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Marker based haplotype diversity of *Saltol* QTL in relation to seedling stage salinity tolerance in selected genotypes of rice

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Abstract

A major QTL for seedling stage salt tolerance, *Saltol* has been mapped on chromosome 1 in rice. The present study was carried out to characterise the haplotype diversity in the *Saltol* region using 20 simple sequence repeat markers, and it association with seedling stage salt tolerance under controlled condition (ECe of 12 dSm^{-1}) in 23 diverse rice germplasm including land races, wild germplasm and improved varieties. Genotypes from *kaipad* ecosystem of Kerala showed salt tolerance and haplotypes of *Saltol* similar to Pokkali, whereas salt tolerant wild rices possessed different haplotypes and therefore may be novel sources for salt tolerance. Altogether, 14 different haplotypes were observed in 23 rice genotypes based on critical markers linked to *Saltol* QTL using Pokkali as the reference. The haplotypes possessing Pokkali alleles at both the markers, RM8094 and RM3412 could discriminate the salt tolerant genotypes from the susceptible genotypes and hence could be useful in marker-assisted selection.

Key words: Rice, *Saltol*, salt tolerance, haplotype, genetic diversity

Introduction

Soil salinity is often accompanied by osmotic imbalance, mineral deficiency and toxicity that have adverse effects on crop growth [1, 2]. It is one of the major factors limiting rice productivity around the world. More than 91% of the world's rice cultivation is confined to Asian sub-continent, spreading across 115 countries. Majority of the rice-grown region in Asia is confined to South and Southeast regions, where salinity is a serious problem in about 20% of the area covering about 47 Mha. These problem areas consist of warm humid coastal regions and marshy inlands [3, 4].

Rice plants are highly salt sensitive, especially at seedling and flowering stages [5]. Yield reduction due to salinity is 12% for every unit (dSm^{-1}) increase in electrical conductivity (ECe) above the threshold tolerance of 3.0 dSm^{-1} [4, 6-8]. In rice, there exists enormous variation for salt response within species, which provides great opportunities to improve salt stress tolerance through genetic means. However, breeding for salt tolerant rice varieties has been difficult task, owing to the complexity in the inheritance of salt tolerance, strong GxE interaction and inherent difficulties experienced in the conventional screening techniques [4, 9-12].

Rapid advancements in the molecular marker technologies and their application in practical plant breeding during last two decades [13, 14] have helped in mapping several rice genes/QTLs for salt tolerance parameters like Na^+ and K^+ uptake, Na^+ and K^+ concentration and Na^+/K^+ ratio in shoot [10, 15-18]. Among all, *Saltol* (for *salt tolerance*) is a major QTL, mapped on the short arm of chromosome 1 by using an F_8 recombinant inbred lines (RILs) developed from the cross of a salt tolerant land race, Pokkali from Kerala and IR29, a salt sensitive rice variety [10]. Later studies identified that *Saltol* controlled Na-K absorption [19] and accounted for substantial phenotypic variation

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for Na^+, K^+ and Na-K absorption ratio amounting to 39.2, 43.9 and 43.2%, respectively. Further SSR marker based fine mapping of *Saltol* locus using RILs of Pokkali/ IR29 saturated this region with more than 20 microsatellite markers spanning a 5Mb region [20, 21, 22, 23]. Since the original *Saltol* donor, Pokkali possessed several undesirable characters, a highly tolerant RIL from the IR29/ Pokkali cross, IR 66946-3R-178-1-1 (FL478) was identified as the donor source of seedling stage salt tolerance [24]. Since then, FL478 has been widely used in transfer of *Saltol* though marker assisted backcross (MABC) breeding into elite varietal backgrounds such as BR11, BRRI dhan 28, IR64, AS996 and Pusa Basmati 1121 [25-28]. However, it possesses red pericarp colour of endosperm, which is an undesirable trait. Therefore, there is a need to identify new donors with improved grain and cooking quality traits and normal pericarp colour.

In addition to the *pokkali* tracts of south Kerala, *kaipad* is a unique coastal organic wetland rice ecosystem from north Kerala, which is naturally saline prone and unfamiliar to the scientific world [29, 30]. *Kaipad* ecosystem is characterised with marshy lands rich in biodiversity of flora and fauna embedded with a traditional rice production system. However, *kaipad* soils are coarser than *pokkali* soils [30]. Cultivation of traditional low yielding saline tolerant rice landraces is the most common practice of the *kaipad* region. The most popular varieties are Kuthiru and Orkayama [31] along with other landraces such as Mundon, Kandorkutty, Orpandy, Odiyan, Orissa, Punchakayama and Kuttadan [29, 30]. Recently, high yielding rice varieties such as Ezhome 1 and Ezhome 2 were developed for *kaipad* tracts by Kerala Agricultural University utilizing the traditional landraces [31, 32]. Although there are few reported studies of SSR based survey of *Saltol* region on diverse germplasm sets [11, 12, 33] to our knowledge, there is no such attempt so far on *kaipad* landraces, improved lines and wild germplasm. Therefore, the present study on SSR based haplotyping of *Saltol* region was carried out in a set 23 diverse genotypes including accessions of *Oryza rufipogon* and *kaipad* land races and improved varieties with special focus (i) to evaluate the haplotype diversity of *Saltol* region in rice genotypes, (ii) to select most discriminating SSR markers for salt tolerance and (iii) to identify putative novel genotypes that possess distinct *Saltol* haplotypes, as new sources of salt tolerance in rice.

