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Research Article

GROWTH, YIELD, AND YIELD COMPONENT CHANGES IN RESPONSE TO METHANOL APPLICATION ON RICE (*ORYZA SATIVA* CV. SHIROUDI)

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ABSTRACT
In order to study the effects of methanol foliar application on growth analysis on rice, an experiment was performed using completely randomized block design in 3 replications at Rice Research Station in Tonekabon, north of Iran, during 2012 growing season. Aqueous solutions were 0%, 6%, 12%, 18% and 24% (v/v) methanol. Methanol solutions were sprayed three times on foliage parts of rice with two week intervals (45 days after transplanting). The results from these experiments indicated that, in general, methanol did not affect on growth and yield in rice and therefore, seem to be ineffective as a growth enhancer and foliar sprays of aqueous methanol cannot be recommended for rice. Also, there was no significant difference among four levels of methanol application and control in the growth indices.

Keyword: *Rice, Methanol, Yield, Total Dry Matter, Leaf Area Index*

INTRODUCTION
Rice is a principal staple food for half of mankind and a major portion of the world's population obtains more than half of its daily calories from rice (Counce *et al.*, 2000). It is estimated that by the year 2025, farmers in the world should produce about 60% more rice than at present to meet the food demands of the expected world population at that time (Thakur *et al.*, 2011). Consequently, rice research and its application potentially affect the well being of a large part of the world's human population.
Methanol is a small organic compound that can naturally be emitted by plants. Considerable amounts of methanol are poured forth by forest areas. A likely source of methanol in leaves is pectin demethylation in the cell walls (Obendorf *et al.*, 1990) in a reaction catalyzed by pectin methylesterase (PME) and producing methanol as a byproduct (Jarvis, 1984). It is likely that pectin demethylation in plant cell walls is the major source of most of the methanol in the atmosphere (Fall and Benson, 1996). These results might be explained by higher rates of pectin demethylation being required during leaf expansion, a period of rapid cell wall synthesis, followed by declining demethylation and methanol production in older leaves. However, a direct correlation between pectin methylesterase activity and methanol release from leaves has not been demonstrated (Ramırez *et al.*, 2006). Gout *et al.*, (2000) demonstrated that methanol readily entered sycamore (*Acer pseudoplatanus* L.) cells to be slowly metabolized to serine, methionine, and phosphatidylcholine. They concluded that the assimilation of methanol occurs through the formation of glutamate and S-adenosyl-methionine, because feeding plant cells with serine, the direct precursor of pteroylpolyglutamate, can perfectly mimic methanol for folate-mediated single-carbon metabolism. On the other hand, the metabolism of methanol in plant cells revealed assimilation of label into a new cellular product that was identified as methyl-b-d-glucopyranoside.
Nonomura and Benson (1992) have reported that 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. Comparable enhancements of growth of wheat, radish, pea and tomato have been reported (Devlin *et al.*, 1994; Rowe *et al.*, 1994). However, some investigators have failed to see such growth enhancements (Mitchell *et al.*, 1994; Esensee *et al.*, 1995). Hemming *et al.*, (1995) suggest that the reproducibility of plant responses to methanol treatment could be a result of experimental variables such as exposure time, amount of

methanol absorbed, tissue morphology and accumulation of methanol in the root zone. Comparable enhancements of growth of wheat, radish, pea, and tomato have been reported (Devlin *et al.*, 1994; Rowe *et al.*, 1994). Some research has shown the 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); peppermint *(Mentha* x *piperita* L.) (Mitchell *et al.*, 1994); potato *(Solanum tuberosum* L.) (James *et al.*, 1994; Feibert *et al.*, 1995); muskmelon (*Cucumis melo* L.), tomato or watermelon (Hartz *et al.*, 1994); or sugar beet (*Beta vulgaris* L.) (Rykbost and Dovel, 1994).

