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Full Length Research Paper

Validation of rice markers tagged to salinity stress

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Validation of rice markers tagged to salinity stress revealed five (5) quantitative trait loci (QTLs) for salinity tolerance. One QTL for tiller number was revealed with RM286, two QTLs for root length were tagged with RM587 and RM589 in chromosome 6. Two QTLs for leaf diameter were tagged at chromosome 6 and 11 with RM508 and RM286, respectively. A novel QTL for tiller number located in chromosome 11 (qSTN-11) was tagged with RM286. Phenotypic variance explained by the QTLs ranged from 4.0 to 10.06%. RM286 tagged tolerant genotypes with similar tillering obtained from an F_2 breeding population (IR88399-B) and from the rice species evaluated. Consequently, RM286 could be utilized in marker assisted selections (MAS) for salt tolerant genotypes.

Key words: RM286, rice markers, QTL, salt tolerance.

INTRODUCTION

Progress in rice breeding for salt tolerance constitutes the identification of the major locus conferring a salt tolerance gene at different growth stages (Gregorio and Senadhira, 1993). With the recent development in the field of molecular marker analysis, it is now feasible to analyze simple inherited traits and quantitative traits and then identify the individual genes controlling salinity tolerance which could facilitate selection in rice for this low heritable trait (Gregorio, 1997). The markers are used to partition the mapping population into different genotypic groups based on the presence or absence of particular marker locus and to determine whether significant difference exist between groups with respect to the traits being measured (Tanksley, 1993). Marker assisted selection (MAS) may greatly increase the effectiveness of selection in plant breeding compared to conventional breeding methods. Once markers that are tightly linked to genes or specific DNA marker alleles are identified, they can be utilized as a diagnostic tool to identify plants carrying the genes or QTLs of interest (Ribaut and Betran, 1999). The goal of this study was to validate the predictive value and reliability of tagged salinity tolerant rice markers to predict phenotypes in a MAS scheme.

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Abbreviations: QTL, RM, MAS etc.

MATERIALS AND METHODS

Plant materials

One hundred and fifty (150) rice genotypes of diverse species (*Oryza sativa, Oryza glaberrima and Oryza barthii*) and an F_2 breeding population (180 lines) with parental designation IR88399-B were obtained from the International Rice Research Institute, Las Banos and the Africa Rice Center (AfricaRice) Ibadan station, Nigeria.

Phenotyping for salt tolerance at seedling stage

Rice seeds were cleaned and placed in an oven for three to five days at 30°C to break seed dormancy. The seeds were surface sterilized with 1:5 benlate solution. Sterilized seeds were soaked in water in a Petri-dish lined with Whatman's filter paper and incubited for 48 h at 30°C. Pregerminated seeds were sown in a hydrophonic system and salinized at an electrical conductivity of 12 dsm^{-1} according to the method described by Gregorio et al. (1997). The nutrient solution was maintained daily at a pH of 5.2±0.1 by adding either NaOH or HCl and maintained at 27/21°C day/night temperature with a minimum relative humidity of 70%. The nutrient solution was replaced fort-nightly for 28 days. Unsalinized control treatment was also setup and maintained. The seedlings were phenotyped for four plant phenotypic characteristics (root length (cm), leaf width (cm), tiller number and seedling height (cm)) based on the standard

Table 1. Haplotypes produced by SSR markers with reference to POKKALI and FL487.

Marker	Haplotype	Allele frequency
RM84	5	99.3
RM286	24	95.3
RM501	40	91.3
RM508	95	93.3
RM520	31	94.01
RM587	89	95.3
RM589	80	90.14

evaluation score (SES) (IRRI, 1997) for salinity tolerance at seedling stage.

Genotyping the rice accessions

Isolation of rice DNA for PCR array

Genomic DNA was isolated from 150 rice genotypes of varying species and fromIR88399-B separately. DNA was extracted from a 21-day old seedling leaves collected from at least two to three seedlings according to the microprep protocol (Wang et al., 1993) with little modifications. Quantified DNA from each genotype was subjected to PCR amplification with 34 SSR primers. For each marker, allelic bands were scored on a 1 (present) and 0 (absent) binary code. Only prominent and unambiguous bands were scored for data reliability.

Data analysis

Genetic similarities were evaluated using Jaccard similarity coefficient for pair-wise comparisons based on the proportion of shared bands produced by primers generated using 'SMQUAL' sub-program of NTSYS-pc software (Rohlf, 1993).

