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RICE LANDRACES WITH GENETIC VARIATIONS FOR SALINITY TOLERANCE AND THEIR ASSOCIATION WITH SUBMERGENCE TOLERANCE

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Abstract- In coastal regions, both salinity and submergence stress alternatively or simultaneously become severe constrain to the rice cultivation by climate changes. This situation necessitates the search of combined tolerance to salinity and submergence stress from the source of germplasms lines. In this study, eight rice landraces collected from coastal region of eastern India were screened for salinity and submergence tolerance at phenotypic and genotypic level. In salinity screening, six rice landraces (Rupsal, Marishal, Polai, Talmugra, Kamini and Raspangar) were identified as salinity tolerant. In PCR screening using SSR markers located in *Saltol* locus, Pokkali alleles (tolerant check) for AP3206 and RM3412 markers and non-Pokkali alleles for RM8094 marker were detected in salinity tolerant genotypes. The results of RM8094 coincided with that of salinity screening. Moreover, none of the landraces showed significant tolerance as FR13A (tolerant check) in submergence screening. In PCR screening using SSR and gene-specific markers of *Sub1* locus, FR13A alleles were documented in intolerant genotypes also. However, the results of *Sub1*BC_2 (InDel) marker coincided with that of submergence screening.

Keywords-Salinity, Submergence, Rice landraces, Microsatellite (SSR) markers, *Sub*1 and *Saltol* locus.

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Introduction

Rice (*Oryza sativa* L.) is under threat of many abiotic stresses due to global warming. Among them, salt stress is very severe constraint to rice particularly in coastal rice farming and in inlands also due to unlimited fertilizer applications. Salt stress disrupts plant ion homeostasis, resulting in excess toxic Na^+ in the cytoplasm and a deficiency of essential ions such as K^+ [1]. To manage salt stress, a major quantitative trait locus (QTL) named as *Saltol* was detected in rice landrace Pokkali. This locus helps rice plants to manage seedling stage stress [2]. In salinity tolerant rice plants, the salt overly sensitive (SOS) pathway maintains the favorable ion ratios in the cytoplasm by limiting Na^+ entry into and exit out of plant cells, regulating Na^+ compartmentalization in the vacuole, and selectively importing K^+ over Na^+ into plant cells. Likewise, submergence stress by flash flooding is another constrain to rice production in rainfed lowland areas and irrigation lands. During submergence stress, intolerant rice plants die by either anoxia or lack of sufficient stored starch materials if plants were submerged completely for more than 1-week. In this context, a major QTL, *Submergence* 1 (*Sub1*) was detected in a rice line, Flood resistant (FR) 13A for managing submergence stress problem. This locus supports the rice plants to survive more than 10 days under submerged condition. Besides, presence of three putative ethylene-responsive transcription factor (ERF) genes, SUB1A, SUB1B and SUB1C within *Sub1* locus has been identified [3]. Among them, SUB1A gene plays a significant role in submergence tolerance. Furthermore, SUB1A gene was identified for having two types of alleles (A1 and A2) in which A1 allele is most important in high survival rate of rice plants under stress condition rather than A2 allele. In tolerant plants, SUB1A gene expression inhibits the accumulation of gibberellic acid (GA) by increasing the accumulation of the GA-signaling suppressors repressors *Slender Rice-1* (*SLR1*) and *SLR1 Like 1* (*SLRL1*) and leads to reduce plant growth under submergence condition and increases the survival rate [4].

In breeding programme, *Saltol* and *Sub1* locus play significant role in improvement of rice cultivars for salinity and submergence tolerance, respectively, through marker-assisted backcrossing (MABC) worldwide [5-8]. However, farmers in coastal region face the problem by salt or submergence stress or sometimes both salt and submergence stress by intrusion of sea water by cyclones or raising sea level to global warming into rice cultivating areas. In this context, rice cultivars should be improved for both salinity and submergence tolerance for cultivating in this areas. Generally, traditional rice landraces exhibit particular properties or characteristics such as early maturity, adaptation to particular soil types, resistance or tolerance to biotic and abiotic stresses. India is home to many such landraces and particularly, West Bengal and North Eastern States of the country exhibits diverse morphologically and genetically rich rice germplasms. Moreover, molecular markers, especially DNA-based markers, have been used extensively for the study of useful traits in germplasms [9,10]. In the present study, we screened eight rice landraces collected from coastal region of eastern India for identification of landrace lines associated with salinity and submergence tolerance at phenotypic and genotypic level.

