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BREEDING AND ANTHER DERIVED LINES OF RICE (*ORYZA SATIVA* L.) FOR SALINE COASTAL AREAS OF BANGLADESH

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Abstract

Twenty seven anther culture lines (AC-lines) from rice (*Oryza sativa* L.) were produced from saline tolerant F_2 progeny after crosses between salt tolerant IRRI-derived lines and Bangladesh Rice and advanced up to Double Haploid 3 (DH_3) generation. Similarly crossed material, which did not respond to anther culture, was also advanced up to F_7. The AC-lines were fully characterized for various salinity tolerance parameters at seedling and reduction division stages. The breeding lines were selected for tolerance again at F_4 and advanced on site in the coastal area. DH_3 plants were also evaluated for agronomic performance in normal soil. The best performing breeding as well as AC-lines in terms of salinity tolerance and agronomic properties were planted in farmers fields in the *Boro* season, when salinity levels are the highest in the coastal zones of Satkhira. One anther derived line AC-1 and three breeding lines, BR7076, BR7077 and BR7098 produced high yield under stress conditions, whereas AC-1, BR7076 and BR7077 showed moderate reduction in grain yield compared to non-saline conditions. Considering both tolerance and yield performance in the saline area, AC-1 showed more stability compared to the breeding lines.

Introduction

In Bangladesh about 1 million hectares of cultivable land in the coastal regions are affected by varying degrees of salinity. Satkhira is one of these coastal areas, where 70% is highly saline with soil conductivity levels ranging from 4 - 16 dS/m (Karim and Iqbal 2001). In general, coastal salinity levels start gradually increasing from November at the beginning of the dry or 'Boro' season and peaks in March until June till October. In the moderate to highly saline southwest coastal areas of Satkhira, farmers can only grow a single rice crop during the monsoon season when the salinity levels are relatively low (Panaullah 1993). Breeding for salt tolerance in rice has been moderately successful (Senadhira *et al.* 2002). The genetic base for salinity tolerance in internationally released cultivars has originated mainly from two common salt tolerance donors, Pokkali and Nona Bokra, particularly in case of improved genotypes released for coastal salinity (Gregorio *et al.* 2002). Initial breeding programs using these donors resulted in moderately salt tolerant, improved cultivars. More complex crosses using a somaclonal variant of Pokkali, TCCP 266-2-49-B-B-3, which had better agronomical properties, resulted in improved cultivars with salt tolerance levels comparable to their donor parents (Gregorio *et al.* 2002, www.cgiar.org/irri/iris). The Bangladesh Rice Research Institute (BRRI) has so far released only three moderately salt tolerant modern varieties, BRRI dhan40, BRRI dhan41 and BRRI dhan47 originating from advanced IRRI lines and suitable for growth in the coastal areas of Bangladesh in the monsoon and dry season, respectively (BRRI 2008). BRRI dhan47 is suitable for the Boro season. Bangladesh needs to increase its food production by at least 40% over the next 40 years to keep up

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with the population growth. Cultivation of areas which remain fallow in specific seasons would not only result in an increase in production but also considerably improve the livelihood of the resource-poor farmers of the coastal areas. This paper describes developing salt tolerant rice varieties suitable for the Boro season having reasonable agronomic characteristics by using anther culture and conventional breeding.

Materials and Methods

Crosses for incorporating salinity tolerance in boro lines: IRRI-derived salt tolerance donor lines having lineage from the popular landrace Pokkali. These lines were IR52724, IR50184, IR60494, IR58440, IR65195 and IR51491-AC5-4 (Table 1 and www.irri.org/iris). One BRRI derived line, BRRI dhan40, having similar lineage was also used (Lisa *et al.* 2004). The recipient varieties were BRRI dhan28, BRRI dhan29 and BRRI dhan36 as well as Purbachi. F_2 generation were raised and screened for salinity tolerance in hydroponics at the seedling stage. For each cross, 2000 seeds were collected out of which 600 - 800 were screened for tolerance to salinity at 12 dS/m. Plants were selected on the basis of good plant type and selected plants advanced in bulk in yearly boro seasons up to F_6 or until there was no segregation. Plants were selected from six breeding lines, BR7071, BR7076, BR7077, BR7078, BR7079 and BR7098.