Material and methods

Plant material

A set of 23 diverse genotypes including accessions of *Kaipad* land races, Basmati rice genotypes, induced mutants, improved varieties and *Oryza rufipogon* were screened for seedling stage salt tolerance and haplotypes diversity in *Saltol* region. The origin of genotypes along with their characteristics is presented in Table 1.

Screening for seedling stage salt tolerance

Genotypes were screened for seedling stage salt tolerance under controlled environment in National Phytotron Facility at Indian Agricultural Research Institute, New Delhi during *kharif* 2012. Pre-germinated (3 days after germination) seeds were sown in punch holes made on extruded polystyrene foam floats fitted with a nylon wire mesh on the bottom side and suspended on trays filled with Yoshida nutrient solution [34]. Each tray carried 12 entries and controls, Pokkali (salt tolerant) and IR29 (salt sensitive) and these trays constituted a replication. Two replications were used for each set of genotypes, with nine individual plants per line evaluated for each replication. In order to avoid border effect, one of the controls, FL478 was sown along the border on all sides to normalize competition for light and space for next rows. Salt stress was imposed 14 days after germination by adding 60mM NaCl (ECe of 6 dSm^{-1}) and salt concentration was increased to 120mM (ECe of 12 dSm^{-1}) after 3 days in Yoshida nutrient solution and was maintained until final phenotypic scoring. The pH of the nutrient solution was adjusted daily to 5.0, and the culture solution was replaced every 7 days. Sixteen days after imposing salt stress, entries were scored based on visual symptoms using the modified score scale, [35] with scores ranging from 1 (highly tolerant) to 9 (highly sensitive).

Molecular marker analysis

Twenty polymorphic SSR markers in the *Saltol* region were used to study the diversity in the *Saltol* haplotypes in rice genotypes. Total genomic DNA from 23 genotypes was extracted by the micro-extraction protocol of Prabhu et al. [36]. Polymerase chain reaction (PCR) was performed in a thermal cycler (G-Storm, Somerset, UK) using a 10 µl total reaction volume as described previously [37].

SSR allelic composition for each genotype at every marker locus was determined by counting the

Table 1. Origin, characteristics and salt tolerance reaction of 23 rice genotypes

S. No.	Genotype	Source origin	Characteristics	Score	Reaction to salinity*
1	FL478	IRRI	Salt tolerant NIL of IR29	3	T
2	IR 29	IRRI	Salt susceptible	9	HS
3	Pokkali	South Kerala, India	Salt tolerant landrace (*Pokkali*)	1	HT
4	Kuthiru	North Kerala, India	Salt tolerant landrace (*Kaipad*)	1	HT
5	Okayama	North Kerala, India	Salt tolerant landrace (*Kaipad*)	1	HT
6	Jaiphula	Orissa	Landrace	9	HS
7	Nipponbare	Japan	Landrace (*japonica*)	5	MT
8	PB1121	IARI, India	Basmati variety	7	S
9	PB1	IARI, India	Basmati variety	9	HS
10	Pusa1734-4	IARI, India	Salt tolerant NIL of PB1121	3	T
11	Pusa1734-24	IARI, India	Salt tolerant NIL of PB1121	3	T
12	Taraori Basmati	India	Basmati landrace	9	HS
13	N22	India	Drought tolerant genotype	5	MT
14	N292	India	Mutant line of N22	5	MT
15	N295	India	Mutant line of N22	5	MT
16	CSR 10	CSSRI, India	Salt tolerant variety	3	T
17	CSR 30	CSSRI, India	Salt tolerant variety	5	MT
18	Ezhome 1	North Kerala, India	Salt tolerant variety (*Kaipad*)	3	T
19	Ezhome 2	North Kerala, India	Salt tolerant variety (*Kaipad*)	7	S
20	NKSWR19	Kevali, UP, India	wild rice (*Oryza rufipogon*)	1	HT
21	NKSWR20	Rawak, UP, India	wild rice (*O. rufipogon*)	3	T
22	NKRWR32	Lokmanpur, UP, India	wild rice (*O. rufipogon*)	5	MT
23	NKSWR35	Bariin, UP, India	wild rice (*O. rufipogon*)	3	T

*HT = highly tolerant; T = tolerant; MT = moderately tolerant; S = sensitive; HS = highly sensitive

number of alleles per locus and the allele frequencies and polymorphism information content (PIC) was determined using the formula,

$$PIC = \left(1 - \sum_{i=1}^{k} \hat{p}_i^2\right) \frac{2n}{2n-1} - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2\hat{p}_i^2 \hat{p}_j^2$$

where $\hat{p}_i$ was the estimated allele frequencies of k alleles (i = 1 to k) and n was the number of individuals sampled [38]. Cluster analysis was performed on a dissimilarity matrix of simple matching coefficients [39] using unweighted neighbour joining algorithm using DARwin version 5.0.158 [40] with 30000 permutations. Haplotype analysis was conducted according to Bai *et al*. [41] and Liu and Anderson [42] using six tightly linked SSR markers, i.e., RM1287, RM8094, RM10720, RM3412, RM10748 and RM493 [11, 12, 33, 43] to compare with Pokkali as a reference. Graphical haplotype of the entire *Saltol* region was constructed using GGT2.0 software [44], and the PIC heat map of the region was drawn using Microsoft Excel.

