Australian Journal of Crop Science AJCS
AJCS 8(3):356-362 (2014) ISSN:1835-2707

Diversity and validation of microsatellite markers in *Saltol* QTL region in contrasting rice genotypes for salt tolerance at the early vegetative stage

Krishnendu Chattopadhyay[1*], Disharee Nath[1], Rajib Lochan Mohanta[1], Somnath Bhattacharyya[2], Bishnu Charan Marndi[1], Amaresh Kumar Nayak[1], Devendra Pratap Singh[1], Ramani Kumar Sarkar[1] and Onkar Nath Singh[1]

[1]Central Rice Research Institute, Cuttack, Odisha – 753 006, India
[2] Department of Genetics, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India
***Corresponding author: Crop Improvement Division, Central Rice Research Institute, Cuttack, Odisha – 753 006, India**

Corresponding author: chatterjee2@yahoo.com; krishnendu_c@sify.com

Abstract

The diversity in microsatellite markers in the *Saltol*-QTL region among 30 accessions from saline tracts was examined and validated by using 37 breeding lines that were salt tolerant at the seedling stage. The diversity was assessed in terms of morpho-physiological traits related to salt stress (at 12 dS m-1) and polymorphism of molecular marker alleles in the *Saltol* QTL region. Principal component analysis of the data on microsatellite markers showed that all moderately tolerant accessions collected from coastal areas in two Indian states, West Bengal and Odisha, were distant from a *Saltol*-introgressed line, namely FL478. That polymorphism of molecular markers in the *Saltol* QTL region did not generate clusters based on salt tolerance suggests the involvement of other QTLs. The specific marker alleles RM3412 and RM10745 were also found in 'Pokkali' and 'Chettivirippu' and FL478-specific marker alleles for 11 polymorphic primers in the *Saltol* region (10.8–12.7 Mb) were found in F_7 lines derived from the cross Annapurna × FL478. The most tightly associated marker, RM10772, was found in a 12.1 Mb region. However, the absence of marker alleles specific to FL478 in the *Saltol* region of three of the phenotypically tolerant lines clearly suggests that we should look, through whole-genome graphical genotyping, for QTLs other than *Saltol* for markedly higher salt tolerance at the seedling stage.

Keywords: coastal regions; *Oryza sativa*; principal component analysis; rice breeding; seedling stage.
Abbreviations: LR_Landrace; MI_marker index; MS_moderately susceptible; MT_moderately tolerant; NIL_near isogenic line; PCA_principal component analysis; PIC_polymorphic information content; QTL_quantitative trait loci; RIL_recombinant inbred line; S_susceptible; SES_standard evaluation system; T_tolerant; UPGMA_unweighted pair group method of arithmetic mean.

Introduction

Although rice is sensitive to salt stress at the seedling stage, it is a preferred crop in salt-affected coastal areas. Rice can withstand water-logging, and standing water helps in diluting and leaching salts from surface soil (Ismail et al., 2008). Therefore, despite their low yields, many landraces are still preferred by farmers in coastal areas. The degree of salt tolerance varies widely in these landraces, and the variability offers an opportunity for varietal improvement for salt tolerance. The Pokkali cultivars have been recognized since long as highly potential salt-tolerant donors and extensively exploited in genetic as well as physiological studies because the cultivars are more salt tolerant than other cultivars. Pokkali refers to a system of rice cultivation under saline conditions in Kerala, a state in southern India along its western coast. These tracts are characterized by prolonged partial flooding, and farmers alternate rice cultivation with shrimp farming (Shylaraj and Sasidharan, 2005). Limitations of conventional breeding have prompted many mapping studies to identify QTLs associated with salt tolerance, a highly polygenic trait in rice (Ammar et al., 2009; Haq et al., 2010; Singh and Flower, 2010). Salt tolerance is governed by several physiological traits, and ion homeostasis which ensures a low Na^+-K^+ ratio, is one of the major mechanisms for salinity tolerance at the seedling stage (Munns and Tester, 2008). A major QTL for salt tolerance at the seedling stage, namely *Saltol*, was mapped on the short arm of chromosome 1 in between RM23 and RM140 (10.7–12.2 Mb). The QTL explains 43% of the variability in shoot $Na^+:K^+$ ratio (Bonilla et al., 2002). Thomson et al. (2010) reported the presence of different 'Pokkali' alleles in the *Saltol* region between 11.0 Mb and 12.2 Mb and suggested that *Saltol* is controlled by the same gene that controls the *SKC1* QTL located at 11.46 Mb and first detected in Nona Bokra (Ren et al., 2005). However, lack of tightly linked markers in this region may be the reason why marker-assisted selection based on this QTL has not been widely successful. Many other salt-tolerant varieties besides Pokkali are cultivated along India's eastern coast, especially in Odisha and West Bengal. The coastal saline tract of Odisha lies along the state's 480 km coastline, extending 10–20 km inland. In West Bengal, on the other hand, Sundarbans (22° N, 89° E; 0–10 m above the mean sea level), covering parts of India and Bangladesh, is the largest single block of tidal halophytic mangrove forests in the world, a site declared by the UNESCO as a world heritage site. People in Sundarbans depend mostly on rain-fed paddy cultivation and they continue to grow traditional rice varieties because of their stable yields and preferred grain quality. However, coastal areas are increasingly vulnerable to frequent cyclones, high tidal waves, and erratic rainfall. The super cyclone (wind