Table 1. Parents in selected anther-derived lines and breeding populations.

Cross combinations	BRRI name	Salt tolerance donors
BR40 / BRRI dhan29	BR7091-117-AC25	Pokkali
IR50184-3B-18-2B-1 / BRRI dhan36	BR7076	Pokkali and SR26B
IR50184-3B-18-2B-1 / Purbachi	BR7077	Pokkali and SR26B
IR51491-AC5-4 / BRRI dhan28	BR7078	Pokkali
IR51491-AC5-4 / BRRI dhan29	BR7079	Pokkali
IR52724-2B-6-2B-1-1 / BRRI dhan36	BR7084-310-AC1/13/16	Pokkali
IR65195-3B-13-2-3 / Purbachi	BR7098	Nona Bokra + TCCP226

Anther culture: Only two crosses (IR52724/BRRI dhan36 and BRRI dhan40/BRRI dhan29) gave a positive response to anther culture. All boots were collected early in the morning. After washing with sterilized water, the boots were placed at 8º C for cold treatment for eight days. Anthers were then plated in callus induction media (modified B5) and placed directly in the dark incubator at 24º C. Anthers were plated in Petri plates with 75 - 90 anthers per plate. After induction from anthers, calli were transferred into MS regeneration media for green plant regeneration. The green plants were then transferred into soil for hardening. In all cases, modified B5 media (Gamborg *et al.* 1968) was used for callus induction with the following supplements: $K_A = B_5$ + 2,4-D (1.0 mg/l) + IAA (0.5 mg/l) + BAP (0.2 mg/l). $K_B = B_5$ + 2,4-D (1.0 mg/l)+ Na_2SO_4 (7.5 g/l). $K_C = B_5$ + 2,4-D (1.0 mg/l) + NaCl (5.0 g/l). For regeneration MS media with K (1 mg/l) + NAA (1 mg/l) were used.

Screening for salinity tolerance at seedling stage: The germinated seeds of all tested entries along with checks BRRI dhan29 (sensitive) and Pokkali (tolerant), in three replicates, were sown on netted Styrofoam sheet floating on culture solution. The seedlings were allowed to grow for five days in normal culture solution, then in solution of EC 6 dS/m, followed by 12 dS/m, six days later. A complete set under normal culture solution was used as control. Scores for tolerance were given based on the performance of whole plants as described by Ponnamperuma (1977) (Table 2).

Different parameters such as per cent survival of plants, per cent leaf area affected, percent reduction of shoot and root length, shoot and root dry weight were recorded from this screening. For the measurement of sodium and potassium concentration in shoot and root, after scoring plants were washed in flowing tap water for 30 sec. and 10 oven-dried plants from each replicate were pooled, ground and analyzed by a flame photometer (Jenway, UK) after 48 h of extraction with 1N HCl following the procedure described by Yoshida *et al.* 1976. All the data were analyzed statistically and means were separated using Duncuns' Multiple Range Test when a significant F-value was obtained.

Table 2. Method of scoring salt injury (Adapted from Ponnamperuma 1977).

Dead leaves (%)	Score	Rating
0 - 20	1	Tolerant
21 - 35	2	Tolerant
36 - 50	3	Tolerant
51 - 70	5	Moderately tolerant
71 - 90	7	Moderately susceptible
91 - 100	9	Susceptible