Biological understanding and crop growth stages are important in matching management decisions and inputs with plant development. Crop growth staging are meant to be an aid in information transfer for crop management. The physiological and morphological characteristics of plants often change in response to the amount of access to the resources (Karimi and Azizi, 1994). Plant physiologists apply growth indices as useful tools for quantitative analysis of growth in different subjects such as plant breeding, plant ecology and physiology (Poorter and Garnier, 1996). Identification of growth physiological indices in analysis of factors affecting yield and its components has a great importance and its stability determines the dry matter production which is a criterion of yield components and in this regard leaf area index (LAI), total dry weight (TDW) and leaf dry weight (LDW) should be measured in periodic intervals during the growing season (Gardner *et al.*, 1985). The above indices plus crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), leaf area duration (LAD), leaf area rate (LAR), leaf weight rate (LWR) and specific leaf area (SLA) are indices which often use for evaluation of plant productivity capability and environmental efficiency (Anzoua *et al.*, 2010).

Numerous experiments have shown that by increasing the CO_2 content in air, the crops yielded better (Devlin *et al.*, 1994), flowering was accelerated (Fisher *et al.*, 1996) and plants accumulated more carbohydrates (Abdel-Latif *et al.*, 1996). According to Nonomura and Benson (1992) methanol treated plants showed increased turgor, higher growth rates and consequently gave higher yield than the control plants.

For increasing of total dry weight (TDW) in plants, we required to high leaf area by methanol because it demonstrated that leaf area index (LAI) has important effect on canopy photosynthesis (Nadali *et al.*, 2011). According to view of Makhdum *et al.*, (2002), methanol treated cotton showed increased leaf area index and turgidy. Also in another experiment on sunflower (*Helianthus annuus* L.) methanol increased stem length, leaf area index, stem dry weight, number of floret primordial and accelerated completion of floral development by 5 day (Hernandez *et al.*, 2000). Methanol has also been shown to retard senescence (Saltveit, 1989), which prolongs the duration of active photosynthesis in leaves, possibly improving CO_2 fixation and thereby increases biomass production. Results showed that foliar application of methanol increased total dry matter at 130 day after planting in comparison with control about 40 percent, whereas leaf area index was increased between levels of solutions in comparison with control (Nadali *et al.*, 2011). The rapid oxidation of methanol to CO_2 and its following assimilation by the Calvin–Benson cycle were shown to be closely related to its influence on increased biomass production (Theodoridou *et al.*, 2002). Methylotrophic bacteria may be associated with plant nitrogen metabolism through bacterial urease production (Holland and Polacco, 1994), therefore methanol application increase nitrogen assimilation and this causes increasing of CGR (Abanda-Nkpwatt *et al.*, 2006). Leaf area index can have importance in many areas of agronomy and crop production through its influence: light interception, crop growth, weed control, crop-weed competition, crop water use and soil erosion (Sonnentag *et al.*, 2007). As far as methanol act as a C source for C3 crops to enhance yield, the main objectives of our experiments (1) to evaluate the effect of foliar application of methanol on the yield some quantity properties (2) to determine the efficacious alcohol concentration for foliar application of methanol.

MATERIALS AND METHODS

Experimental Site and Design

This experiment was conducted at the experimental farm of Rice Research Station in Tonekabon (36°51' N, 50°46' E; -20 m above sea level), north of Iran, during 2012 growing season. The soil properties of the experimental site is a Silty clay loam, with 3.2% organic matter, 30% clay, 50% silt, 20% sand, 7.61 pH and 29.9 cation exchange capacity (CEC) (meg 100 g). To simplify the comparison of growing season weather, we consider monthly total precipitation and temperature from May through August in Rice Research Institute of Tonekabon (Table 1).