Results and discussion

Seedling stage salinity tolerance

Seedling stage salt tolerance of the 23 rice genotypes under evaluation indicated varied responses to salt stress (Table 1). Altogether, 11 genotypes were identified as tolerant to highly tolerant (scores 1 and 3), 6 were moderately tolerant (score 5) and 6 susceptible (scores 7 and 9). Kaipad landraces, Kuthiru and Orkayama were found to be as tolerant as Pokkali but possessed white endosperm and lustrous grains in contrast to the red endosperm of Pokkali and FL478. Therefore, these two land races from *kaipad* region

can be good alternate sources for seedling stage salinity tolerance. Two improved *kaipad* lines showed tolerant (Ezhome 1) to sensitive (Ezhome 2) reaction to salt exposure at seedling stage. Among the *O. rufipogon* (wild) accessions, NKSWR19 showed high tolerance response, while NKSWR20 and NKSWR32 were found to be tolerant. All the Basmati cultivars, Pusa Basmati 1121(PB1121), Taraori Basmati and Pusa Basmati 1 (PB1) were salt sensitive while two *Saltol* introgressed PB1121 NILs (Pusa1734-4, Pusa1734-24) exhibited tolerance to salt stress. The EMS (Ethyl Methane Sulphonate) induced mutant lines of N22 (N292, N295) did not show any significant variation for salt tolerance from that of N22.

Genotype grouping based on molecular diversity

Genotypic cluster analysis based on the allele pattern of twenty SSR markers divided the genotypes into six clusters (Fig. 1). First cluster comprised of four salt tolerant genotypes with a bootstrap value of 82%. All the *kaipad* landraces and improved lines except Ezhome 2 were grouped with Pokkali into this cluster indicating the common allelic profile of markers within the *Saltol* region. Jaiphula, a highly salt sensitive genotype shared common allelic profile for 13 markers and showed distinct alleles for seven markers, namely RM1287, RM8094, RM10720, RM10843, RM10852, RM7075 and RM10927 (Fig. 2). This indicated that these distinct markers may form the basis for identifying key markers associated with salt tolerance. Highly salt sensitive genotypes, IR29 and PB1 formed the third cluster with a high bootstrap value of 96% along with FL478 and NKSWR32, both salt tolerant. IR29 is one of the parents of the RIL, FL478. Looking back to the pedigree of PB1, its lineage can be easily traced back to IR22 (IR579-160-2) as the female parent of Pusa 150, from which PB1 was derived by crossing to Karnal Local. IR579-160-2 falls in the lineage of IR29 as one of its immediate grandparents; hence this grouping has lines that are identical by descent (IBD). However, the genetic proximity of NKSWR32 with the group members could not be explained. Interestingly, looking at the allele pattern with respect to salt tolerance within this group, an apparent departure from similarity can be found for two markers RM8094 and RM10793, relative to other marker loci that are predominantly similar across the members. The fourth cluster constituted three wild *O. rufipogon* lines namely, NKSWR19, NKSWR20 and NKSWR35 indicating that they have different allelic configuration at the *Saltol* region. This is well substantiated by the unique allele pattern observed for at least eight markers. Six

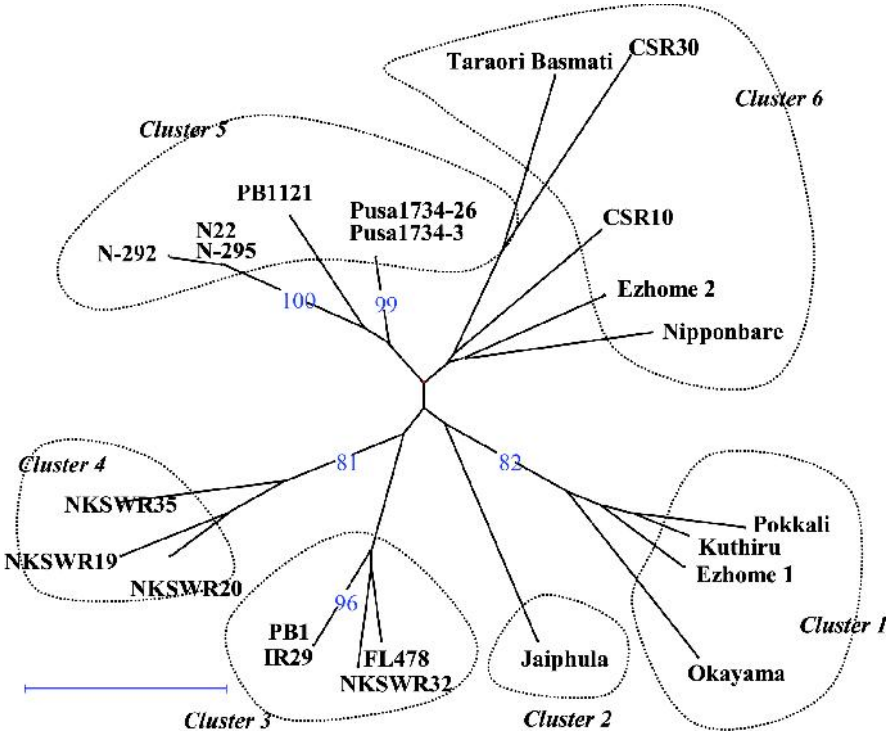


Fig. 1. Radial dendrogram of 23 rice genotypes based on 20 polymorphic SSR markers on *Saltol* region of chromosome 1 according to the un-weighted neighbour joining using dissimilarity matrix of simple matching coefficients

