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Short Communication

Linkage Disequilibrium in Salt Tolerant Genotypes of Rice (*Oryza sativa* L)

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Genome wide linkage disequilibrium (LD) was investigated in a set of 32 genotypes representing salt tolerant improved varieties and landraces and six salt sensitive genotypes of rice with 64 microsatellite markers to identify the genomic regions that are associated with salt tolerance in rice. Out of 64 markers analyzed, 36% SSR pairs exhibited significant LD at 0.05. A few regions were identified as targets of selection in 10 chromosomes with high r^2 values. The model-based groups from Bayesian clustering analysis are largely consistent with known pedigrees of the lines. The increased percentage of association of SSR loci in the improved varieties indicated the role of selection in linkage disequilibrium especially for salt tolerance. LD was extended as far as 100 cM in the present study. Most of the markers (43.8%) with significant LD values were observed in the genomic regions of reported QTL for salt tolerance in rice.

***Key words**:* linkage disequilibrium, microsatellites, salt tolerance, rice.

Improving the salt tolerance in rice has been one of the most important objectives of rice breeding programs. The development of new varieties with a high level of salt tolerance requires the understanding of the genetic control mechanisms for salt tolerance (1). Genetic dissection of salt tolerance in rice has been attempted by several groups through quantitative trait loci (QTL) analysis (2, 3). With the public availability of rice genome sequence information, there is growing interest in identifying and characterizing genes associated with both qualitative and quantitative forms of phenotypic variation. Of particular interest to rice breeders is the possibility of using existing germplasm resources for gene and allele discovery on the basis of association mapping strategies (4). Linkage disequilibrium (LD) is the non random association of alleles at different loci, and it can result from population structure, selection, drift or physical linkage. The basic approach is to identify marker loci at which some alleles are more frequent among affected genotypes than among unaffected genotypes (5). Because rice is largely self-pollinating, it is expected to have higher levels of LD and homozygotes, both of which greatly facilitate LD mapping. Significantly elevated levels of LD were detected among cultivated and wild species of rice (6, 7). Selection by humans to improve the agronomic traits of crops is expected to produce characteristic signatures of selection at loci underlying those traits (8).

Several landraces have been characterized based on their inherent physiological mechanisms for salt tolerance and were used as donors for the development of improved varieties for salt tolerance. In rice, highly polymorphic nature of SSR motifs coupled with a low level of homoplasy provides an appropriate tool for population genetic studies (7). The objective of the present study was to investigate the extent of linkage disequilibrium among 38 salt tolerant and salt sensitive genotypes and to identify the loci/segments that have been targets of selection using 64 rice microsatellite markers.

Seeds of 38 genotypes comprising salt tolerant landraces (15), salt tolerant improved varieties (17) and six salt sensitive gentypes were collected from Central Soil Salinity Research Institute, Karnal and from Directorate of Rice Research Hyderabad (Table 1). The DNA was isolated following modified potassium acetate method (9). A total of 64 microsatellite markers (SSR) covering all the chromosomes were used (Research Genetics, Huntsville). The average markers per chromosome were 5.3 and the average distance between the markers was 25.5 cM based on the linkage map of McCouch *et al* (10). PCR conditions for microsatellites were followed as per earlier published

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Table 1. List of rice genotypes,their parentage and response to salt stress

