SALINITY-TOLERANT RICE VARIETY FOR COMBATING CLIMATE DISASTER AND ASSURING FOOD SECURITY

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ABSTRACT

Phenotypi**c** screening of 60 rice genotypes was performed at seedling and reproductive stages in hydroponic system and in sustained water bath respectively. Morphological studies of the genotypes were done with electrical conductivity (EC) $12dSm^{-1}$ and $6dSm^{-1}$ at seedling and reproductive stages respectively. Most important characteristics like: plant height, root-shoot ratio, total dry matter, effective tiller number, number of filled and unfilled grain, panicle length, percentage of fertility, yield per plant and 1000 seed weight were evaluated. Three simple sequence repeat (SSR) markers (viz. RM585, RM336 and RM152) were used for marker assisted selection of rice genotypes tolerant to salinity. Among the 60 rice genotypes, 24 were selected based on salt tolerance. At the seedling stage screening, nine lines were found tolerant at the EC level of $12dSm^{-1}$, where 15 lines were moderately tolerant. Among the 24 tolerant genotypes, three strains viz. Pokkali, PBRC-37 and FL478 were found salinity tolerant, four lines as moderately tolerant among 24 tested entries at the EC level of $6\text{-}12dSm^{-1}$. PBRC-37 gave a higher yield (5.6t/ha) under salinity stress (10-12 dSm^{-1}) during field trial than the released salinity tolerant rice variety Binadhan-8 and released as new salt tolerant rice variety Binadhan-10 by National Seed Board.

Keywords: Screening, Phenotypic, Genotypic, SSR, Field Trial and Binadhan-10

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INTRODUCTION

In Bangladesh, rice is the staple food, occupying 11.0 million ha, with a total production of 338 million tons in 2013[1] where more than one million hectare of rice land including 53% of the coastal areas are salt affected due to global warming and recent adverse climate change[2]. Soil salinity (3-15 dSm^{-1})[3] from seedling to harvesting stage is one of the most brutal environmental factors and a complex phenotypic and physiological phenomenon in plants imposing ion imbalance or disequilibrium, hyperionic and hyperosmotic stress, disrupting the overall metabolic activities due to injurious effect of toxic Na^+ and Cl^+ and thus limiting the productivity of rice plant[4].Though some varieties especially for saline area already developed but these varieties are not suitable to overcome higher stresses of salinity in rice production to ensure food security in Bangladesh. More emphasis needs to be given on vigorous screening of new breeding lines at seedling stage followed by vegetative stage, to develop better adapted varieties for salinity prone area of this country. There exists tremendous variation for salt tolerance within species in rice[5] providing opportunities to improve crop salt-stress tolerance through genetic means. Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level. Molecular markers associated with quantitative trait loci (QTLs) affecting salinity tolerance are identified, which could be used as indirect criteria to increase breeding efficiency via marker- assisted selection (MAS)[6]. SSR markers are proved to be ideal for making genetic maps, assisting selection and studying genetic diversity among genotype[7]. They are playing important role to identify gene for salt tolerance that can be helpful for plant breeders to develop new cultivars. The objectives of this study were (i) to evaluate the response of rice genotypes to salinity at the seedling and reproductive stages (ii) to identify salinity tolerant rice genotypes through MAS (iii) to evaluate the proposed salt tolerant variety through field trial in the coastal saline area (Satkhira, Bagerhat and Khulna) in Bangladesh.

MATERIALS AND METHODS

This experiment was carried out in Bangladesh Institute of Nuclear Agriculture (BINA) where selected 60 rice genotype were evaluated (Table 1) for salinity tolerance through phenotypic and genetic screening.

