BIOCHEMICAL ATTRIBUTES OF MUTANT RICE UNDER DIFFERENT SALINE LEVELS

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Accepted for publication: 07 Jun, 2007

ABSTRACT

Islam, M.Z., Baset Mia, M.A., Akter, A. and Rahman, M.H. 2007. ***Biochemical Attributes of Mutant Rice under Different Saline Levels.*** **Int. J. Sustain. Crop Prod. 2(3):17-21**

A pot experiment was conducted at the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh to find out the biochemical attributes of mutant rice under varied saline levels during T-aman season from July to November 2003. Three rice genotypes viz. Q-31, Y-1281, and MR-219 were used as tests materials. The experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications. Six salinity levels namely 15 dSm^{-1}, 12 dSm^{-1}, 9 dSm^{-1}, 6 dSm^{-1}, 3 dSm^{-1} with a control were imposed in this experiment. Results of the experiments clearly showed that biochemical characters such as total chlorophyll content, nitrate reductase activity, K^+ and Ca^{++} etc. content of straw were reduced with increase levels of salinity compared to control. On the other hand, Na^+ were increased with increasing salinity levels. Among the genotypes, MR-219 showed best performance in respect of salt tolerant up to 6 dSm^{-1} and showed Y-1281 intermediate status. The genotypes Q-31 and Y-1281 showed its susceptibility to salinity stress.

Key words: salinity stress, biochemical attributes and mutant rice

INTRODUCTION

Rice provides food and livelihood security Bangladesh, where it is the principal food of the people. But the yield of rice in Bangladesh is much lower than that of rice in other rice growing countries of the world. Among the various factors limiting rice yield, salinity is one of the oldest and most serious environmental problems in the world (Mcwilliam, 1986). In Bangladesh, over thirty percent of the net cultivable area is in the coastal area. Out of 2.85 million hectare of the coastal and off-shore areas, about 0.833 million hectares are arable lands, which constitute about 52.8 percent of the net cultivable area in 64 thanas of 13 districts (Karim *et al.*, 1990). This area is largely affected by varying degrees of salinity and decreased the agricultural productivity seriously. As reclamation of saline soils is laborious and almost impossible, development or selection of salt tolerant crop species is one of the possible means for extension of crop area. Generally, salinity affects the growth of rice plant at all stages of its life cycle. But it is more pronounced on reproductive stage than on vegetative stage consequently decreased the grain yield (Afridi *et al.*, 1988). Rice is moderately susceptible to salinity, since most rice plants are severely injured at an EC 8-10 dSm^{-1}. Study on the response of rice to salinity stress may be helpful in breeding salt tolerant cultivars by identifying physiological features potential salinity tolerance such as active osmotic adjustment in cells sap, accumulation of toxic Na^+ and Cl^- ions in the older parts of the plant, higher photosynthetic efficiency of the young leaves, escaping ability to uptake Na^+ and Cl^- etc. (Sultana *et al.*, 2002) showed that the Na^+ concentrations of leaf is increased, while Ca^{++} and K^+ concentrations of leaf is decreased with increasing of salinity level. It is generally recognized that K^+ uptake by the plant and deposition in both growing and non-growing tissues is reduced by salinization. Many studies conducted with reasonable Na:Ca ratios in the solutions have suggested that Ca^{+2} uptake, translocation and distribution may be critically affected by salinization. Salt tolerant cultivars had lower Na and higher K content (Won *et al.*, 1992). The effect of soil salinity varies from variety to variety. Yield losses due to salinity are amounted to 30-50 percent. Our farmers normally grow local varieties due to unavailability of salt tolerant high yielding varieties (HYV). Therefore, to keep pace with the population growth and food productions, the yield per unit area needs to be increased for minimizing the yield gap. Grain yield decreased with increasing salinity levels. Appropriate salt tolerant high yielding varieties that can fit into the rice-growing ecosystem in the coastal areas of Bangladesh will boost up the country's rice production. Therefore, the present research work was undertaken to estimate the biochemical attributes (total chlorophyll content, nitrate reductase activity, Na^+, K^+ and Ca^+) of mutant rice under different saline levels.