The layout of experiment was randomized complete-block design (RCBD) with 3 replicates and the following five treatments: 0%, 6%, 12%, 18% and 24% (v/v) methanol solutions. The solutions also included 0.1% Tween 80 (v/v) as a surfactant. Glycine apparently increased the metabolism rate of methanol. Glycine was added to the spray solution to prevent injury at high methanol concentrations or when light intensity was reduced (Nonomura and Benson, 1992). Addition of the glycine to the methanol solution up to 2 g lit^{-1} enabled use of higher methanol concentrations without visible injuries. Rice seeds were disinfected with thiophanate-methyl pesticide in 2 per 1000 dose and then were sown in the nursery. Seedlings were manually transplanted in main field at 2-3 leaf stage with interval planting of 25×25 cm^2. Recommended rate of nitrogen (100 kg ha^{-1}), phosphorous (100 kg ha^{-1}) and Potassium (150 kg ha^{-1}) were applied. One-third amount of nitrogen and whole phosphorous and Potassium were applied as a basal dose at transplanting stage. The Remaining two-thirds of nitrogen were utilized in two split doses, 30 days after transplanting (tiller stage) and panicle initiation stage. Weeds were controlled by hand weeded during growth season. The permanent flood water level was maintained at 10 cm during rice growing period. During the growing season, all weed species were hand weeded. Methanol solutions were sprayed three times on foliage parts of rice with two week intervals. The first foliar application was applied in 45 days after transplanting. These treatments were applied in July 30th June, 13th and 27th July, between 16:00 pm to 19:00 pm during bright sunny days with hot temperature. Methanol spray was carried out in a way that all above ground parts of rice plants were covered. Back engine sprayer with a capacity of 20 L was used for spray and sprinkler was held 40 cm above the plants.

Sampling

At maturity stage, plant height (from the soil surface to the top of the plant canopy) and tiller number were measured. Plants were harvested by hand-cutting at the soil surface and subsequently aboveground biomass of rice. Rice aboveground biomass from each plot was placed in separate paper bags, dried at 72°C for 48 h, and weighed. The agronomic traits included tiller number and 1000- grain weight was all 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.

In order to analyze and calculate the growth indices, stem dry weight, leaf dry weight and total dry matter of four randomly selected hills excluding boarder rows were sampled five times. Sampling of each plot was done with 15 days intervals. Leaf area (LA) was measured with leaf meter (GA-5 model produced by Japan OSK Company). To determine the LDW (Leaf Dry Weight) and TDW (Total Dry Weight), the samples were first air dried in oven at 75ºC for 48 hours. Then, dry weight of leaves and stem was measured by a 0.001g digital scale. Leaf area, dry weight of stem and leaves were recorded four times at 25, 40, 55 and 70 days after transplanting (DAT). Crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR) and leaf area duration were obtained by the computing (Table 2). Growth curves of the data mean of two experimental locations were drawn by the Excel software.

Statistical Analyses

Statistical analysis was conducted using SAS program (SAS Inst., 2001) to make sure that methanol application was significantly different between treatments. Means were compared using fisher's protected LSD test at α=0.05.

RESULTS AND DISCUSSION

Yield and its Components

We recorded yield changes in our experiment following methanol applications to plant foliage. When considering the high yields from the field trials (Table 3), it seems likely that rice suffer significantly from any methanol treatments. The highest grain yield was belonged to 12% (v/v) but there is no significant difference among 12% (v/v) methanol application with other treatments (Table 3). Grain yield and yield components were influenced when rice imposed either to methanol application. Most mean values for grain and biological yield and other plant height obtained at 12% (v/v) with 7440.8 and 12959 kg ha^{-}1and 103.33 cm, respectively (Table 3).

In our study, the quantities of methanol applied to the plants were, however, so small, when compared with carbon fixation of the plant canopy during growing season that it cannot be expected that changes in growth, resulting from alcohol application. Moreover, it is not clear to what extent of the methanol is absorbed and utilized in the plant. Furthermore, Cossins (1964) observed large variation in utilization of methanol when feeding it to different cell tissue of various crop species. It seems that due to the rainy weather on the rice growth period (Table 1) makes no influence of methanol on rice, because of the influence of methanol is more at water shortage and stress conditions.

Nonomura and Benson (1992) reported that foliar applications of aqueous methanol have been reported to increase yield, accelerate maturity, and reduce drought stress and irrigation requirements in crops grown in arid environments, under elevated temperatures, and in direct sunlight. Rajala *et al.,* (1998) in their research on some of C_3 crops including spring cereals (barley, wheat, and oat), pea and summer turnip rape observed that methanol did not affect growth and yield in any of the crop species examined and therefore, seem to be ineffective as a growth enhancer. They stated that the effect less of methanol is for this reason 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. This is especially important at high methanol concentrations. Accordingly, it is likely that plants were able to convert the methanol into other compounds in the field experiments, even though the applications were not carried out in high light intensity. It is also likely that the methanol penetration into the plant is greater when application is conducted 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. According to Nonomura (personal communication), one application was sufficient to improve plant productivity, but multiple applications were required to achieve maximum benefits. Following Nonomura and Benson's treatment protocol, we found that foliar methanol application totally is not effective in enhancing any measure of rice plant performance under irrigated field conditions.