Table 1: List of the genotype used for screening of salinity tolerant rice variety/line

Sl. No.	Name of the rice genotype	Source of collection	Sl. N0.	Name of the rice genotype	Source of collection
1	PBSAL-613	IRRI	31	PBRC-69	IRRI
2	PBSAL_614	IRRI	32	PBRC-82	IRRI
3	PBSAL_655	IRRI	33	PBRC-97	IRRI
4	PBSAL-730	IRRI	34	PBRC-132	IRRI
5	Iratom-24	BINA	35	IR71829-3R-2B-1	IRRI
6	Binadhan-7	BINA	36	IR72049-B-R-22-3-1-1	IRRI
7	Binadhan-4	BINA	37	IR70023-4B-R-12-3-1	IRRI
8	STL-15	IRRI	38	IR72580-B-24-3-3-3-2	IRRI
9	STL-20	IRRI	39	IR77674-3B-8-2-214-4	IRRI
10	FL-378	IRRI	40	Pokkali	IRRI
11	FL-478	IRRI	41	IR84645-308-2-1-B	IRRI
12	Kashrail	Coastal area	42	IR84645-2-11-1-B	IRRI
13	Horkuch	Coastal area	43	IR72593-B-18-2-2-2	IRRI
14	Ashfal	Coastal area	44	IR72593-B-3-2-3-3	IRRI
15	PBRC-37	IRRI	45	IR77664-B-25-1-2-1-3-12-3	IRRI
16	PBRC-83	IRRI	46	Nona Bokra	Coastal area
17	PBRC-90	IRRI	47	Binadhan-5	Coastal area
18	PBRC-110	IRRI	48	Binadhan-6	Coastal area
19	IR84645-308-2-1-B	IRRI	49	Kaliboro 139-2	Coastal area
20	IR45427-2B-2-2B-1-1	IRRI	50	Bara (Boro) Dhan	Coastal area
21	IR68144-2B-2-2-3-3	IRRI	51	Chini Sagar	Coastal area
22	IR72593-B-13-3-3-1	IRRI	52	Bawoi Jhak	Coastal area
23	IR63731-1-1-3-3-2	IRRI	53	Jangliboro 581	Coastal area
24	PBSAL-546	IRRI	54	Kaliboro 109-4	Coastal area
25	PBSAL-656	IRRI	55	Charnock (DA6)	Coastal area
26	PBSAL-731	IRRI	56	Kalo Bhog	Coastal area
27	PBRC-30	IRRI	57	Dhol Kochuri	Coastal area
28	PBRC-56	IRRI	58	Kala Jira	Coastal area
29	PBRC-64	IRRI	59	Dhaliboro 105-2	Coastal area
30	PBRC-67	IRRI	60	Latisail 11-117	Coastal area

PHENOTYPIC SCREENING.

IRRI standard screening method was used for evaluation of genotypic response to salinity stress at seedling stage in a salinized nutrient solution with electrical conductivity $12dSm^{-1}$ for 21 days under controlled conditions in the BINA glass house at 25-30 °C day and night temperatures with relative humidity of 75-85%. Peter's water soluble fertilizer (Urea: TSP: MP=20:20:20) and ferrous sulphate ($FeSO_4$, $7H_2O$) were used as nutrient solution that mixed in plastic containers. To prepare nutrient solution, 1.0 g Peter fertilizer and 200mg/L ferrous sulphate were mixed with per liter tap water. The pH was adjusted to 5.1 by pH meter using HCl and NaOH when necessary. The genotypes were grouped into 1, 3, 5, 7 and 9 score categories for salt tolerance on the basis of relative seedling shoot-root growth and total dry mass 21 days after salinity treatment[8]. The response of genotypes to salt stress at seedling stage was evaluated at the reproductive stage in completely randomized blocks design (RCBD) with two replications. Four pre-germinated seeds were sown in 1-liter capacity perforated plastic pots with nylon mesh lining and filled with pre-fertilized soil (N: P: K 50:25:25 mg kg^{-1}). The pots were kept in glass fiber tanks with non-saline water at first and water level was maintained at the same level as of the soil. Two weeks after seeding, seedlings were thinned to three per pot and water level was raised about 1-2 cm above the soil level. Saline water solution of 6 dSm^{-1} was prepared by dissolving desired amount of crude salt in water in separate tanks. As the genotypes had different flowering periods, for imposing the stress very specific only to the reproductive stage and to eliminate the effect of salinity before flowering, the pots were immediately transferred to salinized tanks when the tip of the first flag leaf of any tiller appeared. Water level and salinity were maintained regularly. Salinity stress was relieved after two weeks from complete flowering as the seed ripening is relatively tolerant stage. At reproductive stage, plant height, number of effective tiller, panicle length, number of unfilled grains, number of filled grains, percentage of fertility and grain yield per plant were recorded under both normal (control) and salinized conditions according to the standard procedure as mentioned by Gregorio (1997).

