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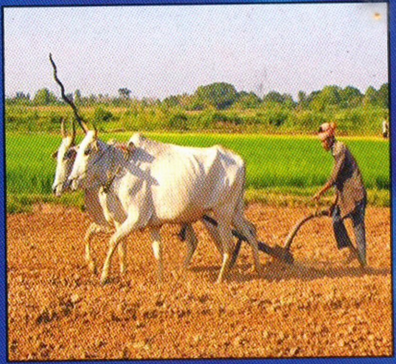

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ANALYSIS OF ANTHER DERIVED RICE (*ORYZA SATIVA* L.) GENOTYPES FOR THEIR TOLERANCE TO SALINITY

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Abstract

Salinity tolerance of 15 different rice genotypes, which had previously been produced at Dhaka University (DU) from anther culture (AC) of F_1 hybrids to combine the salt tolerant trait of traditional salt tolerant rice variety, Binnatoa and high yielding trait of HYV, BR7, was investigated. The salinity screening was done firstly in saline soil and then in hydroponics. In soil the screening was done in two sets, having an electrical conductivity (EC), 10 dSm^{-1}. Each set had four replications. Each replication had four seedlings for each variety. Data was collected after 16 days of salt treatment in soil. Salinity survival score, change in shoot length & shoot dry weight, root length & root dry weight and leaf area were measured. To measure chlorophyll content, chlorophyll stability index (CSI), sodium (Na) & potassium (K) absorption, 3 AC lines among the 15 which performed very well in soil grown in nutrient solution having an EC of 10 dSm^{-1}. The data was subjected to statistical analysis by Duncan's Multiple Range Test (DMRT) using SPSS (Statistical Program for Social Sciences) program. It was observed that 3 AC lines (T7B-2, T7B-3 and T7B-5) showed similarity in all aspects with the tolerant parent Binnatoa in salinity stressed condition. The other 12 anther derived lines differed significantly and therefore were salt sensitive.

Keywords: Anther culture, salinity tolerance, screening and rice

Introduction

Salinity is a serious problem in our country as in other countries of Southeast Asia. The salinity source of Bangladesh is the sea in the South. Sea water surrounding the coastal zones of Bangladesh intrudes the land (about 2.8 million hectare) during cyclone and typhoon. In addition to this, due to heavy withdrawal of surface and ground water for irrigation purpose, the salinity in Khulna, Satkhira, Barisal, Patuakhali and Noakhali (coastal districts of Bangladesh) has become a major problem (Islam *et al* 1993, Panaullah, 1993).

The Bangladesh Rice Research Institute (BRRI) has so far released only 3 moderately salt tolerant modern varieties, BRRI dhan40, BRRI dhan41 for T. Amon and BRRI dhan47 for Boro season (BRRI, 2008). So, the varieties with salt tolerance suitable for the Aus season (March-June) are needed to develop.

Plant cell culture like anther culture strategy may involve crossing high yielding rice cultivars with salt tolerant land races like Binnatoa and Rajashail and culturing anthers of the F_1 population. Some of the double haploid (DH) anther culture regenerants will have a fixation of both the high yielding and salt tolerant characteristics. This approach has been followed by some workers and some regenerant lines are available which have to be screened for salt tolerance and other agronomic characteristics (Seraj *et al.* 1997). Fifteen different rice genotypes were produced at Dhaka University from anther culture of F_1 hybrids among traditional moderately salt tolerant rice variety: Binnatoa, Rajashail, HYV: BR7 and Japonica CV. Taipei-309. This was done in order to incorporate the salinity tolerance of Binnatoa into high yielding but salt sensitive variety, BR7 (Faruque *et al.* 1998). So, the objective of the present study was to screen the above anther culture derived lines to measure survival and physical parameters of salinity tolerance.

Materials and Methods

Anther culture derived lines

In the present study, 15 anther derived lines (Faruque *et al.*, 1998) along with parent and check varieties were used as an experimental material (Table: 1). These used test-lines were obtained from anther of the hybrid of Indica rice varieties, Binnatoa, Rajashail, BR7 with Japonica CV. Taipei-309. The crosses followed by the anther culture of the F_1S, were designed with the objective of combining the salt tolerant trait of Binnatoa and the high yield of BR7 in single line (Faruque *et al.*, 1998). Parent (Binnatoa, Rajashail, BR7) and check (Pokkali, BRRI dhan29) varieties were collected from Bangladesh Rice Research Institute (BRRI), Gazipur and test lines from the Department of Biochemistry and Molecular Biology, Dhaka University.