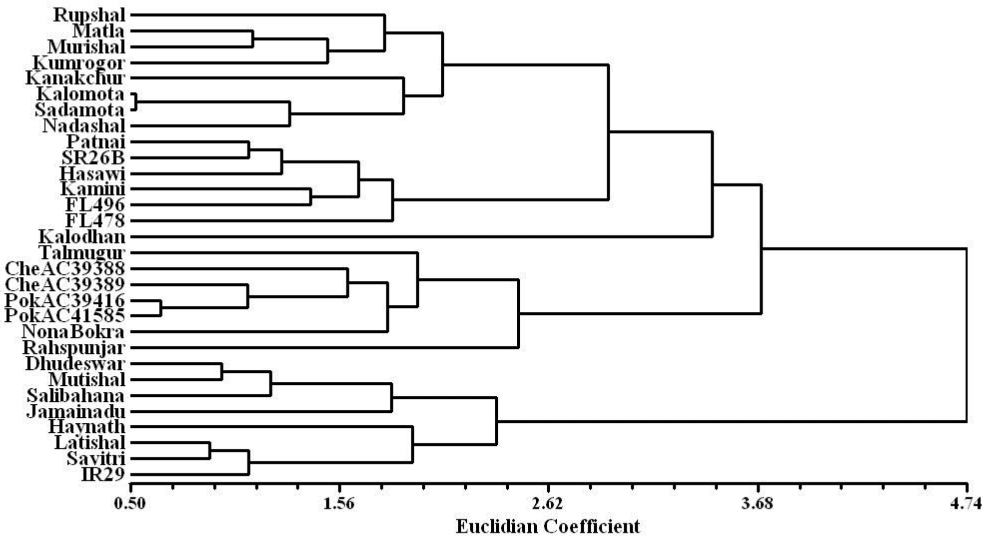

Fig 1. UPGMA dendrogram using pair-wise Euclidian distance coefficients among 30 rice genotypes based on eight phenotypic traits.

speeds greater than 220 km h-1) that hit coastal Odisha and cyclone *Aila* that hit Sundarbans led to the failure of high-yielding rice varieties sensitive to salt stress, resulting in a sharp increase in the extent of uncultivable land (Bhushan, 2012) and, in turn, prompting rice growers and researchers to cultivate and conserve indigenous salt-tolerant lines. New salt- tolerant donors from the rice germplasm available in coastal eastern India are yet to be systematically evaluated based on their molecular and physiological traits (Mahata et al., 2010). As part of this effort, it is necessary to estimate genetic distances in the *Saltol* QTL region of these landraces using landraces from the well-characterized 'Pokkali' and its tolerant derivative, FL478, used frequently (Vu et al., 2012) in the marker-assisted breeding programme to introgress this QTL into a high-yielding background. Therefore, the present investigation sought to (a) assess allelic diversity in the *Saltol* region for using potential donors that have unique marker alleles in breeding for salt tolerance at the seedling stage and (b) validate the *Saltol* QTL with the associated molecular markers in salt-tolerant introgressed lines.

Results and discussion

Phenotypic diversity in levels of salt tolerance

Analysis of variance showed significant genotypic differences ($p < 0.01$) for shoot dry weight (g), K^+ and Na^+ concentration (µg mg^{-1}) in shoot, shoot length (cm), root length (cm), increase in shoot length (%), Na^+-K^+ ratio in shoots, and SES score for salinity tolerance (Supplementary Table 1). The standard evaluation system (SES) score was negatively correlated with shoot dry weight ($r = -0.89$), K^+ concentration ($r = -0.94$), shoot length ($r = -0.88$), increase in shoot length ($r = -0.94$), and root length ($r = -0.47$) but positively correlated with Na^+ concentration ($r = 0.88$) and Na^+-K^+ ratio ($r = 0.94$) in shoots ($p < 0.01$). Some of the observations were in agreement with those reported earlier, recommending low Na^+-K^+ ratio and Na^+ concentration as reliable indicators of tolerance (Lee et al., 2003; Lisa et al., 2004). The tolerant phenotype was associated with nearly normal growth, low Na^+ concentration, high K^+ concentration, and low Na^+-K^+ ratio in shoots. Therefore, based on these phenotypic data, genotypes were grouped by the degree of their salt tolerance. A wide variation in Euclidian distance coefficient values was observed in the matrix derived from these morpho-physiological traits (Supplementary Table 2). The UPGMA dendrogram derived from the coefficient values of all the pairs showed five distinct clusters (Fig 1). The clustering was governed largely by Na^+-K^+ ratio (Supplementary Table 1). Cluster II and Cluster IV contained only tolerant and moderately tolerant genotypes. FL478 was in Cluster II whereas all Pokkali and Chettivirippu accessions were in Cluster IV. The average Na^+-K^+ ratio of moderately tolerant genotypes in Cluster I was considerably higher than that of tolerant and moderately tolerant genotypes in Cluster II and Cluster IV. Cluster V contained all the susceptible lines. Thus, the clustering was clearly in accordance with the degree of salt tolerance at the seedling stage and corroborated the results of an earlier investigation (Theerawitaya et al., 2011).