Screening for salinity tolerance during meiosis: Screening during meiosis was done according to the method described by Gregorio *et al.* (1997). Sprouted seeds of 27 anther culture and two check varieties (Pokkali and BRRI dhan29) were sown in plastic pots, containing about 2 kg of soil within a porous muslin bag made to fit the pot exactly. For easy movement of water and salt, each pot had 138 holes on its surface; pots were prepared by filling with pulverized soil with Urea, TSP (triple super phosphate) and MoP (Muriate of potash) @ 50 g, 20 g and 20 g, respectively. Plants were grown in normal tap water up to reduction division stage. Tap water was withdrawn when the experimental plants reached reduction division stage, visually determined when the auricle distance between the flag leaf and penultimate leaf was between −5 and +5 cm and the pots allowed to dry for 12 hours. NaCl salt solution was added to the plastic bowl instead of tap water in order to obtain a conductivity of 6 dS/m. One bowl was maintained with tap water to serve as control. The salinity level was maintained in the bowl by adding NaCl when necessary, until harvesting. Three plants from a total of five in each of control and stressed were selected randomly for assessment of different morphological characters. Parameters noted were, plant height, total and effective tillers/hill, filled grains/hill, unfilled grains/hill, per cent fertility, panicle length, flag leaf length, 1000 grain weight and yield in g/hill. Analysis of variance was computed and means separated by DMRT.

Field trial during Boro season at Satkhira: Sprouted seeds were sown in the seedbed of the farmer's saline land at Satkhira in the boro season (dry season) during 2004 - 05 and 2005 - 06. Ten advanced breeding lines were tested (where four lines were AC1, AC13, AC16 and AC25, anther derived double haploid lines and other six were conventional breeding lines BR7071, BR7076, BR7077, BR7078, BR7079 and BR7098) along with check BRRI dhan29 and Pokkali for yield performance. Forty days old seedlings were transplanted in 7 m × 2.5 m plot with a spacing of 25 × 15 cm. Identical control sets in normal soil were planted at the same time in BRRI fields, Gazipur. All the cultural operations were done as recommended by BRRI (BRRI 2003). The experiments were laid out in Randomized Completely Block Design with three replications. Soil salinity was checked at the maximum tillering stage. For each replication, soil was collected from ten randomly selected areas within the field. Samples from each location were dried separately and powdered. ECe of a 1 : 1 water extract was measured. The ECe ranged from 5.81 to 9.08 dS/m with an average of 7.36 dS/m. Ten hills were randomly selected for growth and yield components data whereas the grain and straw yield data were taken from pre-selected 5 sq m area in each plot. The data were analyzed by the statistical package IRRISTAT and DMRT.

Results and Discussion

Anther derived lines: Panicles of only two tolerant F_2 plants from the cross of IR52724 and BR36, plant number 310 and 705, responded positively to anther culture in Kc media and produced green plants. All others did not regenerate or produced albino plants only. Average percentage of green plant regeneration was 78, out of which 1/3rd were spontaneous double haploid plants. A total of 25 healthy double haploid plants were obtained from the above cross and named AC1 - AC25. One F_2 plant 117 from the cross BRRI dhan40/BRRI dhan29 produced green plants with 20%. Two healthy DH green plants were obtained and named AC26 and 27. The rice varieties used in this study were all indica. Regeneration of green plants from indica rice is not only known to be poor but is also extremely genotype dependent (Faruque *et al.* 1998).

Characterization of AC-lines at seedling stage against salinity: Leaf area damage of less than 50% and survival rates of more than 50% as well as salinity tolerance scores of 2-3 indicated that AC-lines, 1, 6, 7, 8, 10, 13, 16, 18, 20 and 27 were tolerant. Seedling stress scores for only the best performing plants AC-1, 13, 16 and 25 along with check varieties have been shown in Table 3.

Table 3. Screening of 15-day-old seedlings of anther-derived lines for tolerance to salinity stress at 12 dS/m with Pokkali and BRRI dhan29 as tolerant and sensitive checks, respectively. (Only the best performing AC-lines data were shown in the table).