GENOTYPIC SCREENING.

Three SSR primers (RM585, RM336 and RM152) for salinity screening rice were used in this study. The PCR were optimized for the three microsatellite loci as necessary to produce scorable amplification products. The annealing temperature was 55^0C and PCR reactions were carried out on a Thermocycler-13 (Biometra). The reaction volume was 10 ul containing 2 ul genomic DNA (100 ng/ ul), 1 ul of each primer (2uM), 1ul of dNTPs (0.25mM each), 1 ul 10× *Taq* buffer A (mixed with $MgCl_2$), 0.2 ul *Taq* DNA polymerase (1Unit) and 3.8 ul double distilled water. The temperature profile consisted of 5 min. initial denaturation at 94^0C followed by 35 cycle of 1 min at 94^0C, 1 min at the respective annealing temperature and 2 min at 72 ^{0}C, ending with 7 min final extension. The PCR products were separated on a 8% denaturing polyacrylamide gel containing 9.45ml 40% acrylamide solution (Fluka Analytical), 0.211gm bisacylamide powder (SIGMA, Life science), 5 ml 10× TBE, 500ul Ammonium per sulphate (APS) and 70ul TEMED (SIGMA, Life science). Electrophoresis was conducted using ELITE 300 PLUS (Wealtec) vertical polyacrylamide gel electrophoresis system. It was run for about 2.5-3.5hr (running time depends on the size of PCR fragments) at 80 Volts. DNA fragments amplified by SSR primers were visualized after ethidium bromide staining and then observed on a UV transilluminator (ASUS, Maxdat, Biometra).

The patterns of bands obtained after amplification with the SSR primers were scored using ALPHA VIEW version 3.2.8 to identify the size of allele of DNA band comparing with DNA ladder. A genotypic data matrix including number of alleles, major alleles and their frequencies, rare alleles, polymorphism information content (PIC), availability, gene diversity, heterozygocity and Nei's (1973) genetic distance were constructed for all SSR loci using POWER MARKER version 3.23[9]. UPGMA (Unweighted Pair Group Method with Arithmetic Means) dendrogram from SSR marker was constructed using the software TREEVIEW 1.1[10]

FIELD EVALUATION.

Evaluation of PBRC-37 (proposed salt tolerant variety, Binadhan-10) and Binadhan-8(salinity tolerant check) were done in Boro season of 2011-2012 in the coastal saline areas in 6 different locations of Satkhira, Bagerhat and Khulna districts where salinity level ranges between 5-12 dSm-1 . The experiment was laid out in a Randomized Complete Block Design where thirty day old seedlings were transplanted in each (5 m × 5m) plot. Besides this, evaluation was also **done** in farmers' field at six locations viz., Dumuria (Khulna); Gutodia (Khulna);Metro (Khulna); Fakirhat (Bagerhat); Sadar (Bagerhat); Bemorta (Bagerhat); Debhata (Satkhira); Shyamnagar (Satkhira) in Boro 2011-2012. Morphological and agronomical characters of the proposed rice line PBRC-37 were evaluated during field trial following IRRI standard protocol.

RESULT AND DISCUSSION

SCREENING OF RICE GENOTYPES AT SEEDLING STAGE

Sixty rice genotypes were evaluated for salinity tolerance using modified protocol in hydroponic system at seedling stage based on IRRI standard protocol (Fig.1).