Growth Indices

One of the most important growth indicators which have been being applied as a measure of total photosynthesis and respiratory tissues is (TDW). TDW is increased over time, so that, at early growth stages, it increases with fewer gradients and in later stages, slope increasing is greater until TDW reaches to its maximum (grain filling) and at the end of the growing season, (TDW) is reduced. The trend of total dry weight (TDW) under methanol application (Figure 1) during the growth season implies that, there was no significant difference among four levels of methanol application and control in the TDW accumulation. Likely, in this period, leaves extension and TDW follow carbon increment and the role of urea is negligible (Fageria and Baligar, 2001). Maximum TDW accumulation in each treatment was occurred at grain filling (100 days after transplanting). Ramirez *et al.,* (2006) reported that foliar application of methanol solutions to *Arabidopsis* plants resulted in significant increases in fresh and dry weight, indicating that this widely used model plant can be useful in the investigation of the molecular mechanisms underlying the response to methanol.

One of the important growth indicators which have been being used as a photosynthetic system measurement is leaf area index (LAI). LAI is related to the biologic and economic yields and increase in LAI causes higher yield (Singh *et al.,* 2009). LAI at early growth stages increased slightly over time and in the later stages, it was raised further. Maximum LAI of rice was observed at flowering stage and then it was decreased due to the wilting and falling of lower leaves. LAI trend in different methanol application levels is illustrated in Figure 2. LAI maximum for all the treatments was obtained at flowering stage (77 days after transplanting) and then it was reduced. Also, M4 treatment (18% (v/v)) had lesser LAI compared with other treatments. It seems that this treatment (M4) has toxic effect on leaves and decrease LAI. Methanol treated leaves increased their fresh and dry weight considerably (Rowe *et al.*, 1994).

Regarding Figure 3, it can be concluded that the computed value of leaf dry weight (LDW) at lower levels of methanol (M2 and M3) was less than higher levels. Application of different methanol levels impact on photosynthesis exchange and plant growth and higher levels of methanol stimulate vegetative growth, reduce storage carbon hydrate and increase allocation of dry matter to the leaves. Maximum LDW in all the treatments was occurred at 100 days after transplanting.

Crop growth rate (CGR) is the most important factor in analysis in plant communities which shows the amount of dry matter accumulation per unit area at a specific time. In the early growth stages, CGR is positive but and during the growth season, CGR is increased until at flowering stage it reaches to the maximum (Table 4). After flowering stage, due to the senescence and abscission of active photosynthesis tissues, CGR is reduced. For this reason, reduction of CGR can be observed after flowering stage in CGR trend (Table 4). When CGR is equal to zero, there is a point in the curve which TDW is fixed and does not change, When CGR is negative, and TDW is reduced. CGR has a direct relationship with photosynthetic and also LAI has a high impact on CGR before canopy closure (Azarpour *et al.*, 2014). The trend of CGR under methanol application (Figure 4) illustrated that there was no significant difference between treatments.

Maximum RGR in the treatments was observed at early growth stage and over the time, it is decreased linearly. There was no significant difference among four levels of methanol application and control in the RGR.