Screening for salinity tolerance in soil

For the screening of salinity tolerance in soil, buckets were prepared for transplantation of seedlings. Six-liter plastic buckets contained puddled soil fertilized with urea, MoP (Murate of potass), TSP (Triple super phosphate) and Gypsum. Four kg soil and 1 gram of each fertilizer were used for each bucket. Eight buckets were used for each line. The soil of 4 buckets was puddled with normal water and 4 with saline water. So, for each line 4 buckets were used as control and 4 as stress. Soil in stressed buckets was salinized with 2 liters of sodium chloride (NaCl) solution having an EC of 10 dSm^{-1}. This created soil salinity of 8-10 dSm^{-1}. Salt water was added to the soil before transplanting of the seedlings. The level of the saline water was maintained 2 cm above the soil surface by adding fresh water daily. Thirty-day-old seedlings of 19 lines (15 AC lines, 2 parent varieties and 2 check varieties) were transplanted in plastic buckets that were prepared before (Table 1). Effects of salinity on growth and visual symptoms were scored (Table 2) as described by Ponnamperuma (1977). The visual symptoms of salt injury or toxicity were recorded. This scoring discriminates the susceptible from the tolerant and moderately tolerant genotypes. Scores were recorded at the end of 16-day salt treatment. In this screening, different parameters such as % of survival of plants, % of change of shoot and root length, shoot and root dry weight, leaf area were determined.

Table 1. Anther culture derived lines, parents and check varieties

A. Anther culture derived lines	
Cross combinations	DU name
Taipei-309 / Binnatoa	TB-1
Taipei-309 / Binnatoa	TB-1A
Taipei-309 / Binnatoa	TB-1B
Taipei-309 / Binnatoa	TB-1C
Taipei-309 / Binnatoa	TB-1D
Taipei-309 / Binnatoa	TB-2
Taipei-309 / Binnatoa	TB-4
Taipei-309 / Binnatoa	TB-4A
Taipei-309 / Binnatoa	TB-4B
Taipei-309 / BR7 / Binnatoa	T7B-1
Taipei-309 / BR7 / Binnatoa	T7B-2
Taipei-309 / BR7 / Binnatoa	T7B-3
Taipei-309 / BR7 / Binnatoa	T7B-4
Taipei-309 / BR7 / Binnatoa	T7B-5
Taipei-309 / Rajashail	TRAJ
B. Parent varieties	
Binnatoa	
BR7	
C. Check varieties	
Pokkali	
BRRI dhan29	

Screening for salinity tolerance in hydroponics

The germinated seeds of 7 varieties (3 AC lines, parents and checks) in three replicates were sown on netted Styrofoam sheet floating on nutrient culture solution (Yoshida *et al.*, 1976) for one day. Then the Styrofoam containing seeds were transferred in plastic tray containing 4 liters nutrient culture solution (Yoshida *et al.*, 1976). The seedlings were allowed to grow here without salt for 16 days. The solution of the trays was changed at every 3 days and pH 5.0 were checked every 2-day during this period and adjusted. After 16 days, the solution was replaced with salinized nutrient solution having an EC of 10 dSm^{-1}. Due to evaporation and transpiration there was loss of solution volume in the trays and the optimum level was maintained with tap water in every 2- day. The seedlings were grown here in salinized nutrient solution for 16 days. Additionally, a complete set under normal condition (without salt) was used as control. In this screening % of change of chlorophyll content, chlorophyll stability index (CSI) (Koleyoreas, 1958), sodium (Na) and potassium (K) content were measured. Chlorophyll content was measured following a procedure described in the Laboratory Manual for Physiological Studies of Rice (Yoshida *et al.*, 1976). For the measurement of Na and K concentration in shoot at 0 dSm^{-1} and 10 dSm^{-1}, plants were washed in floating tap water for 30 sec. and 10 oven-dried plants from each replicate were pooled, ground and analyzed by flame photometer (Jenway, UK) following water extraction method.

Statistical analysis

All the data were analyzed statistically and means were separated using Duncan's Multiple Range Test (DMRT) to determine significant differences of means at 5% level.

Results and discussion

In the present investigation, due to less leaf area damage and high salinity survival, the two anther derived lines, T7B-2 & T7B-3 scored 3 and they were remarked as tolerant. T7B-5 scored 4 and was remarked as moderately tolerant. Here the tolerant check, Pokkali scored 3; tolerant parent, Binnatoa scored 4 (Table 2 and 3).