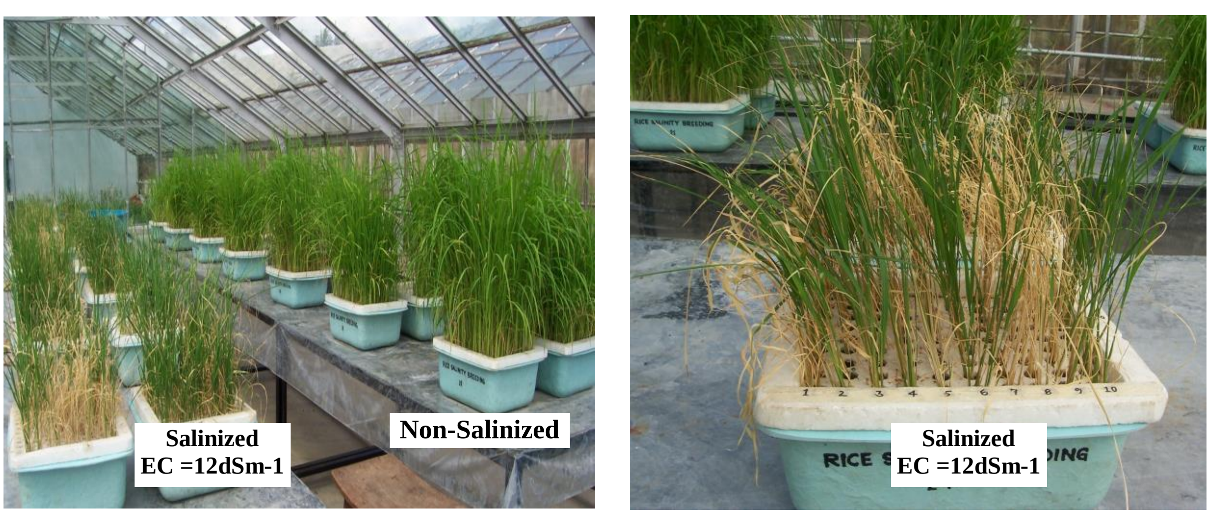


Fig. 1: Screening of rice genotype at seedling stage in hydroponic culture (EC 12 dSm-1)

Out of 60 rice genotype, 24 salt tolerant rice genotype were selected (Table 2) based on modified SES score (Table 3).

Table 2: Modified Standard Evaluation System (SES) score of rice genotypes under salinized condition (EC 12 dSm-1) grown in hydroponic culture at seedling stage

Sl. No.	Name of the rice genotype	SES Scoring	Tolerance	Sl. No.	Name of the rice genotype	SES Scoring	Tolerance
1	PBSAL-613	3	T	31	PBRC-69	7	S
2	PBSAL_614	3	T	32	PBRC-82	7	S
3	PBSAL_655	3	T	33	PBRC-97	7	S
4	PBSAL-730	3	T	34	PBRC-132	7	S
5	Iratom-24	9	HS	35	IR71829-3R-2B-1	5	MT
6	Binadhan-7	7	S	36	IR72049-B-R-22-3-1-1	5	MT
7	Binadhan-4	9	HS	37	IR70023-4B-R-12-3-1	5	MT
8	STL-15	7	S	38	IR72580-B-24-3-3-3-2	5	MT
9	STL-20	3	T	39	IR77674-3B-8-2-214-4	7	S
10	FL-378	3	T	40	Pokkali	5	MT
11	FL-478	3	T	41	IR84645-308-2-1-B	7	S
12	Kashrail	9	HS	42	IR84645-2-11-1-B	5	MT
13	Horkuch	5	MT	43	IR72593-B-18-2-2-2	5	MT
14	Ashfal	5	MT	44	IR72593-B-3-2-3-3	5	MT
15	PBRC-37	3	T	45	IR77664-B-25-1-2-1-3-12-3	5	MT
16	PBRC-83	7	S	46	Nona Bokra	5	MT
17	PBRC-90	7	S	47	Binadhan-5	9	HS
18	PBRC-110	7	S	48	Binadhan-6	9	HS
19	IR84645-308-2-1-B	5	MT	49	Kaliboro 139-2	7	S
20	IR45427-2B-2-2B-1-1	9	HS	50	Bara (Boro) Dhan	7	S
21	IR68144-2B-2-2-3-3	9	HS	51	Chini Sagar	9	HS
22	IR72593-B-13-3-3-1	9	HS	52	Bawoi Jhak	7	S
23	IR63731-1-1-3-3-2	5	MT	53	Jangliboro 581	7	S

