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Full Length Research Paper

Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance

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Selection for salinity tolerance genotypes of rice based on phenotypic performance alone is less reliable and will delay progress in breeding. Recent advent of molecular markers, microsatellites or simple sequence repeats (SSRs) are used to find out salt tolerant rice genotypes. Three selected SSR markers; RM7075, RM336 and RM253 were used to evaluate rice genotypes for salt tolerance. Phenotypic and genotypic evaluation for salinity tolerance was done at the seedling stage. Phenotyping of 11 genotypes was done in hydroponic system using salinized (EC 12 dS/m) nutrient solution. IRRI standard protocol was followed to evaluate salinity tolerance. Large variation in salinity tolerance among the rice germplasms was detected. Plant height and total dry matter of tolerant lines were reduced by 19.0 and 40.6%, respectively under salt stress (EC 12 dS/m), whereas those of susceptible lines were reduced by 46.0 and 73.5%, respectively. The markers showed polymorphism and were able to discriminate salt tolerant genotypes from susceptible. The genotypes having similar banding pattern with Pokkali were considered as salt tolerant. The SSR markers (RM7075, RM336 and RM253) identified 8, 9 and 7 salt tolerant genotypes, respectively. Through phenotypic and genotypic study, three genotypes viz., Pokkali, TNDB-100 and THDB were identified as salt tolerant rice cultivar. These SSR markers might have sequence homology with salt tolerant rice genotypes and consequently the markers could able to identify salt tolerant rice genotypes from susceptibles.

Key words: Rice, salinity tolerance, SSR markers, seedling stage.

INTRODUCTION

Rice is the staple food of more than 50% of the world's population (Aggarwal et al., 2002). By the year 2025, 21% increase in rice production will be needed over that of year 2000 (Bhuiyan et al*.,* 2002). Salinity is one of the major obstacles in increasing production in rice growing areas worldwide, which is an ever-present threat to crop yield. Therefore, development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline prone coastal areas. The response of rice to salinity varies with growth stage. Several studies indicated that rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage, becomes sensitive during pollination and fertilization and then becomes increasingly more tolerant at maturity (IRRI, 1967).

Screening of germplasms at seedling stage is readily acceptable as it is based on a simple criterion of selection; it provides rapid screening difficult at vegetative and reproductive stage (Gregorio et al., 1997). Screening under controlled condition has the benefit of reduced environment effects and the hydroponic system is free of difficulties associated with soil related stress factors. The conventional methods of plant selection for salt tolerance are not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997). This hinders the development of an accurate, rapid and reliable screening technique. However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Recent

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Table 1. Modified standard evaluation score (SES) of visual salt injury at seedling stage.

Score	Observation	Tolerance
1	Normal growth on leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or 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

Source: Gregorio et al. (1997).

progress and technical advances in DNA marker technology permit reduction of time and accuracy of breeding where pronounced effects of environment lead to poor selection efficiency.

SSR or microsatellite markers have been proved to be ideal for making genetic maps (Islam, 2004; Niones, 2004), assisting selection (Bhuiyan, 2005) and studying genetic diversity in germplasms. SSR markers are playing important role to identify gene for salt tolerance that can be helpful for plant breeders to develop new cultivars. The aim of the present study was to screen rice germplasms under salinized and non-salinized conditions and to evaluate microsatellite markers for the identification of salt tolerant genotypes at the seedling stage.

MATERIALS AND METHODS

Plant materials

Eleven rice germplasms with diversified genetic background were used in this study. Of which six were Bangladeshi landraces, four were BINA developed mutants, and one salt tolerant Indian variety 'Pokkali' was used as check.

