Research Journal of Biological Sciences 7 (3): 136-140, 2012
ISSN: 1815-8846

Effect of Methanol and Tetrahydrofolate on Yield and Yield Components of Rice

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Abstract: To examine the effects of foliar application of methanol and tetrahydrofolate on yield and yield components of rice, an experiment was conducted in Water and Soil Farm of Iran's Rice Research Institute located in the city of Rasht in the agricultural year of 2010. Accordingly, an experiment was conducted with factorial arrangement on the basis of randomized complete blocks design with three replications. The 1st agent was the concentration of methanol at 4 levels (control, 10, 20 and 30% by volume) and the 2nd agent was tetrahydrofolate at 4 levels (control, 1-3 mg L^{-1}). The results showed that foliar application of methanol significantly affects the traits of the number of fertile tillers, total number of tillers, number of filled spikelets, number of hollow spikelets, paddy yield and thousand kernel weight at 1% level. In general, the highest values of yield and yield components were observed at concentrations of 20% (by volume) methanol and the highest number of hollow spikelets was in control. The effect of tetrahydrofolate on paddy yield and thousand kernel weight was significant at 1% probability level and the maximum averages belonged to 3 g L^{-1} of tetrahydrofolate.

Key words: Methanol, tetrahydrofolate, yield, yield components, rice, Iran

INTRODUCTION

Rice is the second most important cereal in the world after wheat which is the only grain grown for humans. It has high genetic diversity and adaptability, high yield, the range of global expansion, packaging suitable for storage and transport and protective cover on the seeds. About 70% of human demand for protein is provided directly by consumption of grain or indirectly through their consumption by animals for meat. Today, the results of many research suggest that the use of some simple alcohols (e.g., methanol) increase concentrations of carbon dioxide and ultimately increase yield of three carbon crops (Ramirez *et al*., 2006) and by reducing the effects of stresses induced to crops, it can prevent the increase of photorespiration (Nonomura and Benson, 1992). In general, considering that in most cases, 25% of the carbon in plant is used for photorespiration, the amount of photorespiration can be minimized using foliar application of methanol (Gout *et al*., 2000) because methanol is rapidly metabolized to carbon dioxide and water in plant tissues (Safarzadeh-Vishkaei, 2007). Furthermore, compared to carbon dioxide, methanol is a smaller molecule which is easily absorbed by plants and can be used by the three-carbon plants to increase photosynthesis (Li *et al*., 1995; Kotzabasis *et al*., 1999). Tetrahydrofolate is an essential cofactor for reactions involving the transfer of single-carbon compounds in all living creatures (Hanson and Roje, 2001) which is necessary for the assimilation of methanol in plants (Gout *et al*., 2000; Ramberg *et al*., 2002). Tetrahydrofolate is centeral cofactor for one-carbon transfer reactions which are involved in many cellular reactions such as synthesis of purines, metabolism of amino acids, a glycine to serine conversion, synthesis of methionine and the formation of lignin, chlorophyll and choline and also in the photorespiration cycle (Hanson and Roje, 2001). Mirakhori *et al*. (2010a) reported that foliar application of methanol had a significant effect on grain yield, number of pods per plant, number of seeds per plant, number of branches per plant, total biomass, hundred seed weight and protein content of red beans. In their results, the highest averages were obtained in the treatment of 20-25% by volume of methanol. Concentrations >25% by volume of methanol had no significant impact on their traits and it reduced the amounts of their traits in most cases. The results of Safarzadeh-Vishkaei (2007) showed that foliar application of a solution of 20% (by volume) methanol on the aerial parts of peanuts increased the leaf area index, rate of plant growth, rate of pod growth, efficiency of radiation use, duration of leaf area, increased pod and seed yield, hundred seed weight, number of

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mature pods and amount of protein in seeds of peanut. Furthermore, by examining the effect of foliar application of methanol on soybean. Mirakhori *et al.* (2010b) concluded that the effect of the levels of solutions with 14 and 21% (by volume) of methanol on the traits evaluated was more than other treatments and the methanol used in treatments of 14 and 21% (by volume) can increase the yield by 16.8 and 40.2% compared to control treatment, respectively. Foliar application of methanol increased the yield, height, thousand kernel weight, number of filled pods, leaf area and biomass. Results of Pilehvari-Khomami *et al.* (2008) also suggest that the foliar application of 30% (by volume) methanol increased the pod yield, grain yield, number of pods that can be harvested and the percentage of protein in the peanut plant. In this regard, this study also aimed to evaluate the possibility of using foliar application of methanol and tetrahydrofolate to increase yield and yield components of rice.