Marker-allele diversity in tolerant and susceptible lines

Primers were selected based on their capability to deliver a clear, positive, reproducible, and polymorphic banding pattern in all the genotypes. Although all the five primers designed from the gene and EST clone sequences resulted in clear and reproducible bands, none was found to be polymorphic in agarose gel. A total 284 bands distributed in 32 different marker alleles in 30 rice genotypes were identified using selected polymorphic primers (Supplementary Table 3) located in the *Saltol* QTL region (10.8–12.5 Mb) on the short arm of chromosome 1. Primers RM10682, RM10719, RM10745, and RM3412 proved more informative, as shown by their higher PIC and MI values (Supplementary Table 3) as well as their capability to produce greater numbers of polymorphic bands. The FL478 haplotype for six important *Saltol* marker loci, namely RM493, RM10772, RM10720, RM10745, RM8094, and RM3412, was detected only in Pokkali (AC39416); that for RM3412 marker allele at 190 bp was detected only in the tolerant and moderately tolerant genotypes such as Pokkali (AC41585, AC39416), Chettivirippu (AC39388, AC39389) and Murishal; and that for RM10745 marker at 200 bp was detected only in the tolerant and moderately tolerant genotypes Pokkali (AC39416), Chettivirippu (AC39388), and

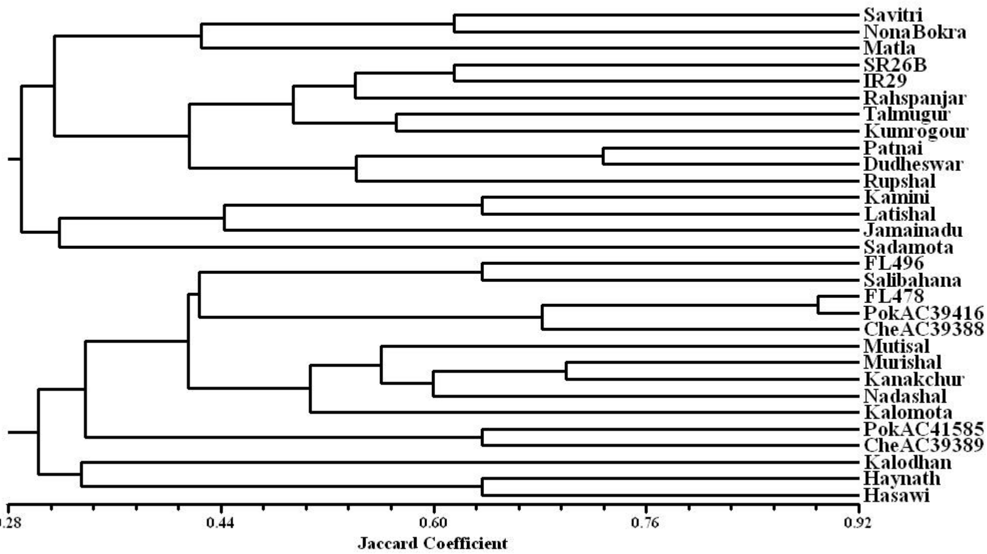

Fig 2. UPGMA dendrogram using Jaccard's similarity coefficients among 30 rice genotypes based on eight microsatellite markers in the *Saltol* QTL region of chromosome 1.

Hasawi. Therefore, the two most important *Saltol* primers for the FL478 haplotype, namely RM3412 and RM10745, were found in Pokkali (AC39416) and Chettivirippu (AC39388). Earlier reports also indicate that these two primers were among the most potential markers that had been used in marker-assisted selection (Mohammadi-Nejad et al., 2010; Thomson et al., 2010). RM10745 produced another marker allele, at 205 bp, which was present only in highly tolerant genotypes such as Pokkali (AC41585) and Chettivirippu (AC39389). A unique band at 415 bp for locus RM10772 was identified in Chettivirippu (AC39416). All these observations point to a remarkable variability in marker alleles in the *Saltol* QTL region even within Pokkali and Chettivirippu accessions. A low average value (0.30) of pair-wise Jaccard's similarity coefficients generated from SSR profiling is another manifestation of the wide variability for this region among the genotypes (Supplementary Table 4). Although FL478 and Pokkali (AC39416) were not phenotypically similar (the Euclidian coefficient was 3.5), we observed the closest similarity in the *Saltol* QTL region of these two genotypes. Single-feature polymorphism in the *Saltol* region showed that FL478 contained a 0.9 Mb fragment from a Pokkali accession at 10.6–11.5 Mb on chromosome 1, flanked by an IR 29 allele (Kim et al., 2009). Therefore, the allelic similarity between them in the present study is probably due to their possessing a similar chromosomal fragment responsible for tolerance. Low allelic similarities in the *Saltol* region were seen in the tolerant genotype pairs Pokkali (AC41585) – Talmugur and Nona Bokra – Chettivirippu (AC39389) (Supplementary Table 4). Such genotypes should be tested further to examine the differences in their genomic region responsible for salt tolerance with correspondingly different mechanisms of tolerance. IR 29, a highly susceptible variety, was 62% similar in the *Saltol* QTL region to the tolerant cultivars SR 26B and Rahspunjar. The similarities of these tolerant lines with their susceptible counterparts are commensurate with their distances from Pokkali (similarity coefficients of 0.18–0.25) in the *Saltol* QTL region. Therefore, some other genes or QTLs or their epistatic interaction may explain the tolerance reaction under salt stress. For this reason, the UPGMA dendrogram (Fig 2), which was based only on pair-wise similarity coefficient values on marker data, failed to match the earlier dendrogram (Fig 1) based on the data for phenotypic traits under salt stress. As a whole, the genotypes fell broadly into nine clusters in the dendrogram (Figure 2). FL478 shared Cluster V with two accessions, a Pokkali (AC39416) and a Chettivirippu (AC39388), and Cluster VII contained only two highly tolerant accessions, namely Pokkali (AC41585) and Chettivirippu (AC39389), whereas the rest of the clusters comprised genotypes with varying levels of tolerance, from tolerant to highly susceptible. Similar observations on the presence of genotypes with varying levels of tolerance in a single cluster based on *Saltol* markers has been frequently noted before (Mohammadi-Nejad et al., 2008; Gregorio et al., 2010), which suggests that the trait is polygenic and also strongly supports the probability that QTLs or genes other than *Saltol* can explain a substantial portion of the phenotypic tolerance or salinity tolerance found among the genotypes tested in this study.