Phenotypic study of salinity tolerance at seedling stage

The genotypes were screened for salt tolerance at seedling stage in hydroponic system using IRRI standard protocol (Gregorio et al., 1997). Salinized and non-salinized setups with 3 replications were maintained. The evaluation was done using Yoshida et al. (1976) nutrient solution at the glass house. The nutrient solution was salinized by adding crude salt to obtain desired EC (12 dS/m). The modified standard evaluation system (SES) was used in rating the visual symptoms of salt toxicity (IRRI, 1997). Visual rating of salinity tolerance was done according to Table 1. This scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. Initial and final scoring was done at 13 d and 22 d after salinization. For phenotypic observation, plant height, root length and total dry matter were recorded at salinized and non-salinized conditions.

CTAB mini preparation DNA extraction

DNA isolation was done from fresh leaf tissues of 14-day old seedlings. DNA was extracted using the CTAB mini preparation method. The leaf sample was ground by extraction buffer and SDS, and then incubated at 65°C for 10 min. 100 µl NaCl and 100 µl CTAB were added sequentially, well mixed and incubated again at 65°C for 10 min. After that the suspensions were transferred to a new tube. 900 µl chloroform (Chloroform: Isoamyl, 24:1) was added and mixed by a shaker. Then the sample was then centrifuged at 5700 rpm for 10 min. After that the supernatant were transferred into new eppendrof tubes. Then 600 µl ice-cold isopropanol was added into the new eppendrof tubes and shaken slowly and then centrifuged at 5700 rpm for 15 min. The supernatant was decanted and air dried for at least one hour. Pellets were washed with 70% ethanol (200 µl), centrifuged for 15 min at 5700 rpm and then air-dried for ½ to 1 h. Then the ethanol was removed and air-dried. The pellets were resuspended in 30.0 µl X TE buffer.

Amplification of microsatellite markers and evaluation of genotypes

Three selected primers were used for this study as those were used previously by Islam (2004), Bonilla et al. (2002), Niones (2004) and Gregorio et al. (2002) in recombinant inbred lines (RILs) of Pokkali X IR29 for tagging salt tolerance genes, where Pokkali was salt tolerant and IR29 was salt susceptible. Among them RM7075, RM336 and RM253 were polymorphic and showed clear bands; these were used to reveal polymorphism. Each PCR reaction carried out with 15.0 µl reactions containing 1.5 µl 10 X buffer, 0.75 µl dNTPs, 1 µl primer forward, 1 µl primer reverse, 0.5 µl taq polymerase, 8.25 µl ddH_2O and 2.0 µl of each template DNA samples. PCR profile was maintained as initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and polymerization at 72°C for 2 min; and final extension by 7 min at 72°C. Banding pattern of the genotypes was scored comparing the banding pattern of Pokkali. The germplasms showed similar banding pattern like Pokkali, were considered as tolerant and had different banding pattern were considered as susceptible.

RESULTS AND DISCUSSION

Screening of genotypes for salt tolerance at seedling stage

All genotypes were grown robustly and showed uniform green colour and height in the non-salinized condition. In salinized condition, the genotypes showed wide variation in phenotypes ranging from score 1 (highly tolerant) and score 9 (highly susceptible) (Figure 1). The most salinity tolerant germplasms were Pokkali, THDB, and TNDB-100. Four moderately salinity tolerant genotypes were identified as RD-2586, PNR-519, Dhol Kochuri and Bara Dhan. The most susceptible salt tolerant genotypes were Kaliboro 139-2 and Kaliboro 109-4. The modified standard