24	PBSAL-546	5	MT	54	Kaliboro 109-4	7	S
25	PBSAL-656	3	T	55	Charnock (DA6)	6	S
26	PBSAL-731	7	S	56	Kalo Bhog	7	S
27	PBRC-30	7	S	57	Dhol Kochuri	7	S
28	PBRC-56	7	S	58	Kala Jira	9	HS
29	PBRC-64	7	S	59	Dhaliboro 105-2	6	S
30	PBRC-67	7	S	60	Latisail 11-117	7	S

T = tolerant, MT = moderately tolerant, S = susceptible, HS = highly susceptible, 1-9 scale, where 1= highly tolerant and 9 = highly susceptible

Table 3: Modified standard evaluation system (SES) of visual salt injury at seedling stage

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

At the seedling stage screening, 9 lines (PBSAL-613, PBSAL-614, PBSAL-655, PBSAL-730, STL-20, FL-378, FL-478, PBRC-37and PBSAL-656) were found tolerant while 15 lines (Horkuch, Ashfal, IR84645-308-2-1-B, IR63731-1-1-3-3-2, PBSAL-546, IR71829-3R-2B-1, IR72049-B-R-22-3-1-1, IR70023-4B-R-12-3-1, IR72580-B-24-3-3-3-2, Pokkali, IR84645-2-11-1-B, IR72593-B-18-2-2-2, IR72593-B-3-2-3-3, Nona Bokra and IR77664-B-25-1-2-1-3-12-3) were moderately tolerant at EC level 12 dSm-1 . On the other hand 10 genotype were found as highly susceptible and rests of the genotypes were susceptible.

SCREENING OF RICE GENOTYPES AT REPRODUCTIVE STAGE

Twenty four rice genotypes were screened for salinity tolerance in sustained water bath at the reproductive stage using modified SES score (Table 4). Two strains (viz., Pokkali and PBRC-37) were found salt tolerant, four genotypes (STL-20, FL-378, FL-478, IR63731-1-1-3-3-2) moderately tolerant and others as susceptible at EC level 6 dSm-1 (Table 5 and Fig.2).

Table 4: Modified standard evaluation system (SES) of visual salt injury at reproductive stage

Score	Observation (Flag-leaf damage %)	Tolerance
1	No flag-leaf symptoms	Highly tolerant
3	1-10% damage	Tolerant
5	11-25% damage	Moderately tolerant
7	26-50% damage	Susceptible
9	More than 51% damage	Highly susceptible

Table 5: Performance of selected rice genotype under salt stress (EC 6 dSm-1) at reproductive stage (modified SES score)

Sl. No.	Designation	Score	Salinity tolerance
01.	Pokkali and PBRC-37	3	Tolerant
02.	STL-20, FL-378, FL-478 and IR63731-1-1-3-3-2	5	Moderately tolerant
03.	PBSAL-613, PBSAL-614, PBSAL-655, PBSAL-730, PBSAL-656 Horkuch, Ashfal, Bara Dhan, IR84645-308-2-1-B, PBSAL-546, IR71829-3R-2B-1, IR72049-B-R-22-3-1-1, IR70023-4B-R-12-3-1, IR72580-B-24-3-3-3-2, IR84645-2-11-1-B, IR72593-B-18-2-2-2, IR72593-B-3-2-3-3 and IR77664-B-25-1-2-1-3-12-3	7	Susceptible

GENOTYPIC SCREENING OF RICE GENOTYPES USING SSR MARKERS

Three microsattelite (SSR) markers RM585, RM336 and RM152 were used to evaluate genotypes for salinity tolerance. The bands (Fig.3) obtained from other genotypes were compared to the band obtained from FL378, FL478, Pokkali, Nona Bokra and Binadhan-8 as which are well known and internationally recognized salt tolerant genotypes. The genotypes having similar banding pattern to FL378, FL478, Pokkali, Nona Bokra and Binadhan-8 were considered as salinity tolerant. Considering the RM585, RM336 and RM152; PBRC-37 was found as tolerant whereas remaining were found as moderately tolerant or susceptible.