Assessment of molecular and phenotypic diversity in indigenous salt-tolerant germplasm

The genetic diversity in the germplasm was presented visually, in the form of a 2-D plot, by principal component analysis on the basis of phenotypic traits. The first two principal components explained 52% of the genetic variation. Two main groups, comprising tolerant to moderately tolerant genotypes, were identified based on positional proximity in the 2-D biplot (Fig 3). Accessions of Pokkali and Chettivirippu along with a few other tolerant and moderately tolerant lines such as Nona Bokra, Rahspunjar, and Talmugur were located close to one another whereas FL478, FL496, SR 26B, Kamini, Patnai, and Hasawi occupied a different location on the 2-D plot. Hosseini et al. (2012) also reported that PCA could group genotypes by their salt tolerance. On the other hand, the first two principal components, which accounted for 65% of the total genetic variability based on SSR marker polymorphism in the *Saltol* QTL region, were the two axes of the 2-D plot (Fig 4). In contrast to the 2-D plot based on phenotypic diversity, in that based on maker-allele polymorphism at the *Saltol* QTL region FL478 was located close to Pokkali (AC39416). Principal component analysis also showed the highly salt-tolerant genotypes Pokkali (AC41585) and Chettivirippu (AC39389) close to

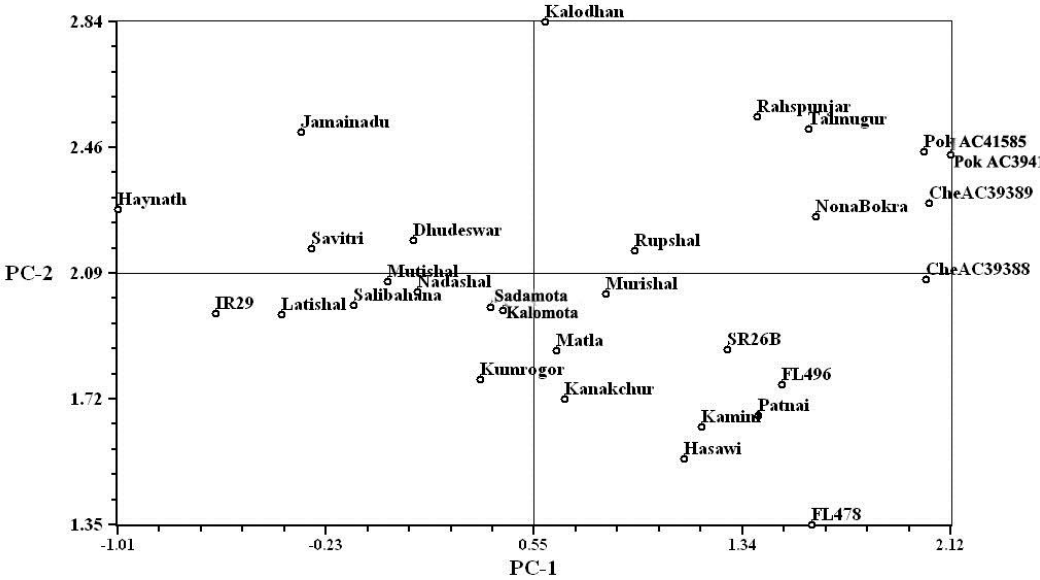

Fig 3. 2-D plot of principal component analysis based on genetic profiles from eight phenotypic traits of 30 accessions of rice germplasm

FL478 in this 2-D plot. The results of PCA were broadly congruent with the grouping observed in the UPGMA dendrogram.