Genotype	Parentage	Response to stress
CSR10	M40-431-24-114 / Jaya	T
CSR11	M40-431-24-114 / Basmati 370	T
CSR13	CSR1 / Basmati370//CSR5	T
CSR23	IR64//IR4630-22-2-5-1-3/ IR9764-45-2-2	T
CSR27	IR51471 - IR5657-33-2 / Nonabokra	T
CSR30	BR4-10 /Pakistani Basmati	MT
CSR31	Somaclonal variant of Pokkali	T
IR4630-22-2-5-1-3	IR4454 / IR4442	T
MK47-22	Malkudai / KR1-24	T
Panvel 1	IR8 / BR4-10	T
Panvel 2	BR4-10 / IR8	T
USAR1	Jaya / Getu	T
CST7-1	Damodar /IR24	T
Sarjoo52	TN1 / Kashi	MT
Jaya	TN1 / T141	MT
Damodar	Local Selection, Sunderbans	T
CSR2	Local Selection, Sunderbans	T
Pokkali-1*	Landrace	T
Pokkali-2*	Landrace	T
Pokkali-3*	Landrace	T
Korgut	Landrace	M
Azgo	Landrace	M
BR4-10	Local Selection, Rata	T
KR1-24 (Kalarata)	Local Selection, Rata	T
Kalarata	Local Selection, Rata	T
Bejhari	Landrace	MT
Nonasail	Landrace	T
Kalanamak	Local Selection	MT
Nonabokra	Landrace	T
SR26B	Local Selection, Kalambank	T
Velki	Landrace	T
Sonasail	Landrace	T
IR28	IR 833-6-2-1-1//IR 2040	S
Pusa Basmati1	PUSA150 / Karnal Local	S
IR36	IR 2042/CR 94-13	S
MI48	Pelita I-1 // H4/H501	S
IR74	IR19661-131-1-2/IR15795-199-3-3	S
HBC19	Local Selection	S

*Collection numbers. T = Tolerant, MT = Moderately tolerant, S = Sensitive

protocols. Amplified products were mixed with bromophenol blue gel loading dye and were analyzed by electrophoresis on a 3% agarose gel using 1x Tris-Borate buffer, pH 8.0. The gels were stained in 0.5mg ml^{-1} ethidium bromide and were documented using Alpha Imager 1220 (Alpha Innotech, USA).

Genetic structure was analyzed using model based program 'Structure' based on Bayesian clustering analysis, allowing for admixture and correlated allele frequencies (11). This method uses multilocus genotypes to infer the fraction of an accession's genetic ancestry that belongs to a population for a given number of populations (k). In this study the data was analyzed using a burn-in of 10,000, run length of 100,000 and an accession was assigned to a cluster if at least 80% of its genome value was estimated to belong to that cluster. The matrix of P values for the pair wise estimates of linkage disequilibrium (LD) between all 64 SSR loci were calculated from the software 'Power Marker' by the permutation version of Fisher's exact test (www.powermarker.net). For each chromosome, r^2 between all pairs of loci were plotted against the genetic distance in cM. The microsatellite markers associated with 'Structure' analysis and high r^2 values were verified for their association in the reported QTL regions for salt tolerance and compared in www.gramene.org/cmap.

A total of 265 alleles were detected among the 38 rice genotypes with an average of 4.1 alleles per locus. The degree of relatedness among genotypes was analyzed with program based on model-based approach 'Structure' and clusters of genetically similar lines were identified. K = 6 were found to converge well and showed comparable or higher likelihoods among K = 4-10 runs of the program. The model-based groups were largely consistent with known pedigrees of the lines. Six groups were formed with 'Structure' analysis. One group consisted of six genotypes mostly comprising the genotypes released from CSSRI, the second group has four salt tolerant genotypes with landraces from Maharashtra and their derivatives. But one improved genotype Panvel1 with the same parents as Panvel 2 (though reciprocal) fell into the mixed group. Four improved genotypes formed another group. The only criterion that appears common to four genotypes was their ideotype with improved plant type characteristics. Three accessions of Pokkali and its derivative IR4630-22 and one landrace accession Korgut constituted one group. Three derivatives of Basmati, specialty rice Kalanamak and Sonasal formed another group.

Table 2. Pair wise LD (r^2) values >0.3 within the chromosomes in 38 rice genotypes

Loci	r^2
Chromosome 1	
RM104-RM449 (105.7)	0.50
RM428-RM220 (9.1)	0.46*
RM220-RM595 (52.6)	0.49*
Chromosome 2	
RM438-RM145 (12.4)	0.61
RM145-RM279 (32.5)	0.49
RM525-RM213 (42.7)	1.00*
RM213-RM250 (16.3)	0.40*
Chromosome 3	
RM60-RM489 (27)	0.34*
Chromosome 4	
RM252-RM470 (16.5)	0.46*
RM470-RM119 (39.4)	0.60*
Chromosome 5	
RM153-RM249 (65.8)	0.46
RM249-RM163 (12.9)	0.61
Chromosome 6	
RM528-RM314 (88.4)	0.46
RM314-RM508 (33.6)	0.46*
Chromosome 7	
RM346-RM11 (0)	0.36*
Chromosome 9	
RM460-RM257 (20.5)	1.00*
RM553-RM219 (65)	0.42

No associations with >0.3 were detected in chromosomes 8, 10, 11, and 12. Markers associated with QTL are indicated by *. Distances in cM are indicated in parentheses.