AC line	Leaf area affected (%) and score		Survival plants (%)	Shoot length reduction (% over control)	Root length reduction (% over control)	Shoot dry weight reduction (% over control)	Root dry weight reduction (% over control)	Shoot Na/K	Root Na/K
AC-1	30.00bc	2	76.66lm	22.98ab	15.24a	29.32a	36.73cde	1.62abc	1.97ab
AC-13	50.00e	3	46.66hi	35.88e	19.52ab	51.24hi	39.41d-g	2.98f	2.41a-e
AC-16	46.66e	3	61.66j	40.40efg	22.50bcd	28.40a	39.50d-g	2.39c-f	1.91a
AC-25	66.66fg	5	33.33ef	44.30fg	28.44efg	29.54a	32.41abc	2.22b-f	3.00de
BRRI dhan29	97.50l	9	7.50a	43.86fg	32.21ghi	60.26j	52.50j	2.84ef	2.40a-e
Pokkali	2.50a	1	98.33n	19.04a	16.78a	27.27a	32.22abc	0.96a	2.64a-e
CV %)	9.40		11.80	8.10	10.00	8.70	7.90	19.80	18.00

Means followed by a common letter(s) are not significantly different at the 5% level by DMRT.

AC-1 did particularly well with leaf damage area of around 30% and survival greater than 76%. AC-lines 4, 5, 15, 17, 25 and 26 were classed as moderately resistant with damage of less than 70%, survival rates of more than 33% and tolerance scores of 5 (Tables 1 and 3). These two groupings were maintained when reduction in shoot length or reduction in dry shoot weight were considered. The tolerant group showed less than 42% reduction in length and less than 50% in shoot dry weight, whereas the moderately tolerant group showed reduction of around 50% in both shoot dry weight and shoot length. The low reduction in root length or dry weight of AC-1, 6, 10, 13, 16 and 18 reconfirmed that these could be called tolerant (Table 3) whereas AC-4, 17 and 25 seemed moderately tolerant. One of the more investigated physiological traits leading to salinity tolerance is the ion content of plants grown in the presence of salt. The tolerance of plants to sodium chloride is commonly, but not uniquely, related to the concentration of sodium in the shoot

(Tester and Davenport 2003). Tolerance may be associated more with the potassium concentration in the shoot under stress, and pathways for net accumulation of the two ions in rice have been shown to be independent (Garcia *et al.* 1997). Tolerance may also be associated with recirculation of Na^+ from xylem vessels to xylem parenchyma mediated by *Arabidopsis thaliana* HKT1 or from shoots to roots in rice mediated by SKC1 (Ren *et al.* 2005). In the present work the shoot and root Na/K ratio was moderately low only for AC-1. These low ratios are indicative of Na^+ exclusion as well as partitioning into older leaves (Moradi *et al.* 2003).

Characterization of AC-lines during meiosis stage against salinity: Selected yield characters (Table 4) such as filled grain/hill, fertility and yield/hill for the best performing line indicated that only AC-1, AC-13, AC-16 were not affected significantly by stress at 6 dS/m and are comparable to Pokkali. Performance of AC-25 is inferior to the above but found better than all the other AC-lines. Under 6 dS/m stress, 1000 grain weight of all four lines was significantly affected at the 1% level; however Pokkali was only affected at the 5% level. When all the 27 AC lines were planted in normal soil at BRRI field in two replicates, all four lines showed acceptable plant type and were therefore further selected for testing on site in the coastal area in Satkhira in farmer's fields.

Table 4. Selected yield components of anther derived lines at reduction division stage in soil at 6 dS/m. (Only the best performing AC-lines data were shown in the table).