Allelic data were analyzed using single marker analysis. The difference between the phenotypic means of tolerance and susceptible genotypes were used to estimate the phenotypic effect of the markers genotypes. The proportion of the trait phenotypic variations explained by the QTL was calculated as R^2 value which is the proportion of sum of square explained by the QTL to the total sum of squares. Additive effect was also calculated as the effect the parental genes exerted as expressed on the progenies.

Primers having similar banding pattern as Pokkali and FL478 (salt tolerant genotype) and lacking IR29 (salt susceptible genotype) alleles with clear bands were tagged as markers that could profile salt tolerant genotypes from a population of susceptible and tolerant genotypes.

RESULT

Tagging of salt tolerance traits to markers

Seven of the 34 markers screened produced the POKKALI and FL487 haplotype. Haplotypes produced ranged from 5 to 89 with RM84 and RM587 respectively. Allelic frequency ranged from 90.14% in RM589 to 99.3% in RM84 (Table 1). The haplotypes produced by these markers were highest in *Oryza sativa* genotypes and comprised of tolerant, moderately tolerant and susceptible genotypes.

Upon association with the phenotypic traits, four markers (RM286, RM508, RM587 and RM589) revealed traits that were associated with salt tolerance at different loci at two chromosomal regions (Table 2).

QTLs tagged for salt tolerance

The markers detected a total of five (5) QTLs in salt stress environment which were located on chromosomes 6 and 11. Two QTLs for root length, two QTLs for leaf diameter and one QTL for tiller number. The phenotypic variation for the tagged markers ranged from 4 to 10.06%. RM286 with QTL identified for tiller number (qSTN-11) revealed the presence of a major gene with a phenotypic variation of 10.06%.

Root length (RL)

Two QTLs were identified for root length (RL) on chromosome 6 with RM 587 and RM589. The QTL with the largest effect was qSRL-6_b. It accounted for 5.2% of the total phenotypic variation and had an additive effect of -0.8 cm. On this chromosome, QTL alleles for decrease in root length were from IR 05A117 parent. QTL qSRL-6_a obtained with RM 587 accounted for 4.3% of the total phenotypic variation and had an additive effect of -0.77 cm obtained from IR 66946-3R-178-1-1. Both QTLs were located at different loci on chromosome 6 (Table 2).

Leaf diameter (LD)

Two QTLs were identified for leaf diameter (LD) on chromosome 6 and 11 with RM508 and RM286, respectively. QTL with the largest effect was qSLD-6 which accounted for 5.6% of the total phenotypic variation and had an additive effect of 0.4 cm. The allele from IR 66946-3R-178-1-1 at this locus could give 0.4 cm increase in leaf diameter in stressed environment. The second QTL obtained for leaf diameter qSLD-11 accounted for 4% of the total phenotypic variation and had an additive effect of -0.05 cm. For each allele of IR 66946-3R-178-1-1 present at this given locus, a reduction of leaf diameter of 0.05 cm resulted. Both QTLs were located at the same locus (0.00 cm) on chromosomes 6 and 11, respectively (Table 2).

Tiller number (TN)

One QTL (qSTN-11) for tiller number was obtained with RM286. This accounted for 10.06% of the total phenoltypic variation and had an additive effect of -0.34 cm. IR 66946-3R-178-1-1 alleles at qSTN-11 caused a reduction in tiller number. The QTL for tiller number was located at 0.00 cm on chromosome 11 (Table 2).

Marker validation

The dendrogram constructed, with IR88399-B based on

Table 2. QTLs obtained using tagged markers.

Trait	Marker	Chromosome	Location (cM)	P-value	%R2	A.E	Parental allele
Root length	RM587	6	10.70	0.05	4.30	0.77	IR 05A117
	RM589	6	3.20	0.03	5.20	0.80	IR 66946-3R-178-1-1
Leaf diameter	RM508	6	0.00	0.02	5.60	0.40	IR 66946-3R-178-1-1
	RM286	11	0.00	0.05	4.00	0.05	IR 66946-3R-178-1-1
Tiller number	RM286	11	0.00	0.007	10.06	0.34	IR 66946-3R-178-1-1

A.E, Additive effect; R^2, proportion of the trait phenotypic variation explained by the QTL.