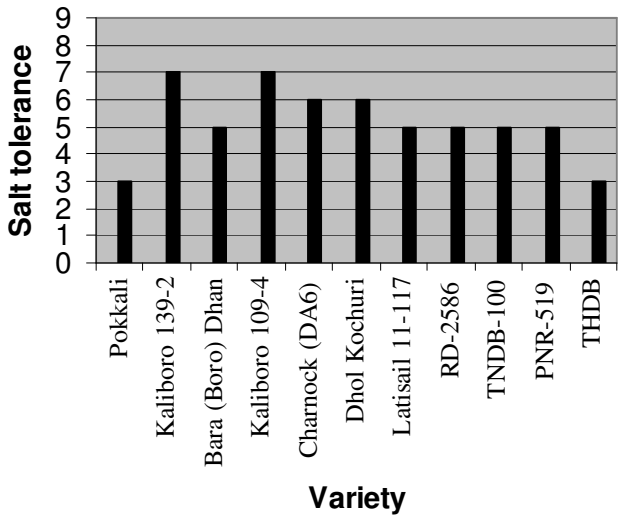

Figure 1. Distribution of 11 genotypes under salt stress at seedling stage were grown in nutrient solution.

evaluation system (SES) of IRRI (Gregorio et al., 1997) was used in rating the visual symptoms of salt injury.

Plant heights were shorter in salinized condition, compared to the plants grown in non-salinized conditions (Table 3). Plant height and total dry matter of susceptible genotypes showed higher percent reduction than tolerant genotypes. Lower percent reduction of plant height was recorded in genotypes Pokkali and THDB followed by genotypes TNDB-100, RD-2586, PNR-519 and Dhol kochuri. On the other hand, higher percent reduction of plant height was showed by genotypes, Kaliboro 139-2 and Kaliboro 109-4. The percent reduction of total dry matter ranged from 40-75. Lower percent reduction of total dry matter was found in genotypes Pokkali, TNDB-100 and THDB. In contrast, Kaliboro 139-2 and Kaliboro 109-4 showed higher percent reduction of total dry matter. Tolerant cultivars showed less growth reduction than sensitive genotypes under salinized conditions (Suplick-Ploense et al., 2002). Eleven genotypes showed wide variation in phenotypes. Salt tolerant seedlings were distinguished from the sensitive seedlings grown in salinized condition. Seedlings grown in salinized condition showed different visual symptoms of salt injury. The symptoms were prominent on the first and second leaves and were visualized by leaf rolling, formation of new leaf, brownish and whitish of leaf tip, drying of leaves and also reduction in root growth, stunted growth, and stem thickness leading to complete cessation of growth and dying of seedlings occurred (Gregorio, 1997). Salinity in rice was associated with Na^+ exclusion and increased absorption of K^+ to maintain a good Na^+/K^+ balance in the shoot under saline condition. It is considered that damage of leaves was attributed to accumulation of Na+ from the root to the shoot in external high concentration (Lin et al., 2004). In several species including rice, salt stress might increase or even include the expression of specific genes and repress or completely suppress the expression of others (Hasegawa et al., 2000).

At the seedling stage, highly significant and positive correlations were found between plant height and total dry matter at salinized condition (Table 2). Whereas, at salinized condition correlations between salt tolerance and plant height, total dry matter and root length were inverse and significant, which imply that salt tolerant genotypes (having lower salt tolerance score) exhibited higher plant height and total dry matter. Peng et al. (1999) reported that increasing plant height would allow greater biomass production. Zhang et al. (2004) found similar result with their studied doubled haploid (DH) population consisting of 81 DH lines. They reported that increase of plant height was responsible for increase of biomass; so as to increase yield potential. It is crucial to note that Pokkali, THDB and TNDB-100 genotypes showed higher plant height and total dry matter and also performed as salt tolerant.

On the basis of SES score and phenotypic performance, three genotypes (Pokkali, THDB and TNDB-100) were identified as salt tolerant and RD 2586, Dhol Kochuri, PNR-519, Bara (Boro) Dhan, Latisail 11-117 and Charnock (DA6) were identified as moderately tolerant at seedling stage.

