

MICROSATELLITE MARKER BASED HYBRIDITY ASSESSMENT FOR SALINITY TOLERANCE IN RICE

Short Communication

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ABSTRACT

Development salinity tolerant rice varieties have been a prioritized research area in rice breeding programs in Sri Lanka. Present study was initiated with the cross of At354, a salt tolerant parent and Bg352, a salt susceptible parent aiming at identifying salinity tolerant quantitative trait loci (QTL) in elite rice background. In rice, as getting true hybrids is a technical problem, F₁ progeny was assessed using microsatellite markers that are close proximity to Saltol QTL in chromosome 1. SSR Markers such as RM1287, RM493, RM10772, RM10852 and RM140 were polymorphic between parents. Six true hybrids were selected by polymorphic markers and subsequent generations were developed successfully by single seed descent method. This study explains the feasibility of utilizing microsatellite markers for verification of hybridity, surveying of polymorphic markers in chromosome 1 of At354 and Bg352 and progress towards Recombinant Inbred Line population development.

Keywords: *Hybridity assessment, Microsatellite markers, Rice, Salinity tolerance*

INTRODUCTION

Salinity is the second most widespread soil problem in rice growing countries next to drought (Gregorio *et al.*, 1997). The productivity of most paddy growing lands in Sri Lanka also declines every year due to soil salinity, which arises due to the introgression of seawater during high tide and the rising of shallow saline ground water particularly during the dry season. This condition has aggregated by the recent tsunami disaster experienced by Sri Lanka by leaving large extent of the croplands in coastal area without cultivation due to considerable increment of saline condition in soil. Therefore, development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline prone areas.

Salinity tolerance in rice is a quantitative trait which is governed by many genes (Islam, *et al.*, 2011). Present study was initiated with the aim of identifying salinity tolerant quantitative trait loci (QTLs) in elite rice background

under Sri Lankan environment. In view of this, development of mapping population for salt tolerance is a prerequisite because mapping populations provide an efficient means for identifying genes and studying their associations with various plant traits.

Generally in self-pollinating species, mapping population is generated by crossing homozygous parents which are distinctly different for the trait that is going to be assessed (Collard *et al.*, 2005). However, in rice, getting true hybrids is a technical problem as self-fertilization may occur before out crossing preventing the transfer of desired traits in progenies. In addition, conventionally, the genetic purity of the rice hybrids is assessed by the Grow-Out Test based on specific morphological traits which is time consuming, restricted to a few characteristics, influenced by environmental condition, and inefficient. Consequently selected plants based on such traits may not be true hybrids and such inaccurate identification can adversely

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affect all stages of future breeding program (Tamilkumar *et al.*, 2009). Therefore, present study reports the use of microsatellite markers for the precise assessment of hybridity in F₁ generation of At354 and Bg352 cross which is then subsequently advanced.

MATERIALS AND METHODS

Experimental site and Plant material

The study was conducted at the Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila in collaboration with the Rice Research and Development Institute (RRDI), Batalagoda and, the crosses were initiated in 2010. At354, derived from Pokkali and Bg94-1, was selected as the salt tolerant parent and Bg352, derived from Bg380/Bg367-4 cross, was selected as the salinity susceptible parent.

Hybridization and development of mapping population

F₁ seeds produced from At354 and Bg352 cross, treated with the fungicide were established in the nursery. Well grown F₁ seedlings were selected and they were assessed by microsatellite markers that were polymorphic between parents. Selected true F₁ hybrids were advanced to subsequent generations through inbreeding towards recombinant inbred line population (RILs) by single seed descent method (SSD).

DNA extraction

Genomic DNA was extracted from putative F₁ individuals and two parents according to the method described in <http://rgp.dna.affrc.go.jp/rgp/protocols/QTL.pdf>. The leaf pieces were homogenized with 300 µl of DNA extraction buffer (1 M KCl, 1 M Tris HCl, 0.5 M EDTA). Homogenized mixtures were incubated at 70°C for 20 min. Extracts were centrifuged at 13,000 rpm for 15 min under room temperature. 100 µl of ice cold iso-propanol

was added into new eppendorf tubes, and the supernatant of the above centrifuged samples were transferred into them. Solutions were mixed gently. After mixing, tubes were kept at 4°C for 15-30 min and centrifuged at 13,000 rpm for 15 min under room temperature. DNA pellets were washed with 150 µl of 70 % ice cold ethanol by centrifuging at 13,000 rpm for 10 min. Supernatants were removed and pellets were air dried and dissolved in 200 µl of 1/10 TE buffer (10 mM Tris, 1 mM EDTA).

