Short communication

Microsatellite marker based diversity analysis for submergence tolerance in some Bengal landraces of rice (*Oryza sativa* L.)

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Abstract

Twenty eight rice genotypes from West Bengal and Assam were studied to assess genetic diversity using three SSR markers linked with QTL of submergence tolerance. Among the three markers, only RM-219 generated polymorphic pattern by producing four alleles, of which two were high frequency alleles. The PIC values derived for this marker was 0.325. All the genotypes exhibited multiple allelism with two alleles, except in 'Panikalas' where three alleles were detected. Markers RM-464A and RM-265 were unable to distinguish any of the 28 genotypes and therefore not suitable for marker assisted selection in the *indica* genotypes. Marker RM-219, however, can be used for monitoring introgression of the *Sub-1* locus responsible for submergence tolerance. However, this marker did not show any polymorphism between known submergence tolerant genotypes and non-tolerant genotypes, which would prevent the use of RM-219 for selection of segregating populations derived from parental combination.

Keywords: Genetic diversity, Polymorphism, Quantitative Trait Loci (QTL).

Rice is a semi-aquatic species typically cultivated under partially flooded conditions. However, flash flooding can submerge the entire plant for prolonged periods, and most rice cultivars die within seven days of complete submergence (Bailey-Serres et al., 2010). To combat this problem, breeders have been searching for new sources of genes and alleles responsible for submergence tolerance. Molecular markers provide a direct measure of genetic diversity and go beyond indirect diversity measures based on morphological traits or geographic origin and facilitate the identification of genomic location linked with the trait of interest. These tasks have become relatively easier after development of nearly saturated molecular [especially with simple sequence repeat (SSR)] map in rice. Quantitative Trait Loci (QTL) of submergence tolerance in rice was mapped by SSR and other Polymerase Chain Reaction (PCR) based markers (Nandi et al., 1997; Xu et al., 2006). These markers are common in rice and are especially suitable for evaluating genetic diversity among the closely related cultivars (Akagi et al., 1997). Although diversity analysis through molecular markers is abundant in rice, allelic diversity linked with submergence tolerance in land races is absent. Keeping this in view, a study was undertaken to assess the genetic diversity of landraces collected from West Bengal and Assam using SSR markers linked with QTL of submergence tolerance and also to identify a perfect marker suitable to study submergence tolerance in a wide range of backgrounds.

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Twenty eight diverse rice genotypes (Table 1) comprising of 17 landraces and two advance breeding lines from West Bengal and one landrace from Assam collected from the Crop Research Unit, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal along with eight released popular high yielding submergence non-tolerant varieties (IR-8, IR-20, IR-64, IR-36, 'Ratna', 'Jaya', 'Rasi' and 'Swarna') were screened for diversity in submergence tolerance. Genotypes were categorized as "tolerant", "susceptible", and "unknown" based on published information (Mohanty and Choudhary, 1986). All the plant materials were grown at the Gayeshpur Regional Research Station (New Alluvial Zone) of Bidhan Chandra Krishi Viswavidyalaya during 2006-07. Genomic DNA was extracted from the leaves of tender seedlings using the method described by Chattopadhyay et al. (2008). Three SSR Primer pairs viz. RM-265, RM-219, and RM-464A (Table 2) were utilized. They are all located on rice linkage group no. 9 and linked with the QTL for submergence tolerance (Xu et al., 2006). The 25 µl reaction volume contained 2 µl (20 ng) DNA, 2.5 µl 10XPCR buffer, 1µl 2.5 mM deoxynucleotide tri phosphates (dNTPs), 1 µl (100 ng) forward primer, 1 µl (100 ng) reverse primer, 0.5 µl Taq

Table 1. Description of 28 rice genotypes, their allelic size for three SSR markers along with the phenotypes for submergence tolerance ability.

Genotypes	Description	RM-464A (bp)	RM-219 (bp)	RM-265 (bp)	Phenotyping
IR36	Released semi-dwarf variety	230	260,210	110	S
IR8	As above	230	260,210	110	S
IR20	As above	230	260,210	110	S
IR64	As above	230	260,210	110	S
'Ratna'	Released variety for irrigated condition	230	260,210	110	S
'Jaya'	As above	230	260,180	110	S
CR-44-1	Advance breeding line	230	260,210	110	UN
'Rasi'	Released variety for up-land situation	230	260,210	110	S
CRM-6-5-90	Advance breeding line	230	260,190	110	UN
'Swarna'	Released photosensitive cultivar	230	260,180	110	UN
'Black patnai'	Photosensitive land race of Bengal	230	260,210	110	UN
'Shunga kalma'	As above	230	260,210	110	UN
'Sunha kalma'	As above	230	260,210	110	UN
'Harijhinga'	Bengal land race for upland situation	230	260,190	110	UN
S-11	Advance breeding line	230	260,190	110	UN
Assam collection	Collection from Assam	230	260,210	110	T
'Pankaj'	Released genotype for lowland eco-system	230	260,210	110	T
'Lalkalmi'	Land race of Bengal	230	260,180	110	UN
'Suryakanta'	As above	230	260,210	110	T
'Kalijalangi'	As above	230	260,210	110	UN
'Jogenchara'	As above	230	260,210	110	UN
'Janglijata'	As above	230	260,180	110	UN
'Panikalas'	As above	230	260,210,180	110	T
'Bhasakalmi'	As above	230	260,180	110	UN
'Nagrasail'	As above	230	260,210	110	UN
'Dinesh'	As above	230	260,190	110	T
'Jitendra'	Released genotype for lowland eco-system	230	260,210	110	T
NC-678	Advance breeding line	230	260,190	110	UN