Screening of salt tolerance through SSR markers

Three markers were used to evaluate germplasms for salinity tolerance. The markers were RM7075, RM336 and RM253. The bands obtained from other genotypes were compared to the band obtained from Pokkali. Pokkali was used as salt tolerant genotype in this study because it is known as salt tolerant genotype. The germplasms, having similar banding pattern to Pokkali, were considered as tolerant and having different banding pattern to Pokkali were considered as susceptible. In case of RM253, Bara (Boro) Dhan, Latisail 11-117, RD-2586, TNDB-100, PNR-519 and THDB were found as tolerant. On the other hand, Kaliboro 139-2, Kaliboro 109-4, Charnock (DA6), and Dhol Kochuri were found as susceptible with RM253 (Figure 2).

Considering the primer RM7075, genotypes Charnock (DA6), Dhol Kochuri, Latisail 11-117, RD-2586, TNDB-100, PNR-519 and THDB were found as tolerant. Whereas Kaliboro 139-2, Bara (Boro) Dhan, and Kaliboro 109-4 were found as susceptible with RM7075. Bara (Boro) Dhan, Charnock (DA6), Dhol Kochuri, Latisail 11-117, RD-2586, TNDB-100, PNR-519 and THDB were identified as tolerant and Kaliboro 139-2 and Kaliboro 109-4 were identified as susceptible with RM336.

The marker RM7075 identified eight tolerant and three susceptible genotypes in comparison with Pokkali. Out of genotypically identified eight salt tolerant genotypes (identified genotypically), three were tolerant and five performed as moderately tolerant based of phenotypic performance at the seedling stage. With regarding RM336,

Table 2. Performance of plant height and total dry matter of 11 rice genotypes at seedling stage grown in hydroponic system.

S/N.	Variety	Plant height (cm)			Total dry matter (g)		
		Non-salinized	Salinized	Reduction (%)	Non-salinized	Salinized	Reduction (%)
1	Pokkali	61	50	18	9.12	5.48	40
2	Kaliboro 139-2	62	34	45	9.17	2.54	72
3	Bara (Boro) Dhan	55	39	29	7.7	3.61	53
4	Kaliboro 109-4	59	31	47	11.73	2.97	75
5	Charnock (DA6)	42	30	29	7.25	3.26	55
6	Dhol Kochuri	59	44	25	10.69	5.28	51
7	Latisail 11-117	55	40	27	8.44	3.75	56
8	RD-2586	44	34	23	6.7	3.85	43
9	TNDB-100	42	33	21	7.38	4.33	41
10	PNR-519	49	37	24	5.78	3.12	46
11	THDB	40	33	18	8.47	4.97	41
LSD $_{(0.05)}$		1.662	1.391		0.4415	0.2081	-

Table 3. Correlation of different traits at seedling stage under salinized condition.

Parameter	Salt tolerance	Plant height	Total dry matter
Plant height	-0.406**		
Total dry matter	-0.74**	0.622**	
Root length	-0.278*	0.115	0.291*

* = Significant at 5% level of probability and ** = Significant at 1% level of probability.