Materials and Methods

Plant materials

Eight rice landraces, Rupsal, Marishal, Talmugra, Kamini and Raspanjar, Nagalmutha,

Ravana, Polai collected from coastal region of eastern India by CRRI gene bank, Cuttack, FR13A (submergence tolerant check), IR42 (submergence intolerant check), Pokkali (salinity tolerant check) and IR29 (salinity sensitive check) were used in the present study.

Phenotypic screening

Salinity

Pre-germinated seeds of Rupsal, Nagalmutha, Ravana, Marishal, Polai, Talmugra, Kamini and Raspangar, FR13A, IR42, Pokkali and IR29 were sown in holes on styrofoam floats with a net bottom suspended on trays filled with Yoshida nutrient solution. Seedling with 5-days old were salinized with NaCl to EC 6 dSm^{-1} in Yoshida nutrient solution for 5 days and then to EC 12 dSm^{-1} until the final scoring. The pH of the nutrient solution was adjusted daily to 5.0 and the culture solutions were replaced weekly. At 15 day after treatment (DAT), survival percentage were calculated based on visual symptoms using IRRI's standard evaluation scale (SES) for rice, with ratings from 1 (highly tolerant) to 9 (highly sensitive) [8].

Submergence

Twenty-one day old seedlings of eight rice landraces, (Rupsal, Nagalmutha, Ravana, Marishal, Polai, Talmugra, Kamini and Raspangar), FR13A, IR42, Pokkali and IR29 were transplanted in two rows (each rice line) in submergence tank, CRRI, Cuttack during Kharif season-2014. Each row consisted of 20 seedlings with 15 x 10 cm gap. Seedlings were allowed 10-days to establish and necessary fertilizers were applied to seedlings. Then, seedlings were submerged and the water level was maintained at the level of 95cm height for 14-days. Following the completion of stress period, seedlings were de-submerged and allowed them 10-days to regenerate and the survival rate was recorded in percentage [7]. This screening was done with 3-replications.

DNA extraction and PCR screening

A crude DNA preparation suitable for PCR screening was prepared using a simplified miniscale procedure [11]. A single piece of healthy young leaf was harvested and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf sample was macerated using thick glass rod after adding 400 µl of extraction buffer (50 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 300 mM NaCl and 1% SDS). The leaf was grounded until the buffer turned into green colour. After grinding, another 400 µl of extraction buffer was added and mixed by pipetting. The contents were centrifuged at 12,000 g in micro centrifuge for 10min. Nearly 400 µl of lysate was extracted with 400 µl of chloroform. The top aqueous supernatant was transferred to another 1.5ml tube and DNA was precipitated with absolute ethanol. The contents were centrifuged for 3 min at full speed and the supernatants were discarded. The pellet was washed with 70% ethanol. The DNA was air dried and re-suspended in 50 µl of TE buffer (10mMTris-HCl, pH 8.0, 1mM EDTA, pH 8.0). One µl of aliquot was used for PCR analysis and the remaining solution was stored at –20ºC for any further use. In the PCR amplification, for screening *Saltol* locus, three best SSR markers (AP3206, RM3412 and RM8094) reported by Thomson et al. [8] and for *Sub1* locus, two closely linked markers (RM8300 and RM219), and four gene-specific markers (IYT1, IYT3, *Sub1*BC_2 and *Sub1*C_{173}) [7] were used.

Results and Discussion

Salinity

Under 12 EC dSm^{-1} concentration of NaCl condition, rice lines were categorized such as Pokkali (87.9%) as highly tolerant (scale 1), Polai (78.3%), Raspangar (64.4%), Marishal (58.3%), Rupsal (57.6%) and Talmugra (57.2%), Kamini (46.7%) as tolerant (scale 3) Ravana (30.2%) and Nagalmutha (21.7%) as moderately tolerant (scale 5), FR13A (10.2%) as sensitive (scale 7) and IR42 (0%) and IR29 (0%) as highly sensitive (scale 9) according to IRRI's SES score [Fig-.1]. But in this screening, submergence tolerant check, FR13A showed sensitive reaction to salt stress. Sensitive checks (IR42 and IR29) completely died of salt stress. In PCR screening using SSR markers located within *Saltol* locus, Pokkali alleles were detected in Polai, Talmugra, Ravana, Nagalmutha and Raspanjar for AP3206; in Nagalmutha, Marishal and Ravana for RM3412. But, none of the tolerant lines showed Pokkali allelic pattern for RM8094 [Fig-.2]. Among SSR markers linked with *Saltol* locus, RM8094 is reported for its association with highly tolerance or tolerance of rice genotypes to salinity stress [12]. For instance, Marishal and Polai which categorized as tolerant were found for possessing Pokkali alleles with RM3412 and AP3206 like Nagalmutha which categorized as moderately tolerant. Significantly, in this screening, we found that all salinity tolerant genotypes possessed same type of allelic pattern for RM8094 maker except Raspangar. Furthermore, this marker differentiated the moderately tolerant rice genotypes (Nagalmutha and Ravana) from highly tolerant (Pokkali), tolerant genotypes (Polai, Raspangar, Marishal, Rupsal, Talmugra and Kamini) and sensitive genotype (IR29). Here, RM8094 marker revealed a new source of rice genotype for salinity tolerance with genetic variability as compared to other SSR markers used. Very recently also, Ali, et al [13] has reported the significant role of RM8094 marker in used 11 SSR markers linked with *Saltol* locus in genetic diversity of rice landrace genotypes. Although AP3206 and RM312 markers used as foreground markers in selection of tolerant genotype in breeding programme [14-16], they showed the contrary results between phenotypic and genotypic screening of rice germplasm lines in this study. Furthermore, AP3206 and RM312 markers did not differentiate the rice genotypes exhibiting different level of salinity tolerance as compared to RM8094 marker.