Table 1: Monthly precipitation and temperature from May to September for growing season (2012) at Rice Research Institute of Tonekabon

Month	**Precipitation (mm)**	**Temperature (^{o}C)**		
		Maximum	Minimum	Average
May	28.3	25.29	18.45	21.87
June	117.9	28.38	21.23	24.81
July	125	29.12	22.81	25.96
August	86.5	30.9	24.22	27.56

Table 2: Analysis of Physiological Index[a]

Parameter	**Equation**	**Unit**
TDW	$Exp(a_1+b_1t+c_1t^{0.5}+d1t^2+e1t^3)$	g/m^2
LAI	$Exp(a_2+b_2t+c_2t^{0.5}+d_2t^2+e_2t^3)$	-
LDW	$Exp(a_3+b_3t+c_3t^{0.5}+d_3t^2+e_3t^3)$	g/m^2
RGR	$(b_1+0.5c_1t^{-0.5}+2d_1t+3e_1t^2)$	g/g.day
CGR	$(b_1+0.5c_1t^{0.5}+2d_1t+3e_1t^2)\times(TDW)$	g/m^2.day
NAR	CGR/ LAI	g/m^2.day

[a]*Azarpour et al., 2014*

Table 3: Mean comparison of determined characteristics in rice as affected by methanol treatments

% alcohol (v/v)	**Grain yield** (Kg ha^{-1})	**Biological yield** (Kg ha^{-1})	**Plant height (cm)**	**Tiller number**
0	6626.5a	11223a	102.27a	21.87a
6	7035.5a	11354a	98.93a	21.13ab
12	7440.8a	12959a	103.33a	21.47ab
18	7246.1a	12070a	101.43a	21.18ab
24	6330.9a	10655a	97.83a	17.87b
$LSD_{0.05}$	1219.6	2470.4	5.39	3.55