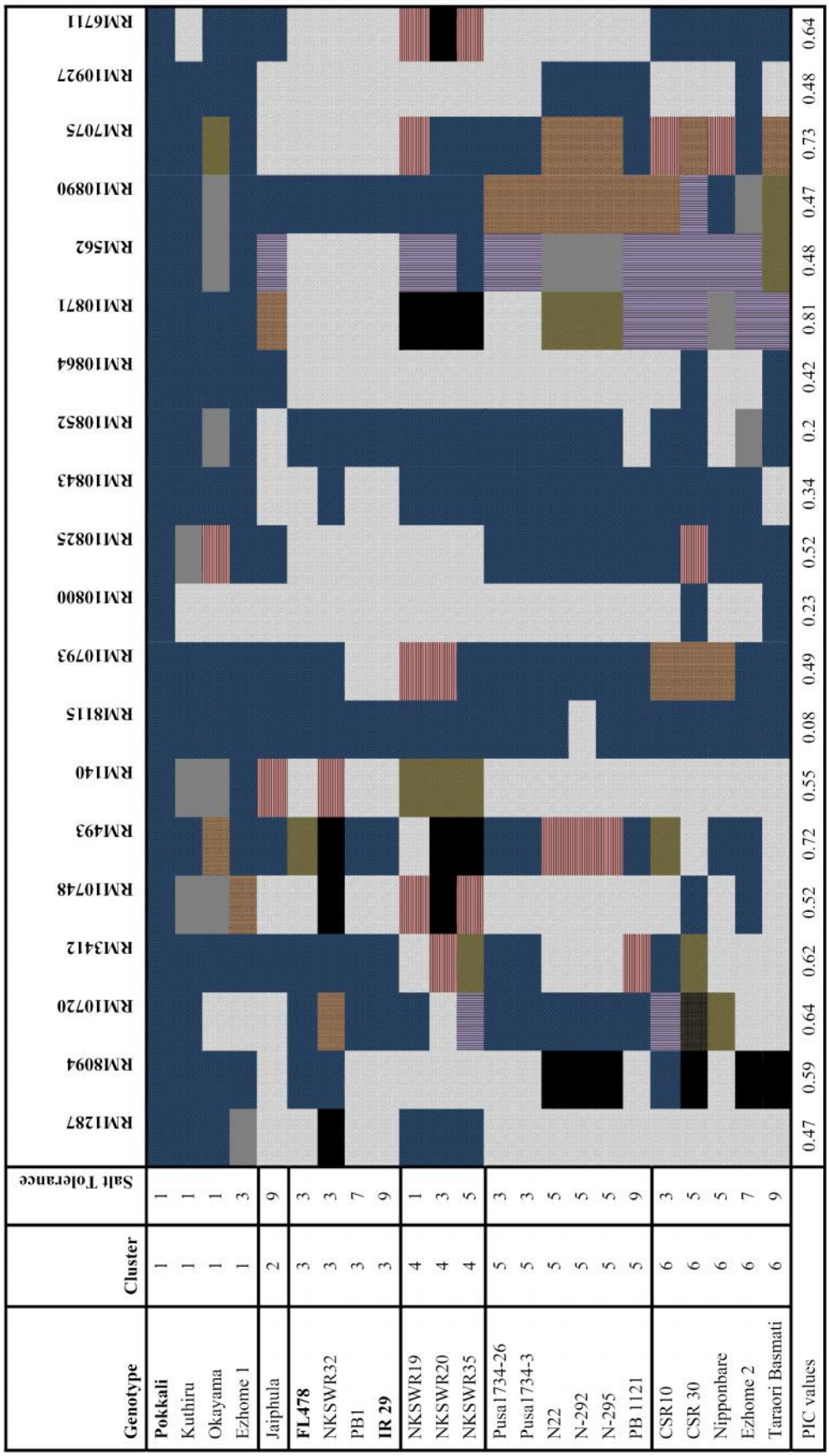


Fig. 2. Haplotypes of the *Saltol* region in 23 rice genotypes

genotypes of cluster 5 had two robust groupings, N22 and its mutant lines forming the first with a bootstrap percentage of 100 and the Near Isogenic lines (NIL) of PB1121 (Pusa1734-3 and Pusa1734-26) forming the second with a bootstrap value of 99% along with PB1121. Both the NILs of PB1121 carrying *Saltol* introgression showed high level of salinity tolerance while PB1121 remained highly sensitive. This provided another opportunity of comparing the *Saltol* haplotypes between near isogenic salt tolerant and susceptible lines. Five genotypes falling in cluster 6 also showed varied level salt tolerance, with CSR10 being highly tolerant. CSR10 differed from other members of this cluster for two markers *viz*., RM8094 and RM3412, for which it possessed Pokkali type alleles. Deducing marker allele pattern associated with salt tolerance among the clusters, we could not find any consistent association for four markers (RM10843, RM10852, RM7075 and RM10927) that were suspected to be associated with salt tolerance in the first group in comparison with Jaiphula. Therefore, we could narrow down to six key SSR markers, namely RM1287, RM8094, RM10720, RM3412, RM10748 and RM493 by genotype grouping based on haplotype heterogeneity of the *Saltol* region *vis-a-vis* response to salt treatment.