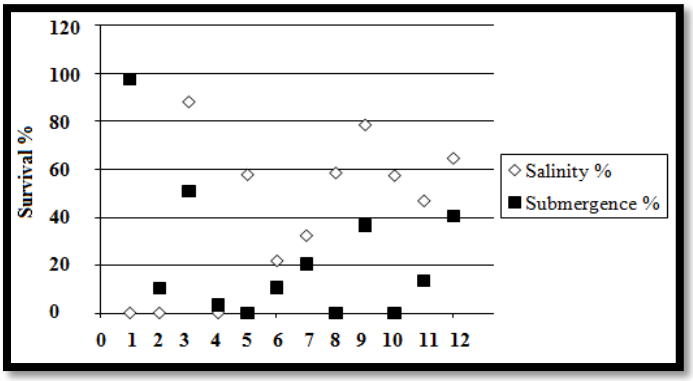

Fig-1. Variations in the survival rate of rice genotypes during salinity and submergence condition.
1-FR13A; 2-IR42; 3-Pokkali; 4-IR29; 5-Rupsal; 6-Nagalmutha; 7-Ravana; 8-Marishal; 9-Polai; 10-Talmugra; 11-Kamini; 12-Raspangar.

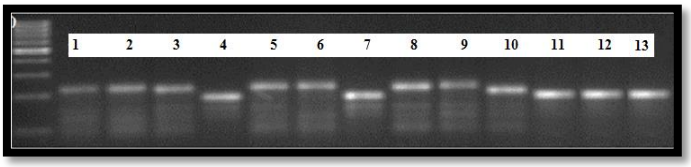

Fig-2. PCR amplification in eight rice landraces with RM8094 marker.
Lane: 1&2-Pokkali; 3-Rupsal; 4-Nagalmutha; 5-Polai; 6-Talmugra; 7-Ravana; 8-Marishal; 9-Kamini; 10-Raspangar; 11-IR29; 12-FR13A; 13-IR42. M-100bp DNA ladder

Submergence

In the submergence screening, the highest survival rate was noted in FR13A (97.5%, scale 1) followed by Pokkali (50.9%, scale 3), Raspangar (40.6%, scale 5), Polai (36.6%, scale 5), Ravana (20.4%, scale 7), Kamini (13.3%, scale 7), Nagalmutha (10.5%, scale 7), IR42 (10.2%, scale 7), IR29 (3.3%, scale 9), Marishal (0%, scale 9), Rupsal (0%, scale 9) and Talmugra (0%, scale 9). In this screening, rice genotypes associated with salinity tolerance at different level have showed tolerance to submergence stress insignificantly [Fig.-1]. However, the survival rate of Pokkali landrace was higher than other salinity tolerant. With SSR and gene-specific markers linked with *Sub1* locus, FR13A alleles were detected in Pokkali, IR29, Nagalmutha, Polai and Raspangar for IYT1 and IYT3; in Pokkali, Nagalmutha, Ravana, Marishal, Talmugra and Kamini for RM219; in Pokkali, Rupsal, Ravana, Marishal, Polai and Raspangar for RM8300 and in Pokkali for *Sub1*BC_2 [Fig.-3]. Moreover, salinity tolerant check, Pokkali possessed FR13A alleles to all SSR and gene-specific markers used in this screening. And also, Pokkali showed the higher survival rate (50.9%) as compared to other salinity tolerant lines in submergence screening. It indicates the