Table 2. Standard evaluation score (SES) of visual symptoms at tillering stage in soil (Adapted from Ponnamperuma, 1977)

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly resistant
2,3	Nearby normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
4,5	Growth severely retarded, most leaves rolled, only a few leaves are elongating	Moderately tolerant
6,7	Complete cessation of growth, most leaves dry, some plants dying	Susceptible
8,9	Almost all plants dead or dying	Highly susceptible

In this study, salt stress caused a significant decrease of shoot length and shoot dry weight of all the cultivars (Table 3). T7B-2, 3 & 5 showed less than 29% reduction in shoot length and less than 37% shoot dry weight. The other 12 AC lines showed significant decrease in the shoot length and shoot dry weight. In case of Pokkali and BRRI dhan29, shoot length reductions were 19 & 55% respectively and shoot dry weights were 31 & 65% respectively. Ashraf and O'leary (1997), Brugnoli and Lauteri, (1991) reported the reduction of plant growth due to salt stress in several plant species.

Root length is one of the most important characters for salt stress because roots are in contact with soil and absorb water from soil. For this reason, root length provides an important clue to the response of plants to salt stress (Kaya and Upek, 2003). In the present investigation, root length increase was detected in the all the test lines as well as parent and check varieties over the control set. We observed the highest root length increase in the case of tolerant parent Binnatoa. Similar result of increase in root length due to salinity stress was reported by Pattanagul and Thitisaksakul (2008). They reported that salt sensitive and moderately salt tolerant cultivars showed an increase in root length in response to salinity stress. This probably reflects the maintenance or even induction of the root elongation at low water potential, which can be considered as an adaptive response to drought and salinity (Perez-Alfocea *et al.*, 1996). In the present

Table 3. Different parameters of salinity tolerance of anther derived lines parent & check varieties measured during screening

AC lines/ parents/ checks	SES, Salinity Tolerance		Shoot length reduction (% over control)	Shoot dry wt. reduction (% over control)	Root length increase (% over control)	Root dry wt. reduction (% over control)	Leaf area reduction (% over control)
	SES	Salinity tolerance					
TB-1	9	HS	52.32 hij	65.93 k	9.78 c	20.08 de	-
TB-1A	9	HS	51.36 fg	65.11 jk	9.49 c	20.47 de	-
TB-1B	9	HS	53.17 j	62.75 h	9.63 c	19.61 de	-
TB-1C	9	HS	51.78 fgh	63.46 hi	9.60 c	20.51 de	-
TB-1D	9	HS	53.05 j	64.40 ij	10.45 c	18.97 de	-
TB-2	6	S	44.79 e	57.81 ef	13.75 d	15.35 c	77.71 d
TB-4	9	HS	51.74 fgh	61.37 g	9.92 c	17.74 cd	-
TB-4A	9	HS	52.59 ij	63.44 hi	9.61 c	19.73 d	-
TB-4B	9	HS	50.74 g	61.38 g	10.12 c	18.85 cde	-
T7B-1	6	S	48.64 f	58.49 f	15.14 e	16.34 cd	83.56 e
T7B-2	3	T	24.22 c	33.28 b	27.88 i	8.69 ab	47.55 a
T7B-3	3	T	21.79 b	36.25 c	25.89 h	9.97 ab	52.67 b
T7B-4	7	S	44.73 e	56.99 e	13.58 d	16.88 cd	81.48 f
T7B-5	4	MT	28.44 d	35.58 c	22.27 f	11.44 b	62.37 c
TRAJ	9	HS	51.39 fgh	63.05 h	7.28 a	20.61 de	-
Binnato a	4	MT	28.98 d	37.47 d	32.29 j	8.01 ab	54.62 b
BR7	9	HS	55.04 k	65.70 k	8.64 b	28.89 f	-
Pokkali	3	T	18.59 a	30.64 a	24.16 g	6.07 a	47.09 a
BRRI dhan 29	9	HS	55.34 k	65.48 jk	10.12 c	22.49 e	-

N. B.: T, Tolerant; MT, Moderately tolerant; S, Susceptible; HS, Highly susceptible; Means followed by common letter (s) are not significantly different at the 5% level by DMRT.