Many genotypes from Sundarbans turned out to be not particularly tolerant; indeed, some of them were highly sensitive to salt stress at the seedling stage. And yet, these genotypes are being cultivated in this region as salt-tolerant cultivars. This anomaly could be explained by their origin and seasonal effects. In Sundarbans, salinity during the wet season occurs initially in patches; at later stages, crop growth is affected mainly by excess water and not as much by salt stress. Therefore, even salt-sensitive genotypes can escape salt stress and yield reasonably well—so long as they can tolerate other forms of abiotic stress such as excess water. The present investigation showed that even the salt-tolerant genotypes were generally only moderately so. Initial growth of Talmugur, Nona Bokra, Rahspunjar, and Patnai under stress was comparable with that of Pokkali and FL478. Similarly, Na^+ uptake and ion homeostasis, manifested as low Na^+-K^+ ratio in shoots in some of the germplasm derived from coastal Sundarbans and Odisha, indicated a physiological similarity in the nature of tolerance although none of these moderately tolerant genotypes was genetically closer to FL478 in the *Saltol* QTL region. Such allelic diversity has been studied frequently (Mohammodi-Nejad et al., 2008; Thomson et al., 2010). Talmugur showed the highest allelic dissimilarity with FL478, indicating its allelic mismatch with Pokkali in the *Saltol* QTL region. On the other hand, the UPGMA dendrogram and the 2-D plot based on PCA showed all the moderately tolerant genotypes, such as Patnai, Rupshal, Matla, Talmugur, and Kamini from Sundarbans, to be genetically closer to one another. These observations suggest that such genotypes can be used for identifying additional QTLs with similar or dissimilar physiological mechanisms that can complement *Saltol* in achieving higher levels of salt tolerance at the early vegetative stage.

Marker validation for Saltol QTL region

Thirty-seven tolerant and moderately tolerant (SES 3–5) F_7 lines derived from the cross Annapurna × FL478 were subjected to SSR analysis for validating the microsatellite markers in the *Saltol* QTL region. The Saltol region on chromosome 1 of all the 37 lines was examined (Fig 5). Marker alleles specific to FL478 at different loci in the 10.8–12.7 Mb region on chromosome 1 were found in all the lines except three, and were either homozygous or heterozygous (Supplementary Table 5). Salt tolerance is reported to be dominant over susceptibility (Lang et al., 2010). The 34 tolerant and moderately tolerant lines sharing a common segment from the donor FL478 probably carry the *Saltol* QTL in homozygous or heterozygous form in this region (Fig 5). However, the absence of marker alleles specific to FL478 in the *Saltol* region of three of the phenotypically tolerant lines clearly suggests that the *Saltol* region did not contribute to salt tolerance in these lines. Similarly, in earlier studies, tolerant lines derived from FL478 had no FL478 alleles in the *Saltol* region (Alam et al., 2010; Thomson et al., 2010; Islam et al., 2012). Their salt tolerance in the absence of the *Saltol* QTL was believed to be due to some other QTLs inherited from Pokkali. However, whole-genome graphical genotyping of FL478 and its tolerant derivatives is required for gaining further insights into different regions inherited from Pokkali and probably responsible for salt tolerance. All the lines developed in this study (Supplementary Table 6), with or without Pokkali and FL478 alleles in the *Saltol* QTL region, showed better crop establishment under salt stress and were found suitable for cultivation in the dry season for ensuring higher productivity and profitability in coastal West Bengal and Odisha. Marker-assisted selection has been similarly used in the past to generate breeding lines with *Saltol* allele from FL478 in the 11.4–12.5 Mb region on chromosome 1 in the background of different popular varieties (Huyen et al, 2012; Vu et al., 2012).

Materials and methods

Plant materials

Thirty accessions were studied, comprising a few germplasm accessions from Sundarbans, a few from coastal Odisha, and the rest from coastal Kerala, along with the salt-susceptible check IR29 and the salt-tolerant check FL478 (Supplementary Table1). In addition, 37 advanced breeding lines derived from the cross Annapurna × FL478 by the

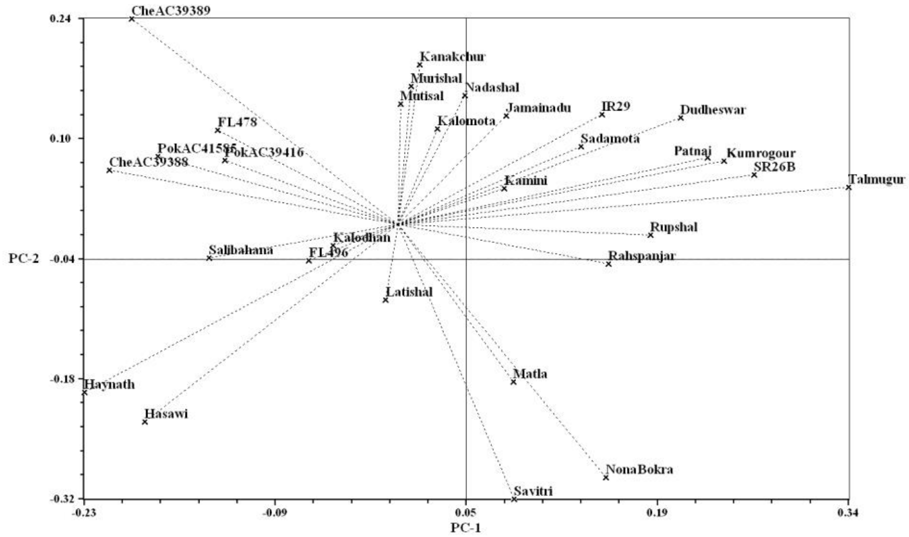

Fig 4. 2-D plot of principal component analysis based on genetic profiles on the polymorphism of microsatellite markers at the *Saltol* QTL region of 30 accessions of rice germplasm.

pedigree method of selection were also employed to validate the *Saltol* QTL. These lines were tolerant to moderately tolerant to salinity stress at the seedling stage and known to be high yielding (3–5 t ha^{-1}) under coastal saline conditions in Odisha during the dry season.