AC line	Filled grains (No./hill)		Fertility (%)		1000 grain weight (g)		Yield (g/hill)	
	Control	Salt	Control	Salt	Control	Salt	Control	Salt
AC-1	1111.00 b	960.33a	75.50 bcd	69.31 ab	19.76 e-i	17.58 hik	21.96 ab	16.67 a
AC-13	1004.33 bc	775.00b	73.19 c-f	66.56 bc	21.13 d	19.42 ef	21.22 b	15.31 ab
AC-16	832.00 def	715.00bc	74.96 b-e	61.38 cd	19.42 ghi	18.71 fgh	16.14 d-g	13.06 bc
AC-25	664.67 g-k	586.33c-f	72.24 c-h	65.77 bc	20.51 d-g	19.16 efg	13.64 g-k	10.35 c-g
BRRI dhan29	584.00 j-m	208.00no	68.76d-k	27.68 m	21.04 d	18.64 f-i	12.31 i-l	3.92 j
Pokkali	586.67 j-m	577.67c-g	82.47 a	75.13 a	27.78 a	26.67 a	16.30 d-g	16.05 a
CV (%)	12.6		6.30		3.10		12.5	

Means followed by a common letter(s) are not significantly different at the 5% level by DMRT.

Field trial of AC and breeding lines: Four AC lines and six breeding lines were tested in two replicates in two different farmer's field on site at Satkhira in two consecutive boro seasons in 2004 - 05 and 2005 - 06 starting from seedling germination in December, transplantation in January and harvesting in April-May. Plants in normal soil at the BRRI fields served as control. The yield data for the ten lines grown in farmer's field in the 2004 - 05 and 2005 - 06 boro seasons, but only 2004 - 05 data is presented in Table 5. The soil conductivity was high at the maximum tillering stage only in this particular boro season. The conductivity averaged 7.36 dS/m with a high of 9.08 dS/m in 2004 - 05, whereas it was only 5.09 dS/m in 2005 - 06, with the highest conductivity of any sample recorded as 6.36 dS/m. The filled grains per hill of all the lines was around 900 grains per hill, although the effective tiller per hill varied considerably, including a low number for the tolerant control Pokkali. Average grain yield of BR7077 and BR7076 was 5.37 and 4.94 t/ha which is statistically superior to others. The yield of the breeding lines BR7098 and BR7078 was 4.93 and 4.38 t/ha which was similar to the yield of the two anther-derived lines AC-1 (4.57 t/ha) and AC-13 (4.47 t/ha) (Table 5). But when yield in the saline zone was taken into account, the lines AC-1, BR7076 and BR7077 showed significantly lower reduction compared to the other tested entries (Fig. 1). Due to the effective tiller number of Pokkali being low, its yield under stress was lowered significantly.

Table 5. Yield, yield components and yield reductions of 4 anther derived and 6 conventional breeding lines at BRRI farm (non-saline zone used as control) and Satkhira (saline zone used as stressed) in the Boro season of the year 2004-05.

AC lines	Life cycle (d)	Plant height (cm)	Effective tiller (No. /hill)		Filled grains (No./hill)		Fertility (%)		Thousand grain weight (g)		Reduction of grain yield (%)
			Control	Salt	Control	Salt	Control	Salt	Control	Salt	
AC-1	156	82f	13.06bc	21.10a	911.13cd	978.50a	73.34d	60.32bcd	22.18e	18.33e	9.17d
AC-13	154	82.4f	14.93ab	19.66ab	1025.20bc	824.43bc	74.32d	55.72de	23.47d	19.37cde	33.12bc
AC-16	156	82.5f	16.06a	19.60ab	1069.77ab	963.47a	73.25d	58.60cde	22.05e	18.26e	25.51c
AC-25	158	111b	10.26de	14.03e	946.37bcd	947.30ab	75.52cd	54.54e	24.52bcd	21.26b	25.20c
BR 7071	150	98.5c	12.06cd	16.20cd	938.30cd	890.60abc	72.97d	63.09bc	24.29bd	18.70de	22.69c
BR 7076	140	88de	13.16bc	18.30b	1008.23bc	805.50c	77.49bcd	58.67cde	24.31bcd	19.76cd	11.74d
BR 7077	141	86e	12.29ed	15.30de	868.63d	928.57abc	75.96cd	63.23bc	25.43b	19.56cd	6.37d
BR 7078	155	99c	11.13cd	15.73de	911.73bcd	944.67ab	83.87a	63.06bc	23.83cd	19.71cd	21.24cd
BR 7079	151	98c	11.67cd	15.30de	1030.80bc	1010.67a	83.32a	64.69b	21.82e	19.94c	24.23c
BR 7098	138	98c	11.83cd	18.66b	990.90bcd	918.93bc	83.39a	62.93bc	24.96bc	20.00c	35.59b
BRRI dhan29	160	90d	12.90bc	18.06bc	1169.20a	509.33d	80.28abc	34.72f	21.96e	16.14 f	34.07bc
Pokkali	160	149a	8.33e	12.00f	699.63e	569.67d	81.14ab	67.74a	33.91a	26.51a	60.21a
CV (%)	-	1.9	7.90		7.50		4.00		2.90		4.80