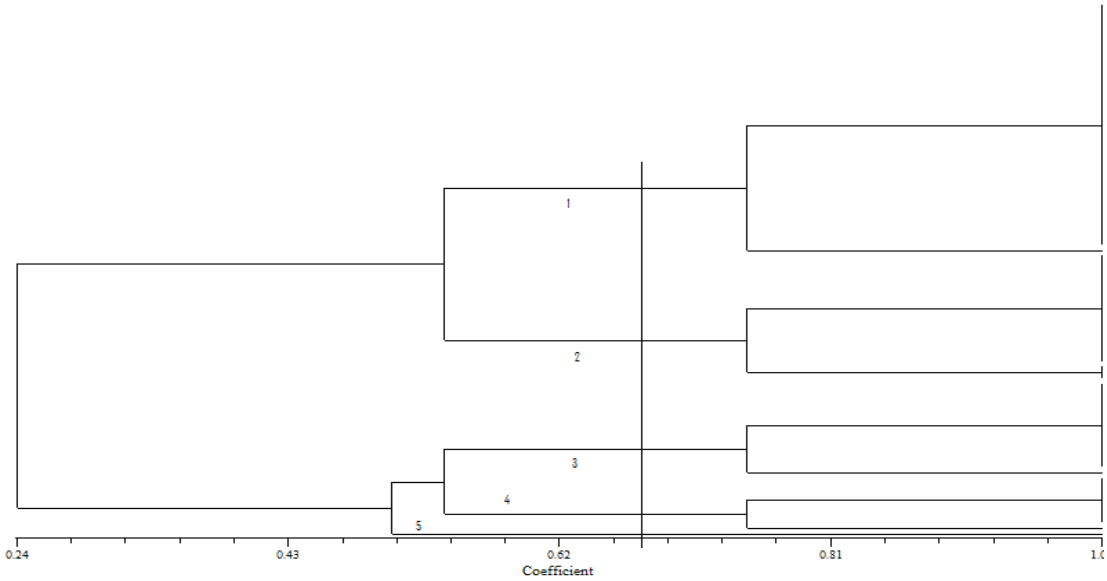

Figure 1. Dendrogram of genotypic relationship in F2 progenies based on RM286 marker, derived from UPGMA cluster analysis using NTSYS (Jaccard Coefficient). Cluster 1, Tolerant, moderately tolerant, susceptible and highly susceptible lines; Cluster 2: Tolerant, moderately tolerant and susceptible lines; Cluster 3: Moderately tolerant, susceptible and highly susceptible lines; Cluster 4: Moderately tolerant and highly susceptible lines; Cluster 5: Moderately tolerant lines.

RM286 revealed five clusters at 68% similarity coefficient. A total of nine tolerant genotypes were identified phenotypically. Five of these were revealed in cluster 1 and four in cluster 2 (Figure 1). Within these clusters, tolerant, moderately tolerant and susceptible genotypes were identified. Clusters 3, 4 and 5 were composed of moderately tolerant to highly susceptible lines.

Upon validation of RM286 on the diverse genotypes, three clusters were obtained at the same similarity coefficient of 68% (Figure 2). The clusters were only able to reveal tolerant genotypes with similar tillering abilities at cluster group 2.

DISCUSSION

Haplotype diversity analysis

In rice, important traits such as salt tolerance are controlled by polygenes with additive and dominant effects that are described by quantitative trait loci (QTLs) (Gregorio and Senadhira, 1993). Haplotypes with Pokkali alleles varied with marker and species. Two third of the genotypes studied (66.6%) did not reveal the Pokkali allele. Allele revealed were highest in *O. sativa* geno-types than other species studied which is expected due to the specie relatedness with Pokkali. The larger numbers of Pokkali haplotypes obtained in *O. sativa* than other species could indicate a positive association bet-ween markers and species. Therefore, markers utilized for screening might be population specific.

The tagged markers (RM84, RM508 and RM520) obtained with Pokkali alleles which was not associated with detectable QTL does not mean that there were no QTL for traits relating to salt tolerance on their respective chromosomes, but merely that they could not be detected because there were no discernible allelic differences with