Molecular diversity and haplotype analysis of Saltol

All of the 20 markers spanning 5.6 Mbp in the *Saltol* region on chromosome 1 used in this study were reported to be associated with salt tolerance in several previous investigations [20, 22, 23]. Because of the large size of the *Saltol* region as derived from Pokkali, it is difficult to consider it with respect to haplotype conservation among the rice gene pool. As expected, the markers used were found to be highly polymorphic among 23 rice genotypes screened (Table 2) indicating that *Saltol* haplotype is not well conserved across rice gene pool. SSR markers based on allele diversity - ranged from seven biallelic markers to one hexaallelic (RM7075) marker. Additionally, there were four triallelic, three quadriallelic and five pentaallelic markers. A gel picture showing the amplification pattern of 3 representative markers in a subset of 8 genotypes is presented in Fig. 3. The polymorphic information content (PIC) varied from 0.08 (RM8115) to 0.73 (RM7075) with an average of 0.51. Since PIC is a function of allelic diversity, this result implies that some loci within *Saltol* region had relatively more frequent recombination and evolutionary reorganisations resulting in more number of alleles and high PIC values. The results indicated that two SSR markers RM493 (PIC = 0.72) and RM7075 (PIC = 0.73) found to be better indicators of genetic diversity within *Saltol* region. It is desirable to have markers with low PIC value with positive association with salt tolerance for use in *Saltol* introgression by marker assisted backcross breeding [45].

Table 2. Number of alleles and polymorphism information content (PIC) value of SSR markers for 23 rice genotypes

Marker	No. of alleles	PIC value	Amplicon size rage (bp)
RM1287	3	0.47	160-190
RM8094	5	0.59	80-220
RM10720	5	0.64	190-260
RM3412	4	0.62	220-250
RM10748	4	0.52	70-110
RM493	5	0.72	220-260
RM140	4	0.55	250-280
RM8115	2	0.08	120-130
RM10793	5	0.49	130-230
RM10800	2	0.23	140-150
RM10825	3	0.52	80-90
RM10843	2	0.34	160-170
RM10852	2	0.2	170-190
RM10864	2	0.42	210-330
RM10871	5	0.81	160-220
RM562	4	0.48	230-260
RM10890	3	0.47	230-260
RM7075	6	0.73	120-180
RM10927	2	0.48	150-160
RM6711	3	0.64	130-150

To compare the presence of six key markers (RM1287, RM8094, RM10720, RM3412, RM10748 and RM493) for salt tolerance, a graphical comparison of haplotypes of Pokkali, IR29 and FL478 was made along with a PIC heatmap of the marker loci obtained for all 23 genotypes (Fig. 4). The allelic distribution clearly indicated that haplotype variability existed only within the key SSR markers, which was further confirmed by the uniform pattern of PIC values for this region ranging from 0.40 to 0.69. These six key SSR markers spanned between 10.8 Mb to 12.3 Mb in the reference rice genome (www.gramene.org).

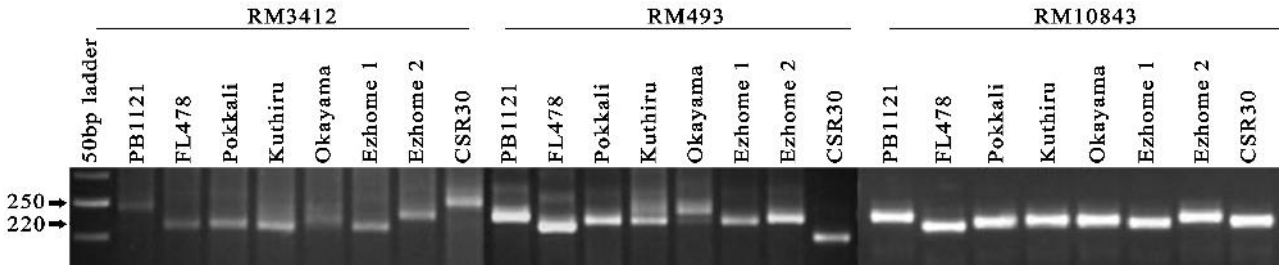


Fig. 3. Amplification profile of three representative SSR markers in the *Saltol* region of four *kaipad* salt tolerant lines, PB1121, Pokkali, FL478 and CSR30

By deciphering the haplotype pattern within these linked SSR markers using Pokkali as reference, fourteen haplotypes were identified among 23 genotypes based on the marker banding patterns (Fig. 5). Kuthiru, a popular *kaipad* landrace had haplotype similar to that of Pokkali, indicating that a complete *Saltol* region was conserved across landraces of two different salt affected rice farming systems (*kaipad* and *pokkali*). However, both these systems being geographically very close to each other, possibility of common ancestry between this landraces could not be ruled out. Eighteen genotypes had different combination of Pokkali alleles at different loci, while three genotypes did not share any allele (Haplotype 14) with Pokkali haplotype. From the comparison of haplotypes with high frequency of Pokkali alleles, it can be deduced that the marker, RM8094 showed association with high salt tolerance response in the present study. However, this marker did not discriminate tolerance found in PB1121 and its NILs, Pusa1734-4 and Pusa1734-24. Pokkali allele at marker RM3412 was present in salt tolerant genotypes such as Kuthiru, FL478, Orkayama, Ezhome 1, Pusa1734-4 and Pusa1734-24. However some of highly sensitive lines IR29, PB1 and Jaiphula also possessed allele similar to Pokkali at this locus. Additionally, genotypes which carried alleles similar to Pokkali at marker loci RM1287, RM10720, RM10843 and RM493 showed differential reaction to salinity stress, which indicated that no single marker had strong