association of Pokkali with both salinity and submergence tolerance at different level of tolerance. Furthermore, we found that IYT1, IYT3, *Sub1*C$_{173}$, RM219 and RM8300 makers are associated with the survival rate in the range of 3.3-97.5%. Among them, *Sub1*BC$_2$ marker linked with SUB1B gene is associated with more than 50% survival rate of rice genotypes. Perhaps, the reason for this association of Sub1BC$_2$ marker with higher survival rate may be the close relationship of SUB1B with SUB1A than SUB1C due to the high amino acid sequence similarity at the N-terminus shared by SUB1A and SUB1B as compared to SUB1C [3]. Even though Sub1BC$_2$ marker played significant role in identification of submergence tolerant germplasms lines, it is also associated with intolerant line when recombination occurs between SUB1A and SUB1B gene during backcrossing [7]. Therefore, only *Sub1*BC$_2$ marker is not reliable in backcrossing programme except identification of F1 plants with heterozygous condition. Therefore, it is needed to screening the rice population using IYT markers, which located in the promoter region of SUB1A gene. However, these markers are associated with both type of alleles (A1 and A2) of SUB1A gene, in which A1 allele is present only in tolerant genotype and A2 allele in intolerant or moderately tolerant genotype. Therefore, only PCR results of IYT markers do not yield the correct results but only after the digestion with restriction enzyme. *Sub1*C$_{173}$ marker is exon of SUB1C gene, which has no role in submergence tolerance, and this gene is present in both tolerant and intolerant genotype. Moreover, SSR marker RM8300 and RM291 are linked with promoter region of SUB1A on one side and on another side with SUB1C, respectively [6]. Therefore, IYT markers, *Sub1C*$_{173}$, RM219 and RM8300 markers did not differentiate intolerant genotype from tolerant genotypes at PCR level and they are not applicable for germplasm screening to identify tolerant lines.

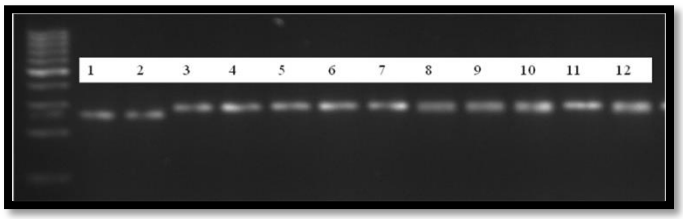

Fig-3. PCR amplification with *Sub1*BC$_2$ marker in *Sub1* locus. 1-FR13A; 2-Pokkali; 3-IR42; 4-IR29; 5-Rupsal; 6-Nagalmutha; 7-Ravana; 8-Polai; 9-Talmugra; 10-Kamini; 11-Marishal; 12-Raspangar. M- 100 bp DNA ladder.

Thus, in this study, we found no genotype with combination of salinity and submergence stress tolerance naturally except Pokkali. Generally, the function of salinity and submergence tolerant genotype differs from each other in energy consumption under stress condition. Because salinity tolerance is determined by several sub-traits when compared to submergence tolerance by SUB1A gene [2]. However, it has been suggested that both *Sub*1 and *Saltol* QTLs can be combined in the same variety in breeding programme [17]. Very recently, Islam, et al. [18] has developed a new rice line with combination of *Sub1* and *Saltol* locus through backcrossing successfully.

In conclusion, in the present study, out of eight rice genotypes, six genotypes (Polai, Raspangar, Marishal, Rupsal, Talmugra and Kamini) were identified as salinity tolerant under 12 EC dmS^{-1} concentrations. These tolerant genotypes were identified for having non-Pokkali alleles (new alleles) with RM8094 marker. These landraces could be used in breeding programme as a new source to enhance salinity tolerance in rice cultivars since these salinity tolerant genotypes have differed from Pokkali in allelic pattern. Addition to this, RM8094 marker is played significant role in differentiating rice lines having different level of tolerance in this study. Other two markers used AP3206 and RM312 did not differentiate the rice genotypes with different level of salinity tolerance at genetic level as compared to RM8094 marker. In submergence screening, none of eight genotypes were identified as tolerant to submergence stress at 14-days stress condition. Moreover, *Sub1*BC$_2$ marker located within *Sub1* locus played a significant role to identify the intolerant rice genotypes in this study. This study reveals the association of rice germplasm lines with salinity at tolerant level as well as moderate and susceptible level to submergence stress and it will help to understand the natural mechanism of rice germplasms lines for salinity and submergence tolerance.

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