Overall, 36% of SSR pairs exhibited significant LD at 0.05 level of significance. Pair wise LD (r^2) values >0.3 within the chromosomes are presented in Table 2. LD was significant at 0.05 between 46.9% of the SSR marker pairs when all genotypes were included in the analysis. When the data obtained from all the salt tolerant genotypes, improved salt tolerant genotypes and landraces were analyzed, LD was significant at 0.05 between 35.9%, 60.9% and 48.4% of the SSR markers, respectively. The increased percentage of SSR loci with significant LD values in the improved varieties clearly indicates the role of selection in linkage disequilibrium. LD was reported to be greatly reduced but not eliminated by grouping lines into three empirically determined subpopulations in maize (12). The selection for salt tolerance in field is actually a process of screening for desired recombinant genotypes in segregating populations responding to the stress. Divergent selection for adaptive traits for salt tolerance may have created LD among chromosomal regions containing major/ minor genes for this trait. Linkage disequilibrium is created in breeding materials when several lines become fixed for a given set of alleles at a number of different loci. Large scale screening by artificial selection allowed identification of genes of potential agronomic importance in maize (13). Though the high values of LD within the chromosome were attributable to the physical proximity, the higher values of LD among the pairs of markers across the chromosomes indicated the association of different loci in trait of the interest. However, the effects of selection are very hard to detect at individual loci, particularly when the number of microsatellites surveyed is not extensive.

Linkage disequilibrium was extended to as far as 100 cM in most of the cases and there was substantial variation of LD decay among the chromosomes (Table 2). Pair wise LD (r^2) values >0.3 within the chromosomes spanned 9.1 cM to 105.7 cM distance. In *Oryza* species, little or no decay of LD was observed as function of genetic distance in the set of 198 accessions of *O. glaberrima* with 93 microsatellite loci and significant associations were observed for many of the comparisons among loci separated by more than 100 cM, (7).

When associations for SSRs with significant LD values of the present study were checked against the reported QTL for salt tolerance, 28 of the 64 SSR loci (43.8 %) were within the estimated map positions of salt tolerance QTL reported in nine studies (Fig. 1). The fact that most of the markers with significant LD values were observed in the reported regions of QTL for salt tolerance validates the strategy of the association analysis in the present study. A high association between RM220 and RM595 loci was observed. This region was reported to be associated with a major QTL “saltol” for salt tolerance (2).

While most of the linkage disequilibrium studies involved either a single genomic locus (4) or population structure (6, 7), in the present study an attempt was made to apply association mapping for identifying genomic