Microsatellite marker analysis

According to the Thomson, *et al.*, 2010, microsatellite markers located at the Saltol QTL in chromosome 1, were selected for the study (Table 01). The primer sequence information was obtained from the published sequence database (www.gramene.org).

PCR was conducted using 8 microsatellite markers with the 15 µl PCR mixture consisted of 5 µl of genomic DNA, 1.5 µl of 10X PCR buffer, 1.2 µl of dNTPs (2.5 mM of each dNTP), 0.8 µl of primer mixture (20 µM of each Forward and Reverse primer) (Integrated DNA Technologies, USA) and 0.25 µl of Taq DNA polymerase (5U/ µl, Sigma, USA). Final volume of the mixture was adjusted to 15 µl by adding sterile distilled water. PCR amplification was performed using thermal cycler, BIORAD (USA) and amplification profile consisted of initial denaturing at 95°C for 5 minutes followed by 35 cycles of 1 minute at 95°C, 30 seconds at relevant annealing temperature (Table 01), 1 minute at 72°C and final extension cycle of 5 minutes at 72°C. Amplified PCR products were analyzed using 3% agarose gel.

RESULTS AND DISCUSSION

F₂, backcross, and RILs are the three primary types of mapping populations used for molecular mapping (Collard *et al.*, 2005). Unlike F₂ or backcross populations RILs produce homozygous or 'true-breeding' lines

that can be multiplied and reproduce without genetic change. In this attempt of producing RILs, as the parents of the cross, At354 and Bg352 exhibited comparatively similar visual appearance and both were in the category of 3 ½ months maturity time, molecular markers were used for screening of segregants. Similar studies have been conducted for the identification of rice hybrids and their respective parents, assessment of plant to plant variation within parental lines and testing the genetic purity of rice hybrids using molecular markers (Yashitola *et al.*, 2002; Nandakumar *et al.*, 2004; Yun *et al.*, 2005; Sundaram *et al.*, 2007).

According to the results of the hybridization, 119 F₁ seeds resulted and only 14 F₁ seeds successfully produced F₁ seedlings exhibiting 11.7% of comparatively low seed viability. Five microsatellite markers of eight, viz., RM1287, RM493, RM10772, RM10852 and RM140 exhibited polymorphism between parents (Figure 01 and 02). These polymorphic markers were used to examine the hybridity of 14 well grown F₁ individuals. Accordingly, hybridity of 6 F₁ individuals was confirmed proving their heterozygosity at the respective loci representing two specific alleles of both

parents (Figure 02). Rest of the 8 F₁ individuals were confirmed as off-types as they exhibited only one of the alleles of parents.

By selfing of selected 6 true hybrids, 350 F₂ segregants were raised successfully followed by advancing of subsequent generations by SSD method as illustrated by Figure 03. Assessment of homozygosity of F₅ population indicated that all tested individuals (96 seedlings) were 100 % homozygous which would be tally with the expected level of 92% homozygosity in Mendelian genetics.

CONCLUSION

In present study, hybridity of 6 F₁ individuals derived from AT354/Bg352 cross was confirmed precisely with the RM1287, RM493, RM10772, RM10852 and RM140 microsatellite markers. By selfing of 6 true hybrids, 350 F₂ segregants were raised successfully followed by advancing of subsequent generations to F₅ with the expected level of homozygosity. As the SSR markers used in this assessment are located in close proximity to Saltol QTL, they would possibly be useful for the identification of Saltol QTL in At354 and Bg352 cross.

Table 01: Selected rice microsatellite markers located at chromosome 1.