Means followed by common letter(s) are not significantly different at the 5% level by DMRT.

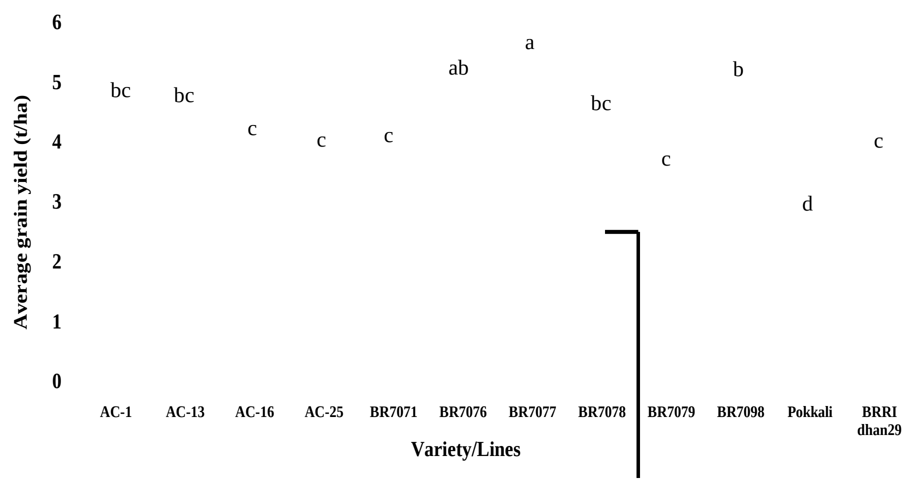

Fig. 1. Two years average grain yield of 4 anther cultured and 8 breeding lines grown in the saline zone of Satkhira. Means followed by common letter(s) are not significantly different at the 5% level by DMRT.

One way Analysis of Variance (ANOVA) analysis for experimental site (Satkhira) versus control (BRRI, normal soil) showed no significant difference between the grain yield of AC-1, BR7076 and BR7077 in control and experimental site (P value equal to 0.213, 0.077 and 0.356, respectively). Similarly there was no significant difference in straw yield for AC-1, AC-25, BR7076, BR7077, BR7078 and BR7079 between control and experimental site. In the 2005 - 06 boro season, when the soil conductivity was lower, AC-1 and BR7076 gave slightly lower yields, whereas, those of AC-25 and BR7077 higher. However the differences were non-significant.

AC-1, showed excellent seedling stress survival as well as moderately low Na/K ratio close to that of the tolerant control Pokkali (variance within 5%). Low shoot Na/K ratios have been shown to be related to rice seedling tolerance (Moradi *et al.* 2003, Elahi *et al.* 2004, Lisa *et al.* 2004) but may not be related to optimal yield under stress. Boro season crops experience maximum salinity stress at the tillering stage during March-April when irrigated water may also be saline (Islam *et al.* 1993). In the present work, at least one of the AC-lines, AC-1, which had moderate shoot Na/K ratios at the seedling stage, showed good yields when stressed at the reduction division stage as well as in field trials in the saline zone of Satkhira. This shows that good seedling health under stress at early stage, does not preclude and also good yields at maturity.

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