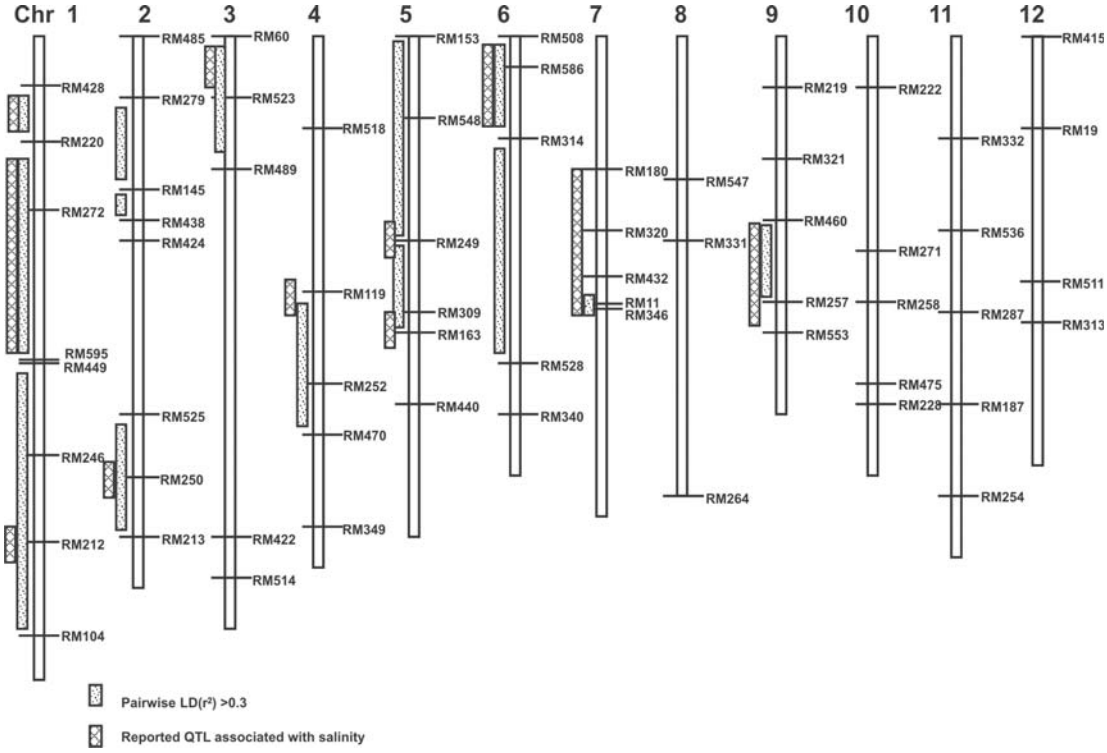

Fig. 1. Representation of microsatellite markers screened in the present study based on IR64 an Azucena linkage map (10), pair wise LD (r^2) values >0.3 and reported QTL for salt tolerance.

regions associated with salt tolerance. Salt tolerant genotypes of rice would have experienced strong selective pressure directed at genes controlling salt tolerance during the selective breeding. Consequently, these genes are expected to exhibit the signature of selection. From the LD mapping and genetic analysis of 38 rice genotypes with 64 microsatellites, a few regions were identified as targets of selection in 10 chromosomes with their high r^2 values. The results confirmed the presence of extensive linkage disequilibrium in the material studied and identified the regions among the chromosomes that might have been subjected to selection for salt tolerance.

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References

1 **Flowers TJ**, *J Exp Bot*, **55** (2004) 307.

2 **Gregorio GB, Senadhira D, Mendoza RD, Manigbas NL, Roxas JP & Querta CQ,** *Field Crops Res,* **76** (2002) 91.

3 **Takehisa H, Shimodate T, Fukuta Y, Ueda T, Yano M, Yamaya T, Camella T & Sato T,** *Field Crops Res,* **89** (2004) 85.

4 **Garris A, McCouch SR & Kresovich S,** *Genetics,* **165** (2003) 759.

5 **Flint-Garcia SA, Thornsberry JM & Buckler ES,** *Ann Rev Plant Biol,* **54** (2003) 357.

6 **Gao L,** *Mol Ecol,* **13** (2004) 1009.

7 **Semon M, Nielsen R, Jones MP & McCouch SR,** *Genetics,* **169** (2005)1639.

8 **Kim Y & Nielsen R,** *Genetics,* **167** (2004)1513.

9 **Dellaporta SL, Wood H & Hicks JB,** *Pl Mol Biol Rep,* **1** (1983) 19.

10 **McCouch SR, Temnykh S, Lukashova A, Coburn J, Declerck G, Cartinhour S, Harrington, Thomson M, Septiningsih E, Semon M, Moncada P & Li J,** In *Rice genetics* IV, (GS Khush, DS Brar, B Hardy, Editors) IRRI and Science Publishers Inc (2001) p 117.

11 **Falush D, Stephens M &. Pritchard JK,** *Genetics,* **164** (2003) 1567.

12 **Remington DL, Thornsberry JM, Matsuola Y, Wilson LM, Whitt SR, Doebley J, Kresovich S, Goodman MM & Buckler ES,** *Proc Nat Acad Sci,* USA, **98** (2001) 11479.

13 **Yamasaki M, Tenaillon MI, Bi IV, Schroeder SG, Sanchez-Villeda H, Doebley JF, Gaut BS & McMullen MD,** *Plant Cell*, **17** (2005) 2859.