Experimental details

The experiment was conducted under a net house at the Central Rice Research Institute (CRRI), Cuttack, India (20°30′N, 85°40′ E) in July–August, 2011. The average maximum and minimum temperatures during the study period were 36.5 °C and 24 °C; relative humidity was moderate to high (72%–94%); and the average sunshine hours were low (4.0). The same experiment was repeated with the same set of materials in 2012. Pre-germinated seeds were placed on styrofoam seedling floats resting on plastic trays filled with Yoshida nutrient solution (Yoshida et al., 1976) containing 40 ppm N, 10 ppm P, 40 ppm K, 40 ppm Ca, 40 ppm Mg, 0.5 ppm Mn, 0.05 ppm Mo, 0.2 ppm B, 0.01 ppm Zn, 0.01 ppm Cu, and 2 ppm Fe.

After three days of seedling growth, NaCl was added to maintain EC of 6 ds m-1for acclimatization and, after another three days, to raise the EC of the nutrient solution to 12 dS m-1. When the susceptible check (IR 29) showed severe symptoms of salt stress, all the genotypes were scored visually on a scale from 1 to 9 (highly tolerant to highly susceptible) based on a modified SES (Gregorio et al., 1997). Mean shoot length of 5 randomly selected plants from each row was recorded when salt stress was introduced (after 6 days of seedling growth as mentioned above) and at the end of the experiment. Plant growth was expressed as the increase in shoot length (as a percentage of the initial length). Shoot samples were dried, powdered, and analysed for sodium and potassium concentration by flame photometry after 48 h of extraction with 1N HCl, following the procedure described by Yoshida et al. (1976).

DNA isolation and amplification

Fresh leaves (0.5–1.0 g), from a pooled sample of five seedlings from each genotype, were cut into small pieces, crushed in liquid nitrogen, and their DNA isolated using the DNeasy Plant Mini kit (Quagen, Valencia, California). Twelve SSR primers in the *Saltol* QTL region (McCouch et al., 2002; IRGSP, 2005) and five primers (SAL-1-5) capable of amplifying intron sequences, designed for the present study using Primer3 (http://www.genome.wiunit.edu.cgi.bin/primer/primer3-www.cgi) from Os01g0756700 and OSISAP1 genes, SKC1 mRNA, and two plasma membrane H^+ ATPase mRNA EST clone sequences derived from the NCBI database were tested for polymorphism (Supplementary Table 3). Further, polymorphism in Annapurna and FL478 was studied using 61 primers from the *Saltol* region and locations adjacent to this region on chromosome 1 (Supplementary Table 7). The polymerase chain reaction was conducted in a solution (25 µl) containing 10 mM Tris-HCl buffer (pH 8.2), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatine, 200 µM dNTPs, 100 ng μL^{-1} primer, 1 unit Taq DNA Polymerase (Promega), and 100 ng of the template DNA. The amplification reaction consisted of pre-heating for 5 min at 94 °C and 35 cycles of 1 min at 94 °C (denaturation), 1 min at 55–61 °C (annealing) and 3 min at 72 °C (elongation), followed by 7 min at 72 °C (extension) in a PCR system (Bio-Rad). The amplified products were separated in 1.5% agarose gel (Promega) containing 0.5 ng mL^{-1} of EtBr (ethidium bromide). The separated PCR products were made visible under UV light and photographed using a Kodak Electrophoresis Documentation & Analysis System.

Data analysis

For microsatellite DNA fingerprinting of the rice genotypes, polymorphism was scored for the presence (1) or absence (0) of bands on agarose gel. Polymorphic information content (PIC) was calculated based on allelic patterns of all the genotypes, and marker index (MI) was calculated by using the formula MI = PIC × proportion of polymorphic bands × average number of loci per assay unit (Powell et al., 1996). This is also called the resolving power of molecular markers that indicates their ability to detect polymorphism for differentiating genotypes. The average proportion of alleles (bands on gels) shared between any two accessions was used for deriving Jaccard’s similarity coefficients matrix, which was then deployed for cluster analysis by UPGMA. A taxonomic distance matrix on standardized morpho-physiological dataset was computed using the SIMNIT function and Euclidian distance coefficients. A dendrogram