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

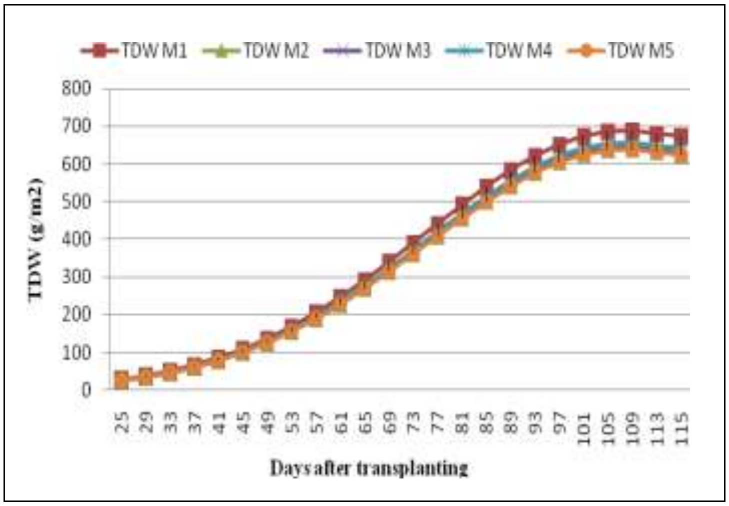

Figure 1: Seasonal change in the TDW at different methanol levels

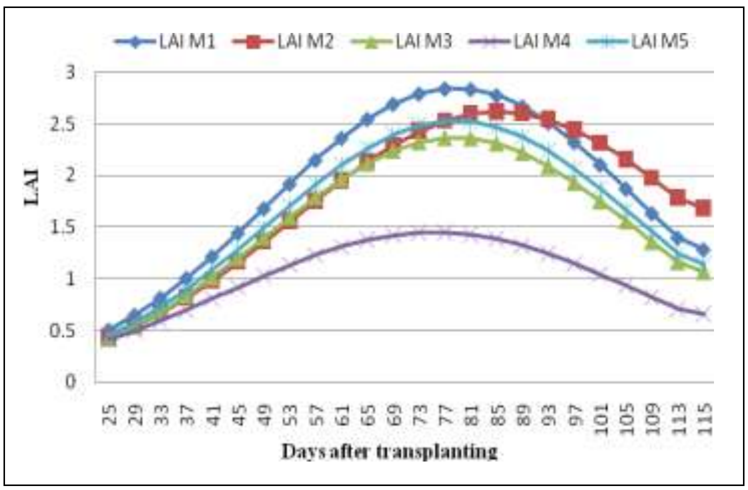

Figure 2: Seasonal change in the LAI at different methanol levels

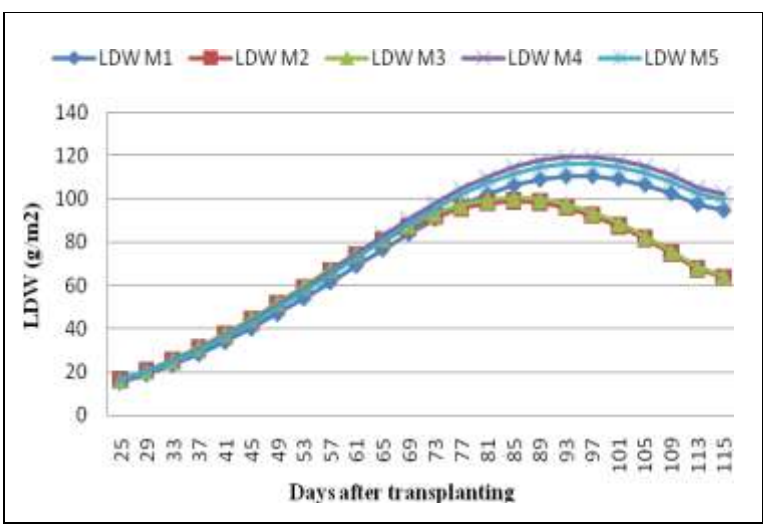

Figure 3: Seasonal change in the LDW at different methanol levels

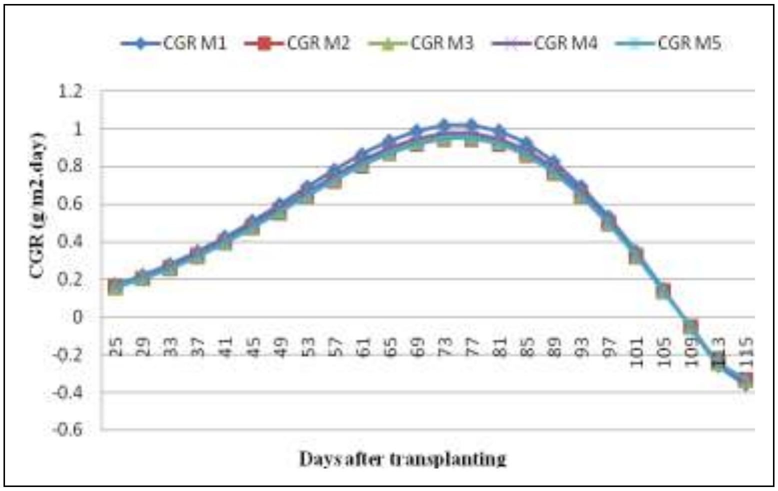

Figure 4: Seasonal change in the CGR at different methanol levels

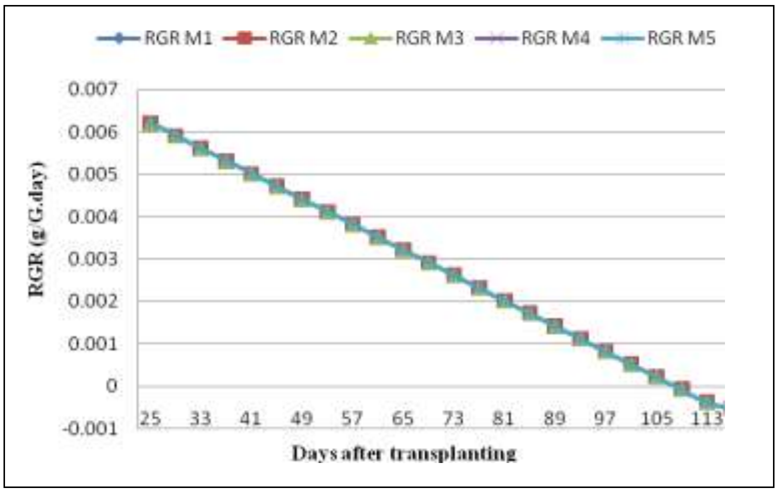

Figure 5: Seasonal change in the RGR at different methanol levels

Conclusion

In conclusion, on the basis of this study, methanol applications do not seem to have any growth promoting effect on the rice in Iran growing conditions. It seems that due to the rainy weather on the rice growth period makes no influence of methanol on rice

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