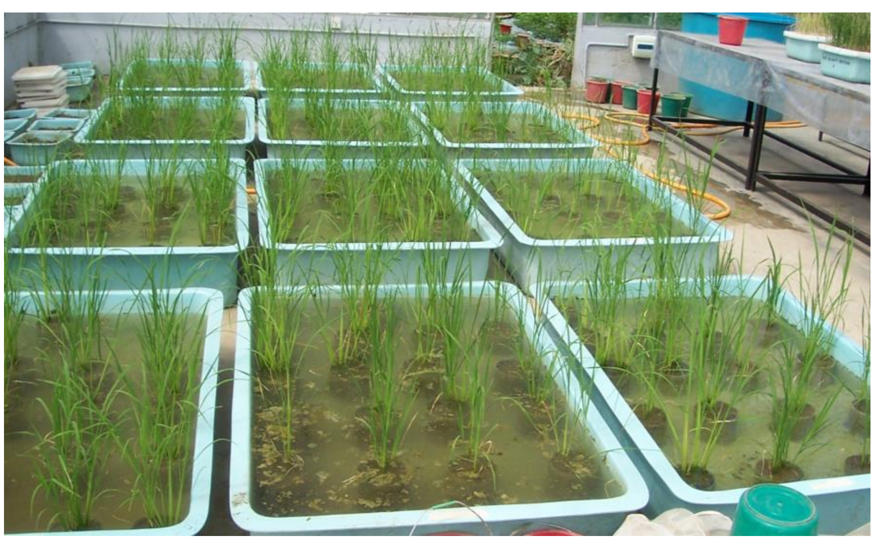
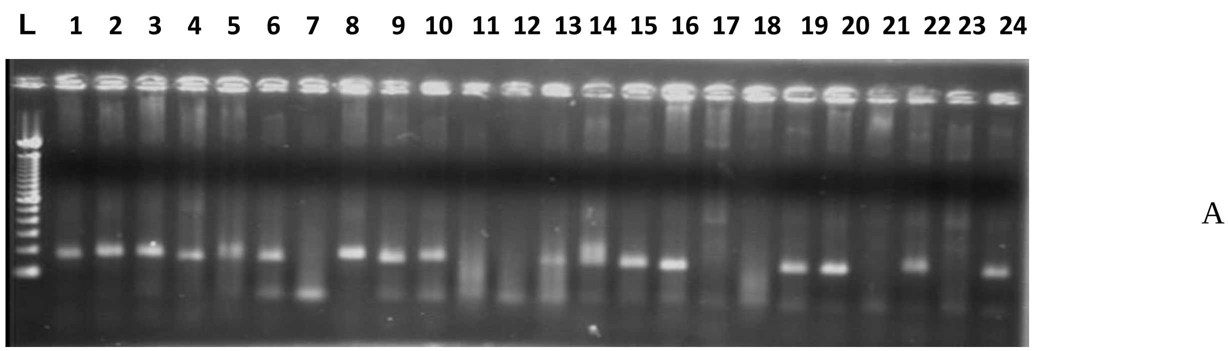
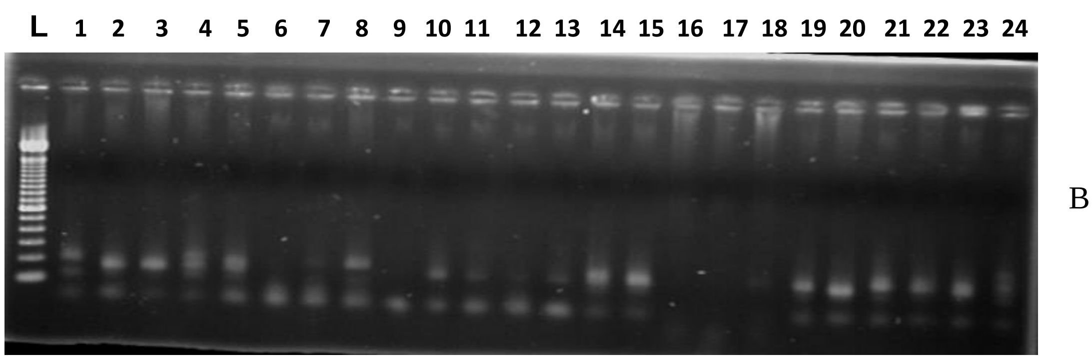