study T7B-2, 3 & 5 showed increase in root length more than 22% and reduction in root dry weight less than 12%; in case of tolerant check, Pokkali those values were 24% and 6% respectively (Table 3).
Here, leaf area decrease of T7B-2, T7B-3 & T7B-5 were 48, 53 & 62% respectively, whereas of tolerant check (Pokkali) & sensitive check (BRRI dhan29) were 47 & 100% respectively. Decrease in leaf area of T7B-2, T7B-3 & T7B-5 were 48, 53 & 62% respectively, whereas of tolerant check & sensitive check were 47 & 100% respectively (Table 3).
Three test AC lines T7B-2, T7B-3 & T7B-5 were then grown in hydroponic nutrient solution to measure the change of chlorophyll content, chlorophyll stability index (CSI), sodium (Na) and potassium (K) content of the salt stressed lines in shoot over the control set. In this study, decrease in chlorophyll content of those three AC lines, T7B-2, 3 & 5 were 47, 49 & 52% respectively and of Pokkali & BRRI dhan29 were 36 & 98% in that order (Fig. 1). Rao and Rao (1981); Sing and Dubey (1995) reported that sodium chloride (NaCl) stress decreases chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: cholorophyllase and by inducing the destruction of the chloroplast structure and the instability of pigment protein complexes.
The leaf chlorophyll stability index (CSI) is an important physiological trait which is directly related to salt stress. In this study, the % of CSI reduction in the shoot of T7B2, 3 & 5 (9, 12 & 15% respectively) including tolerant parent Binnatoa (9%) and check (7%) varieties was lower than the salt sensitive parent

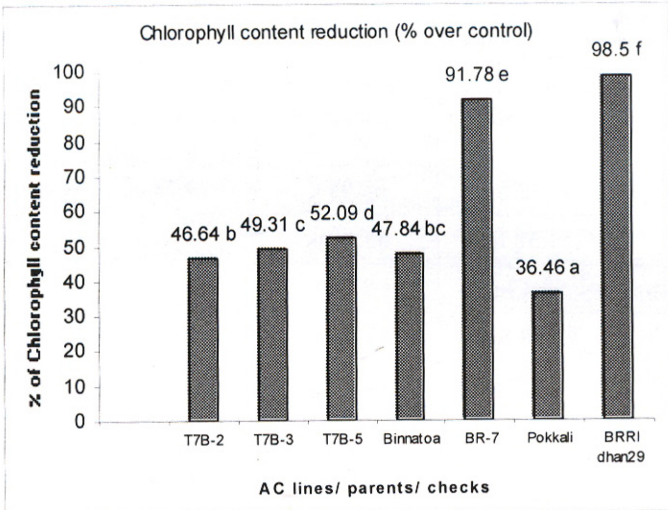

Fig. 1. Percentage of chlorophyll content reduction of AC lines, parents & check varieties compared to control due to salinity stress

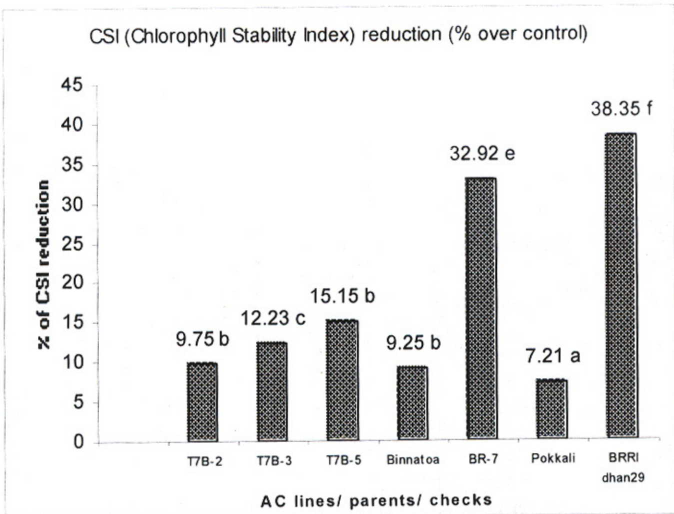

Fig. 2. Percentage of CSI (Chlorophyll Stability Index) reduction of AC lines, parents & check varieties compared to control due to salinity stress

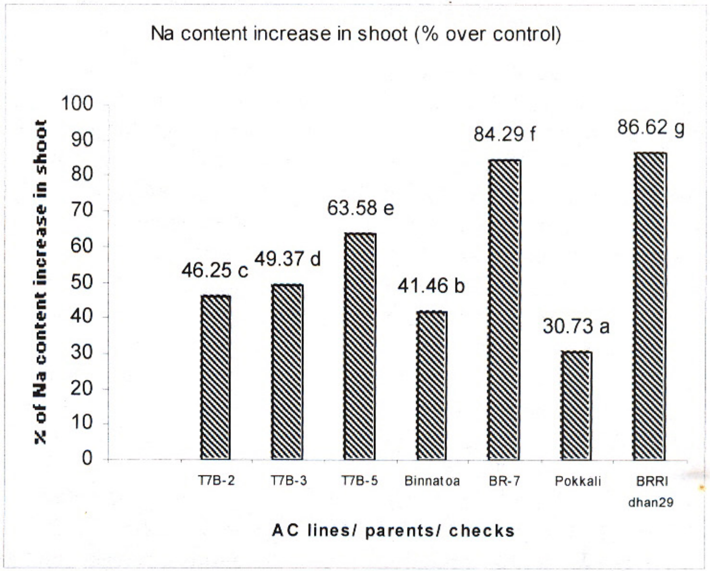

Fig. 3. Percentage of Sodium (Na) content increase of AC lines, parents & check varieties compared to control due to salinity stress