MATERIALS AND METHODS

A pot culture experiment was carried out in Bangladesh Institute of Nuclear Agriculture (BINA) of which each pot contained 8.0 kg of dried soil, belongs to the Sonatola series of Grey Flood Plain under the Old Brahmaputra Agro-Ecological Zone (UNDP and FAO, 1988). The p^H value, cation exchange capacity (CEC) and electrical conductivity (EC) of the soil were 6.44, 6.78meq/100 g soil and 0.6 dSm^{-1} respectively. Three rice genotypes Q-31, Y-1281 and MR-219 were used in the present in this experiment. Earthen pots of 24.5 cm top diameter, 14 cm bottom and 30 cm depth were used, which pot was filled with 8 kg sun-dried soil. A polythene lining was

provided inside the pots. N, P, K and S applied at rate of 60-8-30-4 kg/ha in the form of Urea, TSP, MP and Gypsum according to Fertilizer Recommendation Guide (BARC, 1997). The following salinity treatments namely control, 3, 6, 9, 12 and 15 dSm^{-1} were imposed in the experiment. Salinity treatments were given at 33 days after transplanting (DAT). EC was measured by the electrical conductivity bridge at room temperature and calculated to the standard temperature of 25^0 C. The p^H and CEC soil were analyses following the methods of (Black, 1965). After collecting the leaf of plants, 0.05 g leaf was weighed and chlorophyll content was determined using the method of Coombs *et al.* (1985).

Calculation

The formula for computing total chlorophyll, chlorophyll 'a' and 'b' were Total chlorophyll = $(7.93 A_{663}+19.53A_{645}) \times DF$

Chlorophyll 'a' = $(13.19 A_{663}-2.57A_{645}) \times DF$

Chlorophyll 'b' = $(22.10 A_{645}-5.26A_{663}) \times DF$

Where

A_{663} = Absorbance at 663 nm wave length

A_{645} = Absorbance at 645 nm wave length

7.93, 19.53, 13.19, 2.57, 22.10 and 5.26 are absorption co-efficient

DF = Dilution factor = $\frac{10}{1000 \times 0.05} = 0.2$

Nitrate reductase activity was determined from the leaf samples following the method of Steward and Orebamjo (1979).

Calculation

NRA = CF (Correction factor) × DF (Dilution factor) × OD (Optical density) × Time

The Na^+, K^+ and Ca^{++} contents of straw of the harvested plants were determined by atomic absorption spectrophotometers (model- PERKIN- ELMER, 2380) directly and express in percentages (Jackson, 1967).

The collected data were statistically analyzed by MSTAT-C package program develop by (Russel, 1986).

RESULTS AND DISCUSSION

Total chlorophyll content

The effect of salinity on chlorophyll formation was significant (Table 1a). The chlorophyll content was found to be decreased about 2.83, 4.95, 21.7 and 35.84% lower values when compared with control at 3, 6, 9, 12 dSm^{-1} saline conditions. Decreased in chlorophyll content due to salinity was reported by (Panda and Khan, 2003).

The genotypes showed significant variation with respect to total chlorophyll content (Table 1b). The highest chlorophyll content was found in genotypes Q-31 (3.16 $mg.g^{-1}fw$) and Y-1281 (3.16 $mg.g^{-1}fw$) while lowest in MR-219 (2.89 $mg.g^{-1}fw$).

The interaction effect of genotypes and salinity levels in relation to total chlorophyll was found significant (Table 1c). The results showed that chlorophyll content decreased ranged from 1.4% at 3 dSm^{-1}, 3.08% at 6 dSm^{-1}, 23.18% at 9 dSm^{-1}, 27% at 12 dSm^{-1} as compared to control Q-31. Total chlorophyll contents were decreased in four genotypes of rice at 16.0 dSm^{-1} salt stress reported by (Mandal and Singh, 2001).

Nitrate reductase (NR) activity

The effect of different levels of salinity with NR activity was statistically significant (Table 1a). The nitrate reductase activity was highest in control plants. At 65 DAT, the nitrate reductase activity was found to be decreased 35.0% at 3 dSm^{-1}, 39.0% at 6 dSm^{-1}, 43.5% at 9 dSm^{-1} and 50.5 at 12 dSm^{-1} as compared to control. (Gill and Singh, 1992) found that significantly decreased nitrate reductase (NR) activity in rice under saline condition.