Locus name	Primer sequence	SSR motif	Annealing T. (C°)
RM562	CACAACCCACAAACAGCAAG CTTCCCCAAAGTTTGTAGCC	(AAG) ₁₃	53
RM493*	TAGCTCCAACAGGATCGACC GTACGTAAACGCGGAAGGTG	(CTT) ₉	56
RM8115	TATATAGTAAATTTGTTTGGTGTAGG ACAGATGGATATTATAAGAAGTAACA	(TA) ₁₈	50
RM6711	TAGTGATAGGGGTGGTGTGG TTACAAGCATGGGAGTTGGG	(TAT) ₈	56
RM1287*	GTGAAGAAAGCATGGTAAATG CTCAGCTTGCTTGTGGTTAG	(AG) ₁₇	53
RM10772*	GCACACCATGCAAATCAATGC CAGAAACCTCATCTCCACCTTCC	(CTT) ₁₆	55
RM10852*	GAATTTCTAGGCCATGAGAGC AACGGAGGGAGTATATGTTAGCC	(ATAG) ₅	58
RM140*	TGCCTCTTCCCTGGCTCCCCTG GGCATGCCGAATGAAATGCATG	(CT) ₁₂	58

*polymorphic between parents

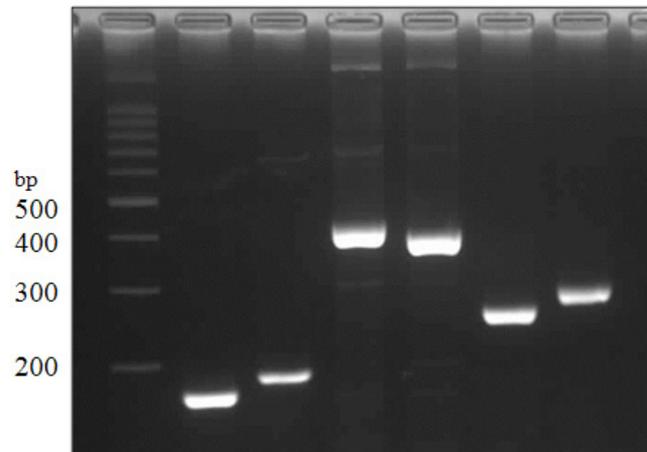


Figure 01: Polymorphism between parents, At354 and Bg352

Lane L- 100 bp DNA ladder, Lane 1,3 and 5- At354 amplified with RM10852, RM10772 and RM140 respectively, Lane 2,4 and 6- Bg352 amplified with RM10852, RM10772 and RM140 respectively

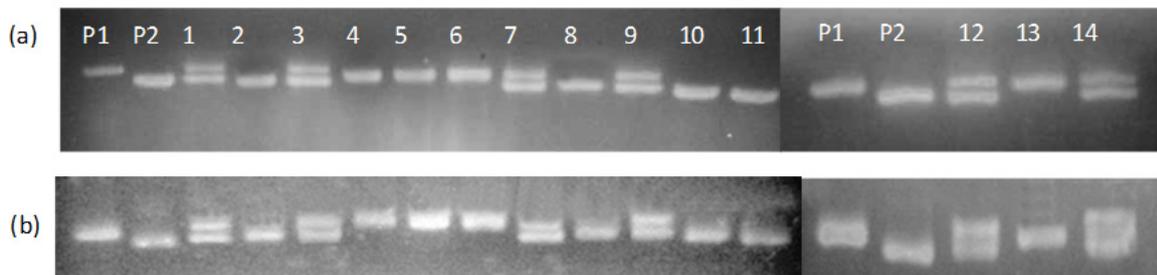


Figure 02: PCR profile generated with F_1 individuals from (a) RM1287, (b) RM493 SSR markers.

Lane P1- At354 parent, Lane P2-Bg352 parent, Lane 1-14- F_1 individuals

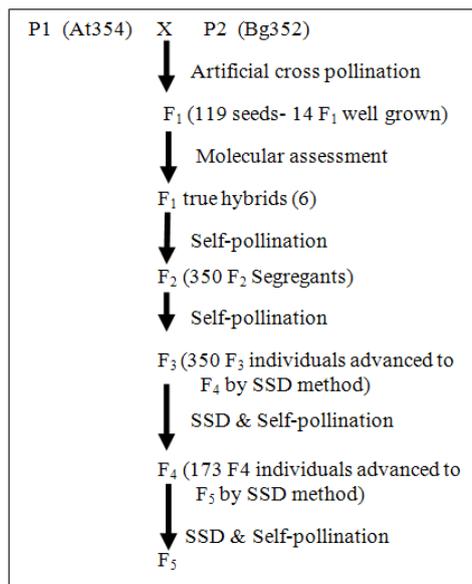


Figure 03: Schematic representation of the progress of developing mapping population

ACKNOWLEDGEMENTS

The authors are thankful to the Research Grants received from the Wayamba University of Sri Lanka (SRHDC/RP/01/10-05) and National Science Foundation (RG/2011/BT/02).

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