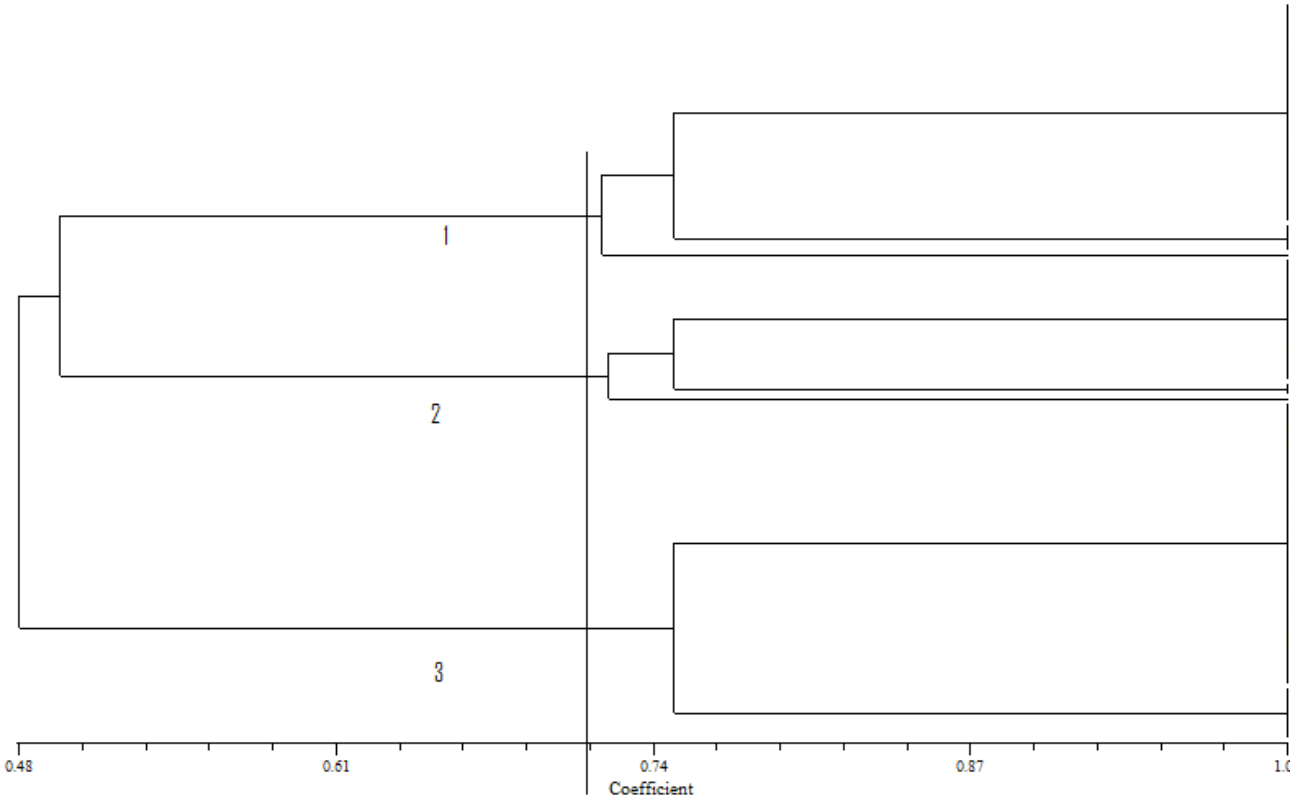

Figure 2. Dendrogram of genotypic relationship in diverse genotypes based on RM286 marker, derived from UPGMA cluster analysis using NTSYS (Jaccard coefficient).

Cluster group 1 - Moderately tolerant, susceptible and highly susceptible lines (A 69-1, ARG 6625, BG 1365, WITA 4, TOX 400-43-1-2-1, BOUAKE 187, BW 293-2, BW 294-5, WAR-115-1-1-2-3-B-B, CARD 176, CICA 8, CK 73, CNTR 800 76-44-1-1, CNTR 85293-47-2-1-1, DR 30, FARO 19, FL 478, FRK 19, GAMBIAKA (NIGERIA), SUAKOKO 8, GIZA 181, IAC 164, PSB Rc 54, PSB Rc 62, IET 3137, IKK 14, IR 20, IR 29, IR 42, NERICA U-1, IR 61642 3B-14-3-3-2, IR 65483-141-2-4-4-2-5, IR 65622-81-5-3-2, ITA 321, ITA 320, ITA 315, IR 72176- 140-1-2, IR 73885-1-4-3-2-1-6, IR 75395-2B-B-18-1-1-1- 11-2, IR 77674 B-22-1-2-1-3-8-B, IR 77674-3B-6B-3-3-2- B, IR 77674-3B-8-1-1-1-B, IR 77674-B-20-1-2-1-3-6-B, IR 77674-B-20-3-3-1-3-13-B, IR 77274-B-20-1-2-1-3-6-B, IDSA 8, TOG 7992, IR 77674-3B-8-1-3-4-5, NERICA L – 4, IR 776389-3B-16-2-2-2-1-2, IR 77645-3B-21-23-14-5, IRGC 104140).