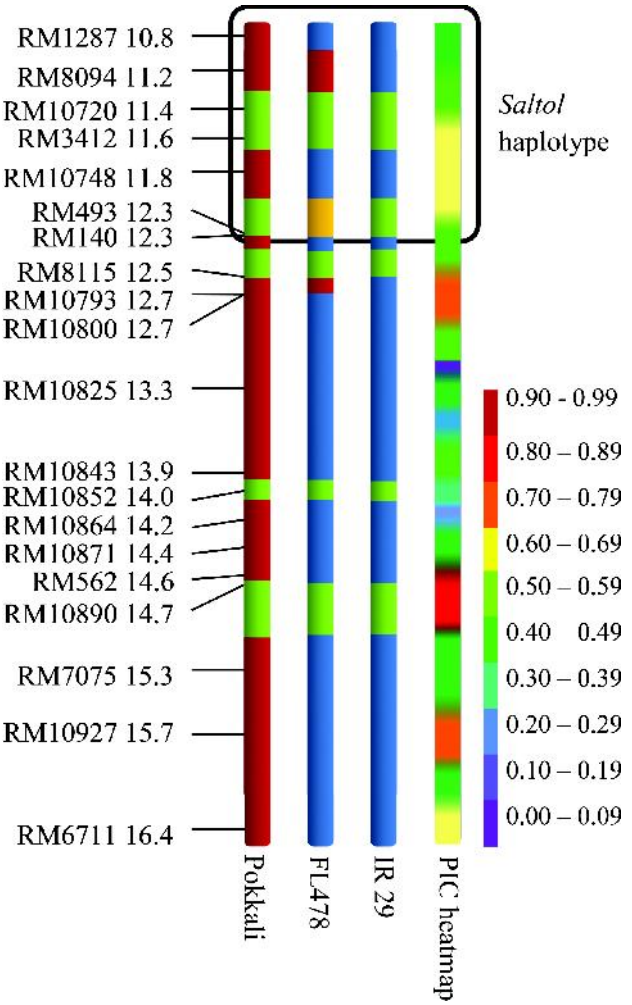
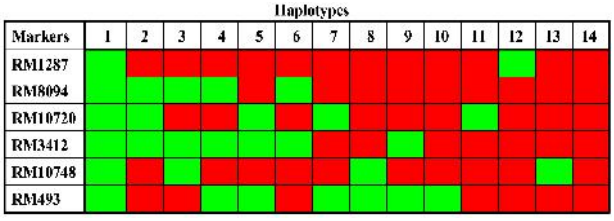


Fig. 4. Graphical representation of Pokkali, IR29 and FL478 showing high level of recombination within the *Saltol* region. The PIC heatmap showing high level of conserved diversity (depicted by similar PIC values) at the *Saltol* haplotype



1. Pokkali, Kuthiru; 2. FL478; 3.Okayama; 4. Ezhome 1; 5. IR29, PB1, Pusa1734-4, Pusa1734-24; 6. NKSWR32, CSR10; 7. PB1121; 8. Ezhome 2; 9. Jaiphula; 10. Nipponbare; 11. NKSWR19, N22, N292, N295; 12. NKSWR35, 13. CSR 30; 14. NKSWR20, Taraori Basmati

Fig. 5. Fourteen rice haplotypes produced by key SSR markers located in *Saltol* QTL region on chromosome 1 with reference to Pokkali

positive association with salt tolerance. However, the combination of two markers namely, RM8094 and RM3412 discriminated the salt tolerant genotypes from sensitive ones with an only exception of PB1. PB1 is a salt sensitive genotype but possessed alleles similar to Pokkali at both these marker loci. Therefore, it is essential to validate the gene linked markers between donor and recurrent parent for its use in marker assisted backcross breeding. Highly tolerant wild rice line, NKSWR19 and tolerant line, NKSWR20 did not possess any allele similar to Pokkali that could explain the tolerance, implying that they may possess novel QTLs/ alleles for salt tolerance.

The present study is a maiden attempt to analyse the salt tolerant landraces of the *kaipad* ecosystem prone to salinity. The SSR based analysis on a set of salt tolerant landraces from *kaipad*, other salt tolerant varieties, wild rice lines and salt susceptible lines showed marked variation within the genomic region encompassing *Saltol* QTL. The results show that the *kaipad* genotypes possess *Saltol* locus similar to Pokkali. The genotypes such as Kuthiru and Ezhome 1 which have normal pericarp colour and high degree of salinity tolerance can be used as new donors for *Saltol*. The salt tolerant wild rice genotypes like NKSWR19, NKSWR20 and NKSWR32 did not seem to possess *Saltol* locus which can become novel sources for mapping QTLs for seedling stage salinity tolerance. Our study further shows that the combination of two markers, RM8094 and RM3412 would provide effective selection for marker assisted transfer for *Saltol* into popular rice varieties sensitive to seedling stage salinity.