Nitrate reductase activity differed significantly among the genotypes (Table1b). The highest nitrate reductase activity was found in genotypes Y-1281 (1.20 µ mol $NO_2^- g^{-1}fwh^{-1}$) and lowest in MR-219 (0.95µ mol $NO_2^- g^{-1}fwh^{-1}$).

The interaction effect of genotypes and salinity in relation to nitrate reductase activity was found to be significantly different (Table1c). Interaction effects between genotypes and salinity showed that salinity decreased nitrate reductase activity ranged from 56.25% at 3 dSm^{-1}, 59.92 at 6 dSm^{-1}, 62.50% at 9 dSm^{-1}, 62.87% at 12 dSm^{-1} as compared to control Q-31. The highest NR activity was recorded in genotypes MR-219 and the lowest in genotypes Y-1281. Increasing salinity levels reduced nitrate reductase activity in rice plants (Pandey and Srivastava, 1989).

Table1a. Effect of different salinity levels on biochemical characteristics of rice plant

Salinity levels ($dS\ m^{-1}$)	Total chlorophyll content ($mg.g^{-1}fw$)	Nitrate reductase activity ($\mu\ mol\ NO_2^-g^{-1}fwh^{-1}$)
	60 DAT	65 DAT
Control(0)	4.24	2.00
3	4.12	1.30
6	4.03	1.22
9	3.32	1.13
12	2.72	0.99
LSD (0.05)	0.32	0.97

Table 1b. Varietal effect on biochemical characteristics of rice plant

Genotypes	Total chlorophyll content ($mg.g^{-1}fw$)	Nitrate reductase activity ($\mu\ mol\ NO_2^-g^{-1}fwh^{-1}$)
	60 DAT	65 DAT
Q-31	3.16	1.17
Y-1281	3.16	1.20
MR-219	2.89	0.95
LSD (0.05)	0.22	0.07

Table 1c. Interaction between genotypes and salinity levels on biochemical characteristics of rice plant

Interaction (genotypes × salinity levels)	Total chlorophyll content ($mg.g^{-1}fw$)	Nitrate reductase activity ($\mu\ mol\ NO_2^-g^{-1}fwh^{-1}$)
	60 DAT	65 DAT
V_1S_1	4.27 a	2.72 a
V_1S_2	4.21 ab	1.19 ef
V_1S_3	4.14 ab	1.09 fg
V_1S_4	3.28 cd	1.02 fg
V_1S_5	3.08 d	1.01 fg
V_1S_6	0.00 f	0.00 h
V_2S_1	4.35 a	2.02 b
V_2S_2	4.34 a	1.46 c
V_2S_3	4.19 ab	1.43 cd
V_2S_4	3.06 d	1.29 cde
V_2S_5	3.03 d	1.04 fg
V_2S_6	0.00 f	0.00 h
V_3S_1	4.25 a	1.28 cde
V_3S_2	3.81 abc	1.26 de
V_3S_3	3.63 bcd	1.15 ef
V_3S_4	3.61 bcd	1.08 fg
V_3S_5	2.06 e	0.93 g
V_3S_6	0.00 f	0.00 h

V_1= Q-31, V_2= Y-1281, V_3= MR-219 S_1 = 0 dSm^{-1}, S_2 = 3 dSm^{-1}, S_3 = 6 dSm^{-1}, S_4 = 9 dSm^{-1}, S_5 = 12 dSm^{-1} & S_6 = 15 dSm^{-1}
In a column figures having similar letter (s) do not differ significantly as per DMRT

Na^+, K^+ and Ca^{2+} content in straw (%)

The effect of salinity on Na^+, K^+ and Ca^{2+} content in straw was significant (Table 2a). Concentration of Na^+ in straw was higher at 6 dSm^{-1}soil salinity levels and lower at control condition. But K^+ and Ca^{2+} content in straw were higher at control condition and lowest at 6 dSm^{-1} soil salinity levels. These results indicated that content of Na^+ increased with increases of salinity levels but content of K^+ and Ca^{2+} decreased with increased of salinity levels (Sultana *et al.*, 2002).

