (Table 3). For 'Hashemi' cultivar, the highest and the lowest root dry weights were obtained from M0 and M28% treatments (Table 3), respectively.

**Table 4.** Mean squares for the combined analysis of variance for aboveground dry weight (ADW), harvest index (HI), root dry weight (RDW), and rice leaf area (LA) as affected by methanol foliar application (M) and cultivar (C).

| Source | df | ADW | HI | RDW | LA |
|---|---|---|---|---|---|
| Year (Y) | 1 | 497.6$^{ns}$ | 0.08$^{ns}$ | 24.1$^{*}$ | 1232466$^{ns}$ |
| R (Y) | 4 | 320.2 | 20.42 | 7.5 | 966324 |
| Methanol (M) | 4 | 10208.9$^{***}$ | 12.73$^{ns}$ | 61.3$^{***}$ | 3930681$^{***}$ |
| M * Y | 4 | 22.2$^{ns}$ | 0.30$^{ns}$ | 0.5$^{ns}$ | 50940$^{ns}$ |
| Cultivar (C) | 1 | 1040.4$^{ns}$ | 1454.45$^{***}$ | 4988.6$^{***}$ | 8424365$^{***}$ |
| Y * C | 1 | 0.4$^{ns}$ | 1.72$^{ns}$ | 2.2$^{ns}$ | 413$^{ns}$ |
| M * C | 4 | 2102.2$^{**}$ | 4.83$^{ns}$ | 12.8$^{**}$ | 1124262$^{ns}$ |
| M * C * Y | 4 | 19.0$^{ns}$ | 0.63$^{ns}$ | 0.5$^{ns}$ | 26700$^{ns}$ |
| Error | 36 | 519.8 | 6.90 | 3.7 | 666642 |

*, **, ***: significant at the 0.05, 0.01, and 0.001 probability levels, respectively

ns, not significant at the 0.05 probability level.

Methanol and cultivar had significant effect on rice leaf area. All 2- and 3-way interactions were not significant (Table 4). 'Hashemi' (6646 cm²) produced grater leaf area than 'Shiroudi' (5897 cm²) over years and methanol concentrations. Regardless of rice cultivar and year, the greatest leaf area was observed in plants which treated with 28%, 14% and 21% methanol, but the lowest one was obtained from M0 and M7% treatments (Fig. 2). This is in agreement the result of Ramirez *et al.* (2006), who reported that methanol foliar application could increase leaves expansion by stimulating genes encoding for pectin methyl esterase, which enhance plant Ca capture for increasing leaf area.

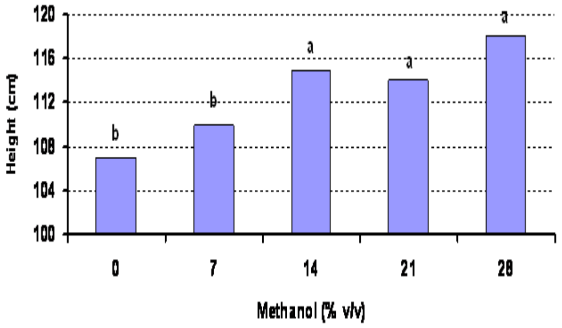


**Fig. 1.** Effect of methanol foliar application on plant height over two years and cultivars.

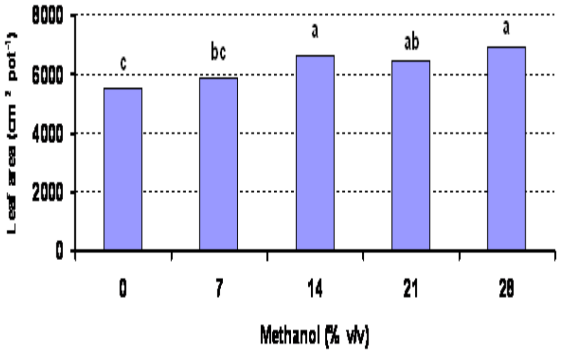


**Fig. 2.** Effect of methanol foliar application on leaf area over two years and cultivars.

## Conclusions

This experiment indicated that 'Shiroudi' and 'Hashemi' had different response to methanol foliar application. For 'Shiroudi' cultivar, the highest grain yield was obtained from 14% methanol concentration, while for 'Hashemi' cultivar, the highest grain yield was obtain from 28% methanol concentration.

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