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References

1. **Asch F., Dingkuhn M., Wittstock C. and Dörffling K.** 1999. Sodium and potassium uptake of rice panicles as affected by salinity and season in relation to yield and yield components. Plant and Soil, **207**: 133-145.
2. **Mantri N., Patade V., Penna S., Ford R. and Pang E.** 2012. Abiotic stress responses in plants: Present and future. In: Ahmad P. and Prasad M.N.V. (ed) Abiotic stress responses in plants: Metabolism, productivity and sustainability. Springer, New York. pp. 1-19.
3. **Abbas S. T., Quraishi A. and Chughtai M. I. D.** 1994. Salt affected soils- problems and prospects. Pak J. Agric. Res., **15**: 176-184.
4. **Vinod K. K., Gopala Krishnan S., Naresh Babu N., Nagarajan M. and Singh A. K.** 2013. Improving salt tolerance in rice: Looking beyond the conventional". In: Ahmad P., Azooz M.M. and Prasad M.N.V. (ed). Salt stress in plants: Signalling, omics and adaptations, Springer, New York. pp. 219-260.
5. **Maas E. V. and Hoffman G. J.** 1977. Crop salt tolerance – current assessment. J. Irrig. Drain. Div. ASCE, **103**:115-134.
6. **Maas E. V. and Grattan S. R.** 1999. Crop yields as affected by salinity. In: Skaggs R.W., van Schilfgaarde J. (eds) Agricultural drainage, Vol. **38**, Agronomy monograph. American Society of Agronomy: Crop Science Society of America: Soil Science Society of America, Madison, pp. 55-108.
7. **Hanson B., Grattan S. R. and Fulton A.** 1999. Agricultural salinity and drainage. University of California Pub 3375, Division of Agricultural and Natural Resources, University of California, Davis, pp. 160.
8. **Maas E. V.** 1990. Agricultural salnity assessment and management. *In*: ASCE manuals and reports on engineering. Tanji K.K. (ed.). American Society of Civil Engineers, New York, pp. 262-304.
9. **Gregorio G. B. and Senadhira D.** 1993. Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). Theor. Appl. Genet., **86**: 333-338.
10. **Gregorio G. B.** 1997. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP). Unpubl. Ph.D. Thesis, University of the Philippines, Los Baños, Philippines.
11. **Mohammadi-Nejad, Arzani A., Rezai A. M., Singh R. K. and Gregorio G. B.** 2008. Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the *SalTol* QTL. Afr. J. Biotech., **7**: 730-736.
12. **Islam M. R., Gregorio G. B., Salam M. A., Collard C. Y. B., Singh R. K. and Hassan L.** 2012. Validation of *SalTol* linked markers and haplotype diversity on chromosome 1 of rice. Mol. Plant Breed., **3**: 103-114.
13. **Mackill D. J., Nguyen H. T. and Zhan J.** 1999. Use of molecular markers in plant improvement programs for rainfed lowland rice, Field Crops Res., **64**: 177-185.
14. **McCouch S. R. and Doerge R. W.** 1995. QTL mapping in rice, Trends. Genet., **11**: 482-487.

15. **Koyama M. L., Levesley A., Koebner R. M., Flowers T. J. and Yeo A. R.** 2001. Quantitative trait loci for component physiological traits determining salt tolerance in rice. Plant Physiol., **125**: 406-422.

16. **Lin H. X., Zhu M. Z., Yano M., Gao J. P., Liang Z. W., Su W. A., Hu X. H., Ren Z. H. and Chao D. Y.** 2004. QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. Theor. Appl. Genet., **108**: 253-260.

17. **Ammar M. H. M., Pandit A., Singh R. K., Sameena S., Chauhan M. S., Singh A. K., Sharma P. C., Gaikwad K., Sharma T. R., Mohapatra T. and Singh N. K.** 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.

18. **Pandit A., Vandna R., Subhashis B., Shikha S., Vinod K., Chauhan M., Gautam R. K., Singh R., Sharma P. C., Singh A. K., Gaikwad K., Sharma T. R., Mohapatra T. and Singh N. K.** 2010. Combining QTL mapping and transcriptome profiling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (*Oryza sativa* L.) Mol. Genet. Genomics, **284**: 121-136.

19. **Bonilla P. S., Dvorak J., Mackill D. J., Deal K. and Gregorio G. B.** 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**: 64-74.

20. **Niones J. M.** 2004. Fine mapping of the salinity tolerance gene on chromosome 1 of rice (*Oryza sativa* L.) using near isogenic lines, Unpubl. M.Sc dissertation, College, Laguna, Philippines: University of the Philippines, Los Baños, Laguna.

21. **Thomson M. J., Ocampo M., Egdane J., Katimbang M., Rahman M. A., Singh R. K., Gregorio G. B. and Ismail, M. A.** 2007. QTL mapping and marker-assisted backcrossing for improved salinity tolerance in rice. Poster presentation, IRRI, Los Baños, Laguna, Philippines.

22. **Thomson M. J., Ocampo M., Egdane J., Rahman M. A., Sajise A. G., Adorada D. L., Raiz E.T., Blumwald E., Seraj Z. I., Singh R. K., Gregorio G. B and Ismail A.M.** 2010. Characterizing the *SalTol* quantitative trait locus for salinity tolerance in rice. Rice, **3**: 148-160.

23. **Alam R., Ahman M. S., Seraj I. Z., Thomson M. J., Ismail M. A., Tumimbang-Raiz E. and Gregorio G. B.** 2011. Investigation of seedling-stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* L. Pokkali. Plant Breed., **130**: 430-437.

24. **Walia H., Wilson C., Condamine P., Liu X., Ismail A. M., Zeng L., Wanamaker S. I., Mandal J., Xu J., Cui X. and Close T. J.** 2005. Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. Plant Physiol., **139:** 822-835.