Among the genotypes, Na^+, K^+ and Ca^{2+} content in straw were significant (Table 2b). The Na^+ and K^+ concentration in straw was higher in Q-31 and lower in MR-219 while Ca^{2+} content in straw was higher in MR-219 and lower in Q-31.

The interaction effect of salinity levels and genotypes in relation to Na^+, K^+ and Ca^{2+} content were found to be significant (Table 2c). Na^+ concentration in straw was gradually increased in all varieties up to 6 dSm^{-1} soil salinity levels. Na^+ concentration was higher in Q-31 at 6 dSm^{-1} soil salinity and lower in MR-219 at control condition. K^+ concentration was higher in Q-31 at control condition, and lower in Y-1281 at 6 dSm^{-1} soil salinity levels. Ca^{2+} concentration was higher in MR-219 at control condition and lower in Q-31 at 6 dSm^{-1} level of soil salinity. The results showed that sodium chloride treatments decreased K^+ content in all varieties. The excess Na^+ restricted the uptake of K^+ by the rice plants. Ca^{2+} content was little affected by the salinity levels. (Islam *et al*., 1995) and (Dionisio and Tobita, 2000) reported that salinity stress increased Na^+ and decreased K^+ in roots, stems and leaves, which partially supports this result.

Table 2a. Effect of elevated salinity levels on Na^+, K^+ and Ca^{++} content in straw of rice genotypes

Salinity levels (dS m^{-1})	Na%	K%	Ca%
Control (0)	0.159	1.58	0.291
3	0.609	1.13	0.245
6	0.978	0.95	0.208
$LSD_{0.05}$	0.03	0.13	0.03

Table 2b. Varietal effect of Na^+, K^+ and Ca^{++} content in straw of rice genotypes grown under different salinity levels

Genotypes	Na%	K%	Ca%
Q-31	0.584 a	1.337 a	0.203 c
Y-1281	0.569 a	1.243 a	0.252 b
MR-219	0.493 b	1.075 b	0.289 a
$LSD_{0.05}$	0.03	0.13	0.03

Table 2c. Interaction between genotypes and salinity levels on Na^+, K^+ and Ca^{++} content in straw of rice genotypes

Interaction (genotypes × salinity levels)	Na%	K%	Ca%
V_1S_1	0.210 d	2.016 a	0.256 abc
V_1S_2	0.543 b	1.046 cd	0.229 c
V_1S_3	0.999 a	0.950 d	0.115 d
V_1S_4	0	0	0
V_1S_5	0	0	0
V_1S_6	0	0	0
V_2S_1	0.146 e	1.550 b	0.293 ab
V_2S_2	0.582 b	1.240 c	0.253 bc
V_2S_3	0.981 a	0.940 d	0.210 c
V_2S_4	0	0	0
V_2S_5	0	0	0
V_2S_6	0	0	0
V_3S_1	0.121 e	1.158 cd	0.317 a
V_3S_2	0.403 c	1.110cd	0.253 bc
V_3S_3	0.955 a	0.957 d	0.299 ab
V_3S_4	0	0	0
V_3S_5	0	0	0
V_3S_6	0	0	0

V_1= Q-31, V_2= Y-1281, V_3= MR-219, S_1 = 0 dSm^{-1}, S_2 = 3 dSm^{-1}, S_3 = 6 dSm^{-1}, S_4 = 9 dSm^{-1}, S_5 = 12 dSm^{-1} & S_6 = 15 dSm^{-1}
In a column figures having similar letter (s) do not differ significantly as per DMRT

At 6 dSm^{-1} no difference in Na^+ uptake were observed between genotypes. However, at 3 dSm^{-1} significantly higher uptake of Na^+ was recorded in two relatively sensitive genotypes Y-1281 and Q-31. The uptake of K^+ did not vary between genotypes with 6 dSm^{-1} salinity levels. However, Q-31 had higher uptake of K^+ followed by Y-1281 and MR-219 at control condition. At 3 dSm^{-1}, there was no difference in K^+ uptake between genotypes. However, at 6 dSm^{-1} Ca^{2+} uptake was higher in MR-219 followed by Q-31 and Y-1281.

Results of the experiments clearly indicated that biochemical characters namely chlorophyll content, nitrate reductase enzyme activity, K^+ and Ca^{2+} content decreased with the increase of salinity and Na^+ increased with the increment of salinity.

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