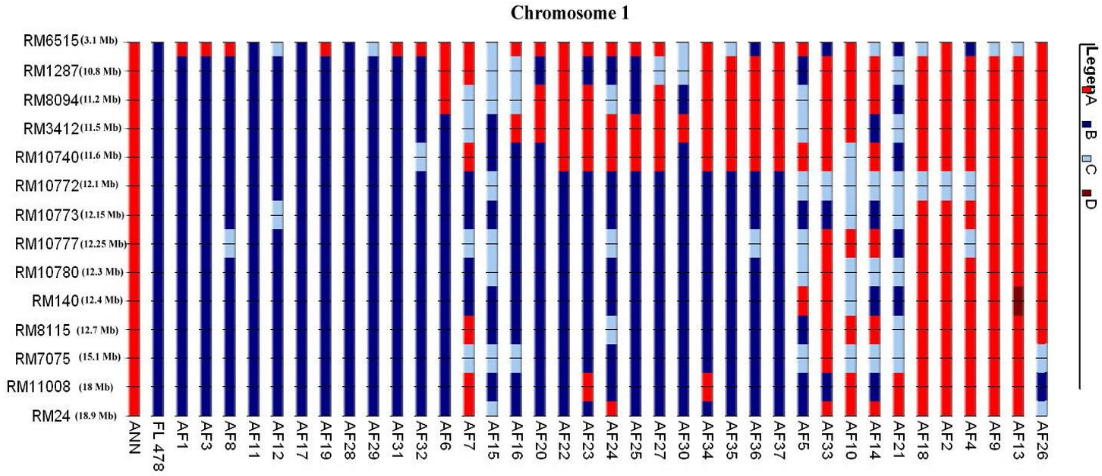

Fig 5. Saltol- QTL region (and the region adjacent to it) on the short arm of chromosome 1 in Annapurna, FL478, and 37 tolerant and moderately tolerant F_7 lines derived from the cross Annapurna × FL478. *Note* A: Annapurna marker-allele, B: FL478 marker-allele, C: heterozygote for both alleles, D: Unidentified.

was generated using UPGMA, based on the genetic distance matrix. Principal component analysis (Jolliffe, 2002) was carried out using eigen vectors and projected genotypes on 2-D scatterplots based on the SSR and morpho-physiological datasets. These analyses were carried out using a software package, namely NTSYS-PC ver. 2.11f (Rohlf, 2000). On the other hand, analysis of variances of all the phenotypic data and correlation coefficients were calculated by standard procedures (Chowdhury et al., 1982). The physical locations of validated molecular markers on the short arm of chromosome 1 in introgression lines from the cross Annapurna × FL478 were marked by using a software package, namely Graphical GenoTyping (GGT 2.0) (Van Berloo, 2008).

Conclusion

Diversity analysis based on marker alleles in the *Saltol* QTL region grouped the salt-tolerant genotypes in a pattern that broadly corresponded with their geographical areas. The observed diversity in landraces from Sundarbans (West Bengal) and Odisha in the *Saltol* QTL region can be used effectively by including a genetically diverse group of salt-tolerant donors in the breeding programme to broaden the genetic base for salt tolerance at the vegetative stage. In the present investigation, salt tolerance in the derived lines was associated with the introgression of a fragment with the *Saltol* QTL region from FL478. The absence of FL478 alleles in the *Saltol* region in the derived lines points to the need for genome-wide mapping to identify additional QTLs even from Pokkali. One of the Pokkali accessions (AC41585) was reported to show relatively high salt tolerance at the reproductive stage (Chattopadhyay et al., 2013), which is desirable, along with tolerance at the vegetative stage, in rice varieties to ensure stable yields. The molecular markers used for validating the *Saltol* QTL region in the lines derived from the cross Annapurna × FL478 can be useful in marker-assisted backcross breeding from this particular cross. The diversity of marker alleles in this region opens up further lines of investigation that can lead to fine-mapping this region, which will certainly be useful in marker-assisted backcross breeding across the parental combination.

Acknowledgements

The authors are grateful to the National Initiative on Climate Resilient Agriculture (NICRA) Project, ICAR, New Delhi, India, for funding and acknowledge the help received from In-Charge, KVK, Nimpith, West Bengal, India, in acquiring seed material from Sundarbans.

References

Alam R, Rahman MS, Seraj ZI, Thomson MJ, Ismail AM, Jumimbang E, Gregorio GB (2011) Investigation of seedling stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* L. Pokkali. Plant Breed 130:430–437

Ammar MHM, Pandit A, Singh RK, Sameena S, Chauhan MS, Singh AK, Sharma PC, Gaikwad K, Sharma TR, Mohapatra T, Singh NK (2009) Mapping of QTLs controlling Na^+, K^+ and Cl^- ion concentrations in salt tolerant Indica rice variety CSR27. J Plant Biochem Biotechnol 18:139–150

Bhushan C (2012) Living with changing climate: impact, vulnerability and adaptation challenges in Indian Sundarban. Centre for Science and Environment, New Delhi

Bonilla P, Dvorak J, Mackill D, Deal K, Gregorio GB (2002) RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. Philipp Agric Sci 85:68–76

Chattopadhyay K, Nath D, Das G, Mohanta RL, Marndi BC, Singh DP, Sarkar RK, Singh ON (2013) Phenotyping and QTL linked marker based genotyping of rice lines with varying level of salt tolerance at flowering stage. Ind J Genet 73(4):434-437

Chowdhury RK, Singh VP, Singh RK, Eberhart SA, Russell WA, Perkins JM, Jinks JL, Freeman GH (1982) Efficiency of various stability models for ranking barley genotypes. Cereal Res Commun 10:95–101

Gregorio GB, Senadhira D, Mendoza RI (1997) Screening rice for salinity tolerance. IRRI Discussion paper series no. 22, International Rice Research Institute, Manila, Philippines

Gregorio GB, Aliyu R, Adamu AK, Muazu S, Alonge SO (2010) Tagging and validation of SSR markers to salinity tolerance QTLs in rice (*Oryza spp*). International conference on biology, environment and chemistry IPCBEE vol.1, IACSIT Press, Singapore, p 328–332