25. **Rahman M. S., Islam M. R., Rahman S., Ferdousi A., Malo R., Thomson M. J., Salam M. A., Ismail A. M. and Seraj Z. I.** 2008. Introgression of *SalTol* QTL into Bangladeshi mega rice varieties BR 11 and BR through marker assisted selection. Plant tissue culture and biotechnology conference: opportunities and challenges of agricultural biotechnology in developing countries.

26. **Ho V. T., Thomson M. J. and Ismail A. M.** 2010. Introgression *Saltol* into IR 64 through marker assisted selection. 3rd international rice congress. Honoi, Vietnam.

27. **Huyen L. T. N., Cuc L. M., Ismail A. M. and Ham L. H. 2012.** Introgression the salinity tolerance QTLs *Saltol* into AS996, the elite rice variety of Vietnam. Amer. J. Plant Sci., **3**: 981-987.

28. **Naresh B. N., Sharma S. K., Ellur R. K., Singh V. K., Pal M., Pathania S., Singh V. K., Singh D., Gopalakrishnan S., Bhowmick P. K., Nagarajan M., Vinod K. K., Singh N. K., Prabhu K. V. and Singh A. K.** 2012. Marker assisted improvement of Pusa Basmati 1121 for salinity tolerance. In: Abstracts of Poster Presentation (*MB52*) International Conference on Plant Biotechnology for Food Security: New Frontiers, Feb 21-24, 2012, National Agricultural Science Centre, New Delhi, India. Pp.72-73.

29. **Chandramohanan K. V. and Mohanan K. T.** 2012. Kaipad rice farming in north Kerala: an indigenous saline tolerant organic farming system. Indian J. Tradit. Know., **11:** 185-189.

30. **Vanaja T.** 2012. KAIPAD – a unique, naturally organic, saline prone rice ecosystem of Kerala, India. Amer. J. Environ. Prot., **2**: 42-46.

31. **Taliyil V. and Mammootty K.** 2010. '*Kuthiru*' and 'Orkayama' – Newly identified genetic resources from Kerala, India for salinity tolerance in indica rice. Available from Nature Precedings, http://hdl.handle.net/10101/npre.2010.4561.1. Accessed 07 August 2013.

32. **Vanaja T., Neema V. P., Mammootty K. P., Rajeshkumar R., Balakrishnan P. C., Naik J. and Raji P.** 2009. Development of first non-lodging and high yielding rice cultures for saline Kaipad paddy tracts of Kerala, India. Current Sci., **96**: 1024-1028.

33. **Aliyu R., Adam A. M., Muazu S., Alonge S. O. and Gregario G. B.** 2010.Tagging and validation of SSR markers to salinity tolerance in rice. International conference on Biology, Environment and Chemistry. IPCBEE Vol **1**. Singapore.

34. **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.

35. **Gregorio G. B., Senadhira D. and Mendoza R. D.** 1997. Screening rice for salinity tolerance. IRRI discussion paper series no. 22. International Rice Research Institute, Metro Manila, Philippines. pp. 3-19.

36. **Prabhu K. V., Somers D. J., Rakow G. and Gugel R. K.** 1998. Molecular markers linked to white rust resistance in mustard, *Brassica juncea*. Theor. Appl. Genet., **97**: 865-870.

37. **Basavaraj S. H., Singh V. K., Singh A., Singh A., Singh A., Yadav S., Ellur R. K., Singh D., Gopalakrishnan S., Nagarajan M., Mohapatra T., Prabhu K. V. and Singh A. K.** 2010. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. Mol. Breed., **26**: 293-305.

38. **Botstein D., White R. L., Skolnik M. and Davis R. W.** 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., **32**: 314-331.

39. **Sokal R. R. and Michener C. D.** 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull., **38**: 1409-1438.

40. **Perrier X., Flori A. and Bonnot F.** 2003. Data analysis methods. *In*: Genetic diversity of cultivated tropical plants. Hamon P., Seguin M., Perrier X., Glaszmann J. C. (ed.), Enfield, Science Publishers, Montpellier. pp. 43-76.

41. **Bai G., Peiguo G. and Kolb F. L.** 2003. Genetic relationship among head blight resistant cultivars of wheat assessed on the basis of molecular markers, Crop. Sci., **43**: 498-507.

42. **Liu S. and Anderson J. A.** 2003. Targeted molecular mapping of a major wheat QTL for *Fusarium* head blight resistance using wheat ESTs and synteny with rice, Genome, **46**: 817-823.

43. **Islam M. R., Gregorio G. B., Salam M. A., Hassan L., Tumimbang-Raiz E., Adorada D. L. and Mendoza R. D.** 2005. Molecular markers to salt tolerance in rice: Is it working? *In*: Abstracts, 5th International Rice Genetics Symposium, IRRI, Manila, Philippines, pp. 115-116.

44. **van Berloo R.** 2008. GGT 2.0: Versatile software for visualization and analysis of genetic data. J. Hered., **99**: 232-236.

45. **Singh A. K., Gopala Krishnan S., Singh V. P., Prabhu K. V., Mohapatra T., Singh N. K., Sharma T. R., Nagarajan M., Vinod K. K., Singh D., Singh U. D., Chander S., Atwal S. S., Seth R., Singh V. K., Ellur R. K., Singh A., Anand D., Khanna A., Yadav S., Goel N., Singh A., Shikari A. B., Singh A. and Marathi B.** 2011. Marker assisted selection: a paradigm shift in Basmati breeding. Indian J. Genet., **71**: 1-9.