MATERIALS AND METHODS

This research was conducted in Soil and Water Farm of Iran's Rice Research Institute located in the city of Rasht in the agricultural year of 2010. Accordingly, an experiment was conducted with factorial arrangement on the basis of randomized complete blocks design with three replications. The 1st agent was the concentration of methanol at 4 levels (control, 10, 20 and 30% by volume) and the 2nd agent was tetrahydrofolate at 4 levels (control, 1-3 mg L^{-1}). Before the operations of preparing the ground, some samples were taken from 0-30 cm soil depth for measuring nitrogen of soil and other soil nutrients. The operations of preparing the reserved ground were conducted a few days before wetting and germination. It was similar to the operations of preparing the original ground and included ploughing, harrowing, troweling and leveling. Before plowing, 80 kg ha^{-1} of phosphate fertilizer was added to the soil from triple super phosphate source (P_2O_5) and 100 kg ha^{-1} of potassium from potassium sulfate source. Urea was added to the soil 3 times: 60 kg after transplanting, 30 kg at the time of maximum tillering and 30 kg during fertility. Cultivar used in this experiment was Hashemi. The planting was done in plots with dimensions of 2.5×4 m with a distance of 20×20 cm. Foliar application of methanol and tetrahydrofolate was performed twice during the growing season. The first foliar application of the solution was made at the time of the emergence of the panicle, i.e., when 50% of the farm had gone the panicle. The second foliar application of the solution was made 10 days after the first foliar application of the solution at the beginning of grain filling in rice panicles. Foliar application of solution was made between 17-19 a.m. on designated days. Foliar application of solution continued until the flow of liquid droplets on rice plants. The solutions provided in all plots were sprayed by a pump sprayer with the same pressure with the amount of 2 L on rice plants. Also, the amount of 2 g L^{-1} of glycine andtoin was added to each of these treatments. To determine the paddy yield at the time of maturing, 1 m^2 in the middle of each experimental plot was completely pruned near the soil surface. After removing the seeds from the rest, they were dried for 24 h at 75°C in the oven and then were weighed. To calculate the number of fertile tillers and total number of tillers at maturity, 10 plants were randomly selected from each plot which were separately counted in each area. To determine the plant height at maturity, 10 plants were randomly selected from each plot and were measured in terms of centimeters.

To determine thousand kernel weight in each plot, 10 panicles were randomly selected which was calculated by counting and weighing seed of the filled kernels dried in oven. Number of filled and hollow spikelets was also the average of counting in 10 samples. Harvest index was calculated by dividing the economic yield by biomass percentage and them multiplying by 100. The software program MSTAT-C was used for statistical analysis and the averages were compared using Duncan Method at 5% probability level.

RESULTS

Plant height: According to the analysis of variance table, none of the treatments at the level of 5% had a significant effect on height of rice plant. With increasing the amounts of methanol and tetrahydrofolate, a constant trend was observed in plant height. Furthermore, the interaction of methanol and tetrahydrofolate was not significant on the height (Table 1). Maximum plant height belonged to 10% (by volume) methanol with an average of 150.91 cm. Also, maximum plant height belonged to 3 mg L^{-1} of tetrahydrofolate with an average of 151.88 cm (Table 2).

Number of fertile tillers: Table of analysis of variance indicates that methanol in a statistical level of 1% had a significant effect on the number of fertile tillers in rice plants. With increasing the amount of methanol by 20% (by volume), there was an increase observed in the number of fertile tillers however nonsignificant reduction was then observed in the number of fertile tillers. The maximum number of fertile tillers belonged to 20% (by volume) methanol with a average of 17.46 fertile tillers (Table 1). With increasing the amount of tetrahydrofolate, a nonsignificant increase was observed in the number of fertile tillers. Also the highest number of fertile tillers

Table 1: ANOVA of measured traits of rice under the influence of methanol and tetrahydrofolate

SOV	df	Plant height	No. of fertile tillers	Total No. of tillers	No. of filled spikelets	No. of hollow spikelets	Paddy yield	Thousand kernel weight	Harvest index
Block	2	46.88	19.73	13.85	11.31	11.31	1303347.95	0.77	131.27
Methanol	3	5.02^{NS}	22.01**	25.83**	25.25**	25.25**	6431512.09**	8.00**	37.50^{NS}
Tetrahydrofolat	3	28.27^{NS}	2.89^{NS}	6.52^{NS}	9.81^{NS}	9.81^{NS}	1346510.73**	10.19**	5.54^{NS}
Methanol x tetrahydrofolate	9	54.03^{NS}	2.97^{NS}	3.81^{NS}	5.94^{NS}	5.94^{NS}	244240.47^{NS}	3.47*	16.97^{NS}
Error	30	43.55	2.09	2.57	4.80	4.80	143920.35	1.27	21.75
CV (%)	-	4.39	9.20	9.20	2.44	24.01	7.44	4.73	11.42