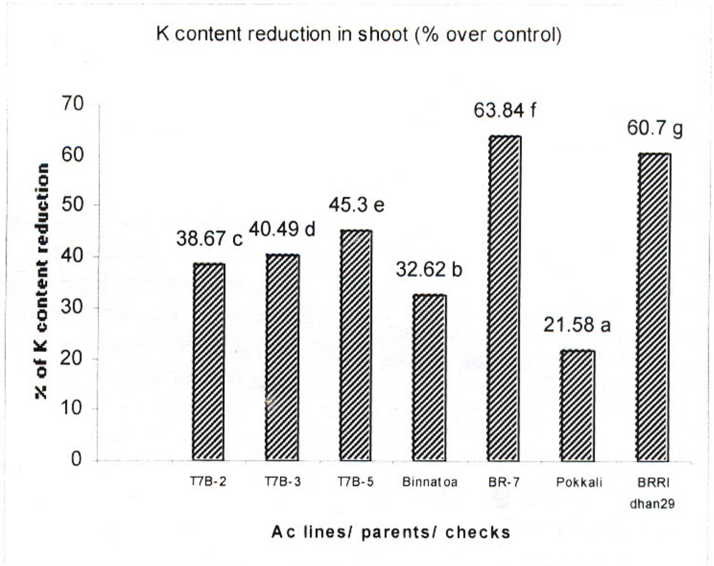

Fig. 4. Percentage of Potassium (K) content decrease of AC lines, parents & check varieties compared to control due to salinity stress

(35%) and check (38%) varieties, verified the tolerance of test lines to salinity stress and these results were supported by the findings of Raja Babo *et al.*, 2005.

In the present study, sodium (Na) content increase in shoots of 3 AC lines, T7B-2, 3 & 5 were 46, 49 & 64% respectively, whereas of Pokkali & BRRI dhan29 were 31 & 87% correspondingly (Fig. 3). But the potassium (K) content was decreased due to salinity stress in all the test lines including parent and check varieties. In this case, T7B-2, 3 & 5 showed the values of 39, 40 & 45% respectively and Pokkali & BRRI dhan29 showed 22 & 61% respectively (Fig. 4). According to Weimberg (1987), high levels of Na^+ inhibit the K^+ uptake causes an increase in the Na^+/K^+ ratio. Generally, tolerant species accumulated lower Na^+ and decreasing of K^+ was lower than sensitive species (Weimberg, 1987; Hagibagheri *et al.*, 1989). Flowers and Yeo (1995) reported that the low sodium & high potassium content means low Na/K ratio which is indicative of Na exclusion as well as partitioning into older leaves. Based on these reports, it may be concluded that T7B-2, 3 and 5 lines maintained less amount of Na in their leaves and hence were tolerant to salinity. The increase in Na content and decrease in

K content are significant in sensitive genotypes in comparison to tolerant.

Screening for salinity tolerance can be done in both salinized clay soil and in hydroponic system. It is very important to check the tolerance level of cultivars in saline soil because farmers will grow them in saline soil. In the present study, the tolerance level of anther derived lines (Table 1) was checked in saline soil in pots and in hydroponic system at BRRI (methods described in methods and materials section). Statistical analysis was also performed against all salinity parameters and T7B2, T7B-3 were found to be superior to their salt tolerant parent Binnatoa in all salt tolerance indicators such shoot length & dry weight, root length & dry weight, leaf area, chlorophyll content, chlorophyll stability index (CSI), Na & K absorption. Another AC line, T7B-5 performed moderately well. Considering all the parameters of salinity tolerance, mentioned above, it was observed that T7B-2 & T7B-3 were superior and T7B-5 was similar to their salt tolerant parent, Binnatoa.

Conclusion

In the present investigation, 15 anther derived lines were used to evaluate their tolerance to the salinity stress in soil in hydroponics. It was observed that among 15 AC rice lines, only 2 lines (T7B-2 and T7B-3) were tolerant and 1 line (T7B-5) was moderately tolerant in respect of various salinity tolerance parameters. From this result, it can be concluded that the objective of anther culture to fix salt tolerance in a single line by producing double haploid is fulfilled. These three lines may prove to be useful for coastal region in the Aus season (March-June).

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