Cluster group 2 – Tolerant, moderately tolerant, susceptible and highly susceptible lines (AR BURKINA, C 168, FARO 31, FAROX 239-3-3-2, IR 74, IRGC 106207, ITA 150, ITA 235, ITA 302, LAC 23, MOROBEREKAN, NERICA L-20, TOG 6595, NERICA L-41, NERICA L-59, NERICA U-3, NERICA U-4, TAINAN 5, NERICA U-5, SAHEL 108, NERICA U-6, POKKALI, NERICA U-7, NERICA L – 1, BG 2765, ITA 212, FKR (4418 x IR 6115-1-1-1), ITA 112).

Cluster group 3 - Moderately tolerant and susceptible genotypes (CG 14, TOG 7943, SIPI 692033, NERICA U-2, TOG 12377, TOG 5318, TOG 5626, TOG 7191, TOG 9524, TOG 7428, TOG 9047, TOG 9281, IRGC 101958, IRAT 144, TOG 9300, IR 77674-3B-8-3-1-1-5, IR 77674-3B-8-1-1-10-4, IRGC 103582, IR 77674-3B-21-1-1-1-2, TOG 9281, TOG 9395, ITA 128, IR 72, IR 71033-4-1-127, IR 68, TOG 8514, ITA 306, IR 62, TOG 5482, IR 45, IR 48, NERICA L-58, NERICA L-3, , SAHEL 202, PSB Rc 84, OS 6, IDSA 92, TOG 8539, NERICA L – 6, IRGC 106226, SIK 131, FRX 73F5-22F6BF8, FRX 73F5-22F6BF8, FRX 23F5B-13F6BF8, NSIC Rc 118, TAINAN 8, TOG 8008, TOG 5601, TOX 3100-44-1-2-3-3, TOG 5270, TOG 5442 TOG 5533, TOG 6584, CL SELECION 70, TOG 6762, CISADANE, TOG 7240, TOG 7886, IRGC 100122, IRGC 89148, IRGC 106489, IRGC 103590, IRGC 101317, IRGC 100941, IRGC 100933, IRGC 86523, IRGC 100936, IRGC 101252, IRGC 101931).

the markers and phenotype. RM216, RM508, RM587 and RM589 with tagged QTLs could be utilized in the profiling of salt tolerant lines within and between species. Gong et al. (2000) reported a major QTL for salt tolerance on chromosome one. He further reported QTLs for plant height on chromosome one. Sabouri and Biabani (2009) have reported 2 QTLs for plant height on chromosome six at vegetative stage.

Prasad et al. (2000) reported QTL for root length ion chromosome 6 at 18.90 cm; the position of the reported QTL differed from that obtained in this study, thus revealing two new QTL for root length ion chromosome 6 with

minor effect. QTL for root length have also been reported ion chromosomes 7 and 9 by Sabouri and Sabouri (2008).

The QTL obtained for leaf diameter qSLD-6 and qSLD-11 have not been reported in any study. Interestingly, these novel QTLs were due to the same parent (IR 66946-3R-178-1-1) at the same chromosomal. The additive effect was positive at chromosome 6 and negative on chromosome 11 suggesting differential effects of parental alleles linked to QTL traits. Three QTLs for tiller number have been reported in chromosomes 1, 3 and 6 at reproductive stage by Gong et al. (2000) in salt stress environment and also in chromosome 1, 2 and 6 by Sabouri and Biabani (2009) at reproductive stage. However, QTL for tiller number have not been reported at seedling stage of rice under salt stress. The QTL obtained from this study for seedling tiller number (qSTN-11) was a major QTL that accounted for 10.06% of the phenotypic variance of the population. The new QTL at chromosome 11 contained a new major gene for salt stress tolerance at seedling stage. This new QTL identified was strongly linked to RM286 and overlapped with qSLD-11 on the same chromosomal region. This could be due to the fact that performance under salt stress at seedling stage seemed derived from leaf diameter and tillering ability. Thus, there is a relationship between these traits which may be controlled by the same gene or linked genes. Chromosomal regions carrying multiple QTLs have been identified on chromosomes 1, 4 and 8 by Gong et al. (2000). Sabouri and Biabani (2009) also reported similar results of multiple effect of QTL on the same chromosomal regions at reproductive stage in rice. The regions of the genome that had effects on multiple traits may have acted through the pleiotropic effects of a single gene or by the chance linkage of multiple genes as salinity tolerance is a complex physiological trait related to several traits (Lin et al., 2004). The association of RM286 to QTL for seedling tillering ability in IR 88399-B breeding population is novel and could be used for indirect selection of salt-tolerant traits to be used in marker assisted selection.

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