**Significant at 0.01 level; *Significant at 0.05 level; NS: No Significant difference

Table 2: Comparison of measured traits in rice under different levels of methanol

Methanol (vol. %)	Thousand kernel weight	No. of fertile tillers	Total No. of tillers	No. of filled grains
0	22.89^{c}	14.24^{c}	15.82^{c}	89.00^{b}
10	23.45^{bc}	15.92^{b}	17.51^{b}	90.50^{ab}
20	24.79^{a}	17.46^{a}	19.34^{a}	92.25^{a}
30	24.03^{ab}	15.23^{bc}	16.98^{bc}	91.75^{a}
Methanol (vol %)	**No. of hollow spikelets**	**Plant height**	**Paddy yield**	**Harvest index**
0	11.00^{a}	149.65^{a}	4175.50^{c}	42.96^{a}
10	9.50^{ab}	150.91^{a}	5263.00^{b}	40.44^{ab}
20	7.75^{b}	150.87^{a}	5892.40^{a}	38.71^{b}
30	8.25^{b}	149.92^{a}	4983.70^{b}	41.24^{ab}

Difference in averages with a common letter is not significant (p>0.05)

belongs to tetrahydrofolate of 3 mg L^{-1} with an average of 16.32 fertile tillers. The interaction of methanol and tetrahydrofolate on the number of fertile tillers was not significant (Table 2).

Total number of tillers: Table of analysis of variance indicates that methanol in a statistical level of 1% has a significant effect on the total number of tillers in rice plants. With increasing the amount of methanol by 20% (by volume), there was an increase observed in the total number of tillers. But, a significant reduction was then observed in the total number of tillers. Maximum total number of tillers belonged to methanol of 20% with a average of 19.34 total tillers. Also, table of analysis of variance indicates that tetrahydrofolate in the statistical level of 1% was not significant (Table 1). But according to the table of comparison of means (Table 2), a significant increase was observed in the total number of tillers with an increase in the amount of tetrahydrofolate and the highest total number of tillers belongs to tetrahydrofolate of 3 mg L^{-1} with an average of 18.38 total tillers. The interaction of methanol and tetrahydrofolate on total number of tillers was not significant (Table 1).

Number of filled spikelets: Table of analysis of variance indicates that methanol in the statistical level of 1% has a significant effect on the number of filled spikelets in rice plants (Table 1). With increasing the amount of methanol by 20% (by volume), there was an increase observed in the number of filled spikelets but then there was no significant change. Highest yield belongs to 20% (by volume) methanol with an average of 92.25% of filled spikelets (Table 2). Table of analysis of variance indicates that tetrahydrofolate in the statistical level of 5% was not significant (Table 1). But according to the table of comparison of means (Table 2), a significant increase was observed in the number of filled spikelets with an increase in the amount of tetrahydrofolate and the highest number of filled spikelets belongs to tetrahydrofolate 3 mg L^{-1} with an average of 92.17% of filled spikelets. The interaction of methanol and tetrahydrofolate on the number of filled spikelets was not significant (Table 1).

Number of hollow spikelets: Table of analysis of variance indicates that methanol in the statistical level of 1% has a significant effect on the number of hollow spikelets in rice plants (Table 1). With increasing the amount of methanol by 20% (by volume), a decrease was observed in the number of hollow spikelets but then there was no significant change. Minimum number of hollow spikelets belonged to 20% (by volume) methanol with an average of 7.75% hollow spikelets (Table 2). Table of analysis of variance indicates that tetrahydrofolate in the statistical level of 5% was not significant on the number of hollow spikelets (Table 1). However, the table of comparison of means shows that (Table 2) with increasing the amount of tetrahydrofolate, a significant reduction was observed in the number of hollow spikelets; minimum number of hollow spikelets belonged to 3 mg L^{-1} of tetrahydrofolate with an average of 7.83% of hollowness. The interaction of methanol and tetrahydrofolate was not significant on the number of hollow spikelets (Table 1).

Paddy yield: According to the analysis of variance table, methanol and tetrahydrofolate have a significant effect on paddy yield in a statistical level of 1% (Table 1). With the increase of methanol to 20% by volume, a significant increase was observed in the amount of paddy yield but then it decreased. The highest paddy yield belongs to 20% (by volume) methanol with an average of 5892.40 kg ha^{-1} and the lowest belonged to methanol of 0% (by volume) or this very control with an average of 4175.50 kg ha^{-1} (Table 2). With increasing the amount of tetrahydrofolate, there was an increase observed in the amount of paddy yield. The maximum paddy yield belongs to tetrahydrofolate 3 and 2 mg L^{-1} with averages of 5439.40 and 5224.80 kg ha^{-1}, respectively and the minimum belongs to tetrahydrofolate of 0 and 1 mg L^{-1} with averages of 4848.50 and 4858 kg ha^{-1}, respectively (Table 2). Furthermore, the interaction of methanol and tetrahydrofolate was not significant on the paddy yield of rice (Table 1).