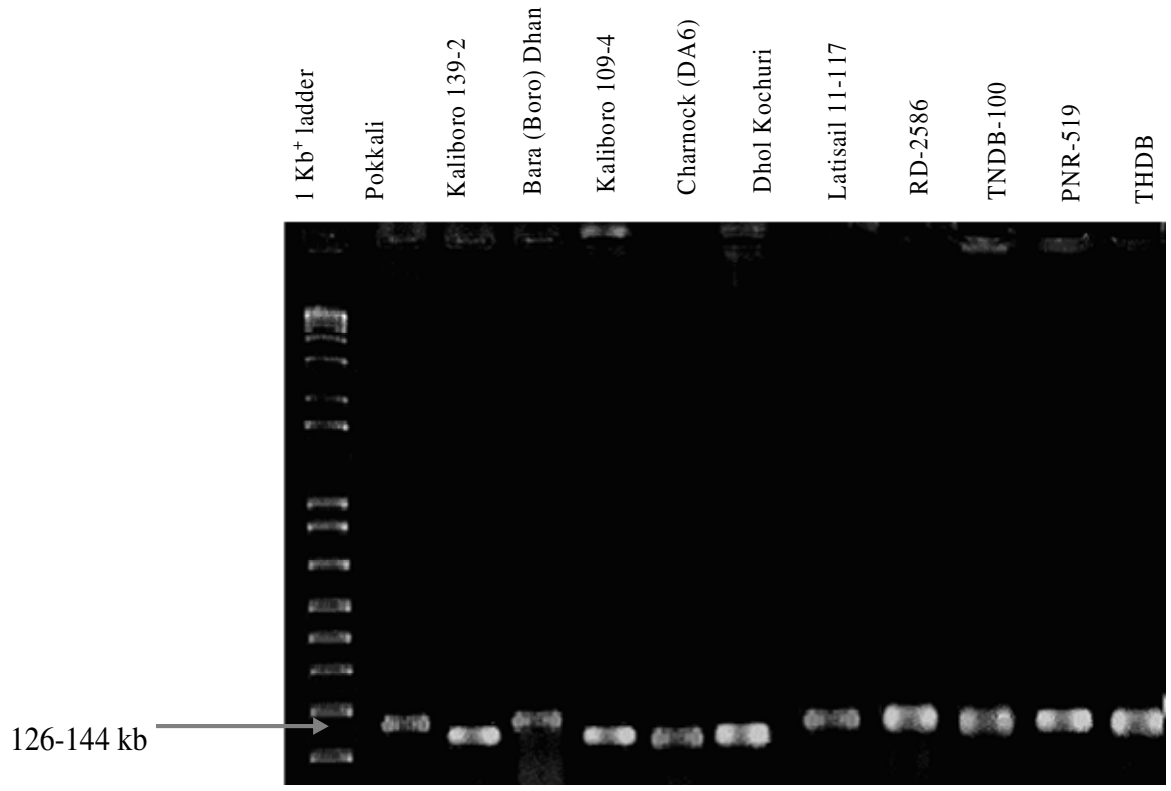

Figure 2. Polymorphism exhibition of RM 253 among eleven genotypes

nine genotypes exhibited as salt tolerant and two genotypes were susceptible. Phenotypically three tolerant, six moderately tolerant at seedling stage were identified amongst the nine genotypically tolerant genotypes. Seven tolerant and four susceptible cultivars were found when eleven genotypes were tested with RM253. Considering these seven genotypically salt tolerant genotypes, there were phenotypically three tolerant and four moderately tolerant at seedling stage. Bhuiyan (2005) identified 158 tolerant individuals of the F2 and F3 population of BRRI Dhan 28 X PSBRc88 with the marker RM493. Moreover, he observed 105 tolerant individuals phenotypically.

Considering phenotypic and genotypic observations, three genotypes TNDB-100, THDB and Pokkali were identified as salt tolerant. Four genotypes i.e. RD 2586, Dhol Kochuri, PNR-519 and Bara (Boro) Dhan were found as moderately tolerant. The selected markers (RM7075, RM336 and RM253) showed polymorphism in eleven rice cultivars. These markers were able to discriminate tolerant genotypes from susceptible. So these markers have relationship with salt tolerance alleles studied in rice genotypes. Molecular marker helps to identify alleles that are associated with key phenotypic traits (Xu et al., 2004). Nguyen et al. (2001) found that the marker RM315 had association with NaCl tolerant alleles at seedling population (IR64/ChengHui 448, IR64/OM1706 and IR64/FR13A) under EC 18 dS/m and salt stress genes were located at loci in chromosomes 1 and 8. Similar result was reported by Lang et al. (2000). They found that RM223 was closely linked to salt tolerance gene in chromosome 8. Since the markers were used in this study showed polymorphism with the genotypes, these markers could be proficiently used in tagging salt tolerant genes, in marker-assisted selection and quantitative trait loci (QTL) mapping. The identified salt tolerant rice genotypes could be used in the improvement of salt tolerant rice genotypes.

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