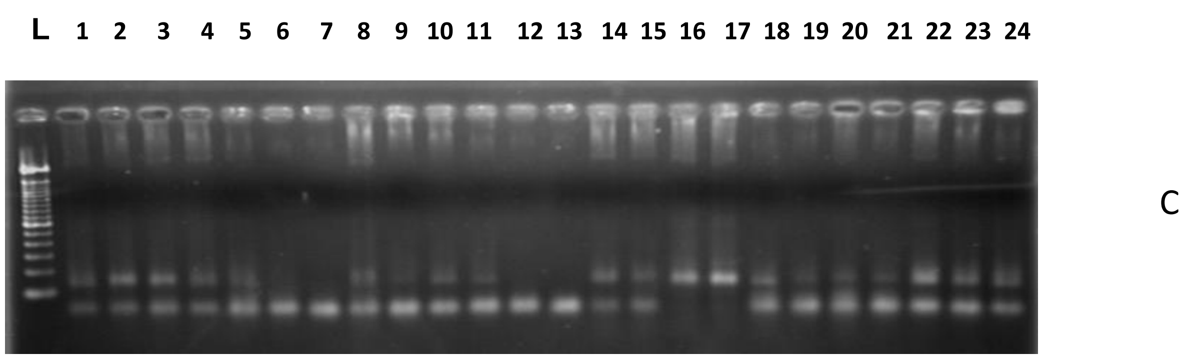
Fig. 2: Screening of rice genotype at reproductive stage (EC 6 dSm-1) using sustained water bath grown at BINA glasshouse



Primer RM585



Primer RM336



Primer RM152

Fig. 3: (A-C): DNA profile of test rice genotype using SSR markers (RM585, RM336 and RM152) at the seedling stage (1= PBRC-37(Binadhan-10), 2= STL-20 (Binadhan-8), 3= Pokkali, 4=FL-478, 5=FL-378, 6= Nona Bokra, 7= BINA dhan-4, 8= Binadhan-7, 9= STL-15 10= PBSAL-613, 11= PBSAL-614, 12= PBSAL-655, 13= PBSAL-730, 14= Iratom-24, 15=Ashfal, 16= Kashrail, 17= PBRC-83, 18= PBRC-90, 19= PBRC -110, 20= IR70023-4B-R-12-3-1, 21=, 22= IR84645-2-11-1-B, 23= IR72593-B-18-2-2-2, 24= IR77664-B-25-1-2-1-3-12-3 and L= 100bp ladder.)

PERFORMANCE OF THE PROPOSED RICE LINE PBRC-37 AT FIELD TRIAL UNDER NON SALINE AND SALINE STRESS CONDITION

Based on seedling and reproductive stages screening, advanced line PBRC-37 (IR64197-3B-14-2) was evaluated under field condition. Grain yield data showed that PBRC-37 gave the highest zonal yield (6.80 ton/ha) which was higher than Binadhan-8 (Table 6) at Fakirhat, Bagerhat. Grain yield (t/ha) in six locations range from 4.0 to 7.48 tons (Table 7) where proposed line PBRC-37 gave the highest yield (7.48 t/ha) at Metro (Khulna) and the lowest (4.2 t/ha) in Dumuria (Khulna) during farmer's field trial. Morphological and agronomical characters of the proposed rice line PBRC-37 were also evaluated with Binadhan-8 (check) under non-salinized condition in boro season, 2011-12. Grain characteristics, viz., milling yield (%), chalkiness, whole grain length, dehulled grain length, breadth, L/B ratio, size, shape and amylose (%) were measured following SES for rice. Milling yield of PBRC-37 was 76%, chalkiness less than 10%, size of the dehulled grain is medium and slender in shape with 26% amylose content (Table 8). The proposed line PBRC-37 was shorter in plant height, 10 days earlier and yielded 0.5 t/ha more than Binadhan-8. Proposed line performed better than Binadhan-8 for all the studied characters (Table 9).