Thousand kernel weight: Table of analysis of variance indicates that methanol and tetrahydrofolate at 1% level and the interaction of methanol x tetrahydrofolate at 5% level have a significant effect on the thousand kernel weight of rice (Table 1). With increasing the amount of methanol by 20% (by volume), the thousand kernel weight showed a significant increase. The highest thousand kernel weight belonged to methanol of 20% (by volume) with an average of 24.79 g and minimum thousand kernel weight belonged to the control methanol (0% by volume) with an average of 22.89 g (Table 2). After tetrahydrofolate was used, the thousand kernel weight showed a significant increase compared to the control but no significant difference was observed between different values. The maximum thousand kernel weight belongs to tetrahydrofolate of 3 mg L^{-1} with an average of 22.65 g. Although, this value was at the same statistical level as other amounts of methanol, their differences with the control was statistically significant and minimum thousand kernel weight belonged to control tetrahydrofolate (or 0 mg L^{-1} with an average of 22.54 g) (Table 2). The highest thousand kernel weight was obtained in the interaction of methanol and tetrahydrofolate, 20% by volume use of methanol and 3 mg L^{-1} of tetrahydrofolate with an average of 26.9733 g and the lowest belonged to the control methanol and tetrahydrofolate of 1 mg L^{-1} with an average of 22.1967 g (Table 2).

Harvest index: According to the analysis of variance table, none of the treatments at the 5% level has significant effect on the harvest index of rice (Table 1). But according to the table of comparison of means (Table 2), a significant decrease was observed with increasing the amount of methanol which continued to 20% (by volume) methanol but then increased. The lowest of harvest index belonged to methanol of 20% by volume with an average of 38.71% and the highest harvest index belonged to 0% (by volume) methanol or this very control with an average of 42.96%. But with increasing the amount of tetrahydrofolate, a constant trend was observed at harvest index and all levels of tetrahydrofolate are at a statistical level. Furthermore, the interaction of methanol and tetrahydrofolate was not significant on harvest index (Table 1).

DISCUSSION

Overall, the results showed that foliar application of methanol significantly affected the traits of the number of fertile tillers, total number of tillers, number of filled spikelets, number of hollow spikelets, paddy yield and thousand kernel weights at 1% probability level. In general, the highest values of yield and yield components were observed in the methanol concentration of 20% by volume and the highest number of hollow spikelets was in the control. The effect of tetrahydrofolate on paddy yield and thousand kernel weights was significant which the highest averages belonged to 3 g L^{-1} of tetrahydrofolate. Foliar application of methanol did not affect other traits. It seems that part of it is for the reason that the traits studied are affected more by genetics than the test environment. Harvest index and plant height at harvest time are among these traits. According to Murali *et al.* (1994), the difference in the results of different studies may be due to differences in methanol concentrations and weather conditions in the test site. Thus, the appropriate concentration for foliar application of solution should be considered for the intended plant. In addition, the stage of plant growth in which foliar application of the solution takes place is of great importance. As Nonomura and Benson (1992) concluded in their experiments, methanol mostly improves plant growth in dry conditions and in fact is a factor in reducing stress. Perhaps this is the reason for the lack of effect of methanol on the part of characteristics of the environmental conditions of Gilan (in Northern Iran) which is somewhat far from dry and drought conditions. It can show well the importance of climatic conditions of the test site. Nonomura and Benson (1992) stated that the use of methanol increases plant growth as a carbon source and can increase the efficiency of plant photosynthesis. Although the nitrogen, phosphorus and potassium fertilizers are commonly used in agriculture and recently no attention has also paid to certan micro elements, no attention was actually given to increase in CO_2 available to the plant and it can be done only at the greenhouse level by CO_2 injection. Since, carbon is a big part of the dry weight, the use of methanol as a factor enhancing the carbon source and photosynthetic efficiency can have a dramatic effect on crop growth and yield. For this reason, the use of foliar application of methanol is of greatly importance. If researchers examine the practical aspect of the methanol use, perhaps its use may be questioned considering the price of methanol and its high consumption at the farm level.

CONCLUSION

This study conclude that considering the issues said, since methanol as described before can provide carbon element which is needed and mostly consumed by plant, methanol can be considered as a fertilizer and as in many countries where subsidy is given to the fertilizer, methanol can also be treated similarly. Of course, this will be achieved when the beneficial use of the methanol in various crops can be well proven the subject which requires further experiments in the future.

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