Haq T U, Gorham J, Akhtar J, Akhtar N, Steele K A (2010) Dynamic quantitative trait loci for salt stress components on chromosome 1 of rice. Funct Plant Biol 37:634–645

Hosseini SJ, Sarvestani ZT, Pindashti H (2012) Analysis of tolerance indices in some rice (*Oryza sativa* L) genotypes at salt-stress condition. Int Res J Appl Basic Sci 3:1–10

Huyen LTN, Cuc LM, Ismail AM, Ham LH (2012) Introgression the salinity tolerance QTLs Saltol into AS996, the elite rice variety of Vietnam. Am J Plant Sci 3:981–987

IRGSP, International Rice Genome Sequencing Project (2005) The map based sequence of the rice genome. Nature 436:793–800

Islam MR, Gregorio GB, Salam MA, Collard BCY, Singh RK, Hassan L (2012) Validation of *SalTol* linked markers and haplotype diversity on chromosome 1 of rice. Mol Plant Breed 3:103–114

Ismail AM, Thomson MJ, Singh RK, Gregorio GB, Mackill DJ (2008) Designing rice varieties adapted to coastal areas of South and Southeast Asia. J Indian Soc of Coast Agric Res 26:69–73

Jolliffe IT (2002) Principal Component Analysis. 2nd edition, Springer Verlag, New York

Kim SH, Bhat P R, Cui X, Walia H, Xu J, Wanamaker S, Ismail AM, Wilson C, Close TJ (2009) Detection and validation of single feature polymorphisms using RNA expression data from a rice genome array. BMC Plant Bio 9:65

Lang NT, Luy TT, Buu BC, Ismail AB (2010) The genetic association between the yield, yield component and salt tolerance in rice. Omonrice 17:99–104

Lee KS, Choi WY, Ko JC, Kim TS, Gregorio GB (2003) Salinity tolerance of Japonica and Indica (*Oryza sativa* L) at the seedling stage. Planta 216:1043–1046

Lisa LA, Seraj ZI, Elahi MF, Das KC, Biswas K, Islam MR, Salam MA, Gomosta AR (2004) Genetic variation in microsatellite DNA, physiology and morphology of coastal saline rice (*Oryza sativa* L.) landraces of Bangladesh. Plant Soil 263:213–228

Mahata KR, Singh DP, Saha S, Ismail AM, Haefele AM (2010) Improving rice productivity in the coastal saline soils of the Mahanadi delta of India through integrated nutrient management. In: Hoanh CT, Szuster BW, Pheng KS, Ismail AM, Nobel AD (eds.), Tropical Deltas and Coastal Zones: Food Production, Communities and Environment at the Land-Water Interface, CAB International, Wallingford, UK

McCouch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Waltol M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q, Kono I, Yano M, Fiellstrom R, DeClerck G, Schneider D, Cartinhom S, Wane D, Stein L (2002) Development of mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

Mohammadi-Nejad G, Arzani A, Rezai AM, Singh RK, Gregorio GB (2008) Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the saltol QTL. Afr J Biotechnol 7:730–736

Mohammadi-Nejad G, Singh RK, Arzani A, Rezaie A M, Sabouri H and Gregorio G B (2010) Evaluation of salinity tolerance in rice genotypes. Int J Plant Prod 4:199–207

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Ann Rev Plant Bio 59:651–681

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238

Ren ZH, Gao JP, Li LG, Cai XL, Huang W, Chao DY, Zhu MZ, Wang ZY, Luan S, Lin HX (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nature Genet 37:1141–1146

Rohlf FJ (2000) NTSYS-pc Numerical Taxonomy and Multivariate Analysis System version 2.1. Applied Biostatistics, New York

Shylaraj KS, Sasidharan NK (2005) VTL 5: a high yielding salinity tolerance rice variety for the coastal saline eco-systems of Kerala. J Trop Agric 43:25–28

Singh R K, Flower T J (2010) The physiology and molecular biology of the effects of salinity on rice. In: Pessarakli M (ed.) Handbook of plant and crop stress, 3rd edn. Taylor and Francis, Boca Raton, Florida, USA

Theerawitaya C, Triwitayakorn K, Kirdmanee C, Smith DR, Supaibulwatana K (2011) Genetic variation associated with salt tolerance detected in mutants of KDML105 (*Oryza sativa* L. spp. *Indica*) rice. Aus J Crop Sci 5:1475–1480

Thomson MJ, De Ocampo M, Egdane J, Rahman MA, Sajise A G, Adorada D L, Tumimbang-Raiz E, Blumward E, Seraj Z I, Singh R K, Gregorio G B, Ismail A M (2010) Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. Rice 3:148–160

Van Berloo (2008) GGT 2.0: Versatile software for validation and analysis of genetic data. J Hered 99:232–236

Vu HTT, Le DD, Ismail AM, Le HH (2012) Marker assisted backcrossing (MABC) for improved salinity tolerance in rice (*Oryza sativa* L.) to cope with climate change in Vietnam. Aus J Crop Sci 6:1649–1654

Yoshida S, Forno D A, Cock J H and Gomez K A (1976) Laboratory manual for physiological studies of rice, 3rd edn., International Rice Research Institute, Manila, Philippines