Table 6: Zonal yield trial of salt tolerant rice genotypes grown in saline (EC 5-12 dSm-1) areas at 6 locations in boro season during 2010-11

Designation	**Grain yield (t/ha)**						
	L1	**L2**	**L3**	**L4**	**L5**	**L6**	**Mean**
PBRC-37	5.91	5.80	6.51	6.71	6.80	5.90	6.27
Binadhan-8 (check)	5.60	5.34	6.45	6.62	6.30	5.80	5.97

L_1 = Shyamnagar (Satkhira); L_2 =Kaligonj (Satkhira); L_3 = Sadar (Satkhira); L_4 =Dumuria (Khulna); L_5 =Fakirhat (Bagerhat); L_6 =Mollarhat (Bagerhat)

Table 7: Performance of proposed rice line under salt stress condition (EC 10-12 dSm-1) in boro season during 2011-12

Designation	Grain yield (t/ha)								
	L1	L2	L3	L4	L5	L6	L7	L8	Mean
PBRC-37	4.2	5.01	7.48	6.85	4.81	5.92	7.37	6.0	5.96
Binadhan-8 (check)	4.0	4.28	7.40	6.30	4.71	5.63	6.43	5.5	5.53

L_1 = Dumuria (Khulna); L_2 = Gutodia (Khulna); L_3 = Metro (Khulna); L_4 = Fakirhat (Bagerhat); L_5 = Sadar (Bagerhat); L_6 = Bemorta (Bagerhat); L_7 = Debhata (Satkhira); L_8 = Shyamnagar (Satkhira)

Table 8: Grain characteristics of the proposed rice line PBRC-37

Proposed lines with std. checks	Milling yield (%)	Chalkiness[a]	Whole grain length (mm)	Dehulled grain/kernel				Amylose (%)
				Length (mm)	Breadth (mm)	L/B ratio	Size and shape	
PBRC-37	76	Wb1	8.5	6.7	2.6	2.58	Medium Slender	26.0
Binadhan-8 (check)	74	Wb1	8.2	6.2	3.0	2.06	Medium	25.0

[a] Wb1 = Less than 10% chalkiness, Wb2 = Less than 20% chalkiness

Table 9: Morphological and agronomical characters of the proposed rice line under salt stress condition (10-12 dSm-1) and normal (non-salinized) in boro season during, 2011-12

Proposed line	Plant height (cm)		Days to maturity		1000 grain wt. (g)		Grain yield (t/ha) (at EC level 10-12 dSm-1)	
	Salinized	Non-salinized	Salinized	Non-salinized	Salinized	Non-salinized	Salinized	Non-salinized
PBRC-37	95	97	121	125	24.3	24.9	5.5	8.5
Binadhan-8 (check)	97	99	130	130	26.5	26.7	5.0	8.0

(Salinized at EC level 10-12 dSm-1)

CONCLUSION

End of the all kinds of phenotypic, genotypic and field based study proposed line PBRC-37 was applied to SCA for variety release and the National Seed Board released the line PBRC-37 as Binadhan-10 which can tolerate up to 12 dSm-1of salinity and can be cultivated in 40-50% of fallow lands in both Boro (dry season) and Aman (wet season) seasons. The farmers can get potential yield of 5.5-6.50 t/ha in saline land and 7.5-8.5 t/ha in non-saline land. Farmers can increase their income per hectare by Tk 45,000 by cultivating Binadhan-10 other than the salinity susceptible variety. This new salt tolerant variety can be cultivated in a large part of saline prone 13 districts in the salinity prone area and more 4-5 million tones of additional rice can be produced a year. Dissemination of this variety in a large-scale would enable farmers to get higher productivity, ensure food security and improve livelihood for combating climate disaster.

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