S: Susceptible; UN: Unknown; T: Tolerant

Polymerase enzyme (1 U/µl) and 17 µl double distilled sterile water. PCR reaction was performed with a Gene AMP PCR System 2400 (Perkin Elmer, Norwalk, CT) and amplified products were separated in 2.5% agarose gel. Banding patterns were visualized and photographed on trans-illuminator (Gel Logic 200, Kodak). The frequency of SSR polymorphism was calculated based on presence (1) or absence (0) of common bands. Polymorphism Information Content (PIC) was calculated as: PIC= 1/nΣ2f (1–f), where f is the proportion of a particular allele among the genotypes.

The distribution of alleles of three SSR primer pairs across the 28 rice genotypes (Fig. 1) revealed that RM-265 and RM-464A did not show any polymorphic pattern. Amplification profile of RM-265 and RM-464A showed the presence of only one allele of 110 bp and 230 bp respectively among the 28 selected genotypes (Table 1). However, screening with RM-219 located on the same rice linkage group no. 9 generated polymorphic pattern by producing four alleles (260 bp, 210 bp, 190 bp and 180 bp). Allele of 260 bp was present in all genotypes with 100% frequency and allele of 210 bp was present in 18 genotypes with 65% frequency and both these were considered as high frequency alleles (Table 2). On the other hand, allele of 180 bp was observed in six genotypes viz. 'Jaya', 'Swarna', 'Lalkalmi', 'Janglijata', 'Panikalas' and 'Bhasakalmi' whereas allele of 190 bp was present in five genotypes viz. 'CRM-6-5-90', 'Harijinga', 'S-11', 'Dinesh' and 'NC- 678'. All genotypes exhibited multiple allelism with two alleles except 'Panikalas' where three alleles were detected. The PIC values derived from allelic diversity and frequency among genotypes for this primer was 0.325.

Submergence tolerance is controlled by a single major QTL, *Sub1* on chromosome 9 along with a number of minor QTLs (Nandi et al., 1997; Toojinda et al., 2003). By using RFLP marker, Xu et al. (2006) found a submergence tolerance QTL located 4 cM from the RFLP marker C-1232, which accounted for 69% of the phenotypic variance for

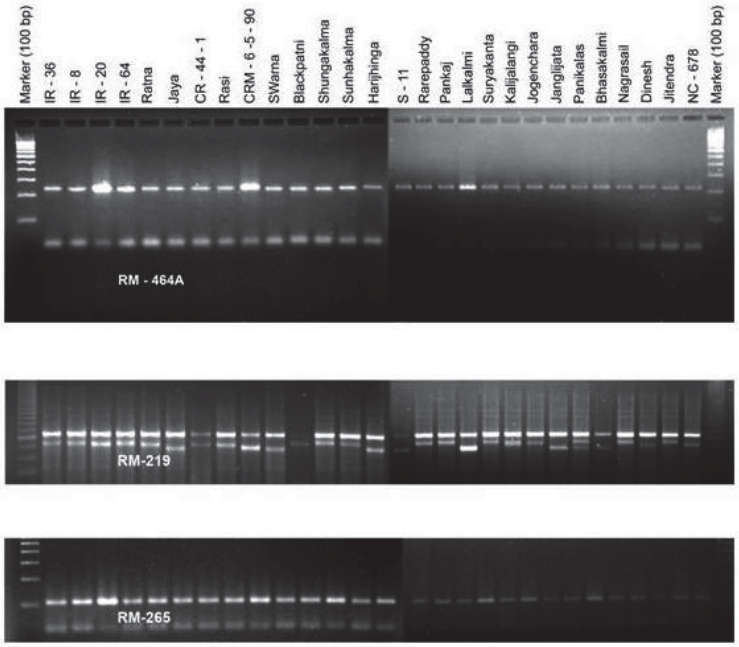

Figure 1. Amplification profile obtain from 28 rice genotypes using 3 SSR primer pairs (RM-265, RM-219 and RM-464A) in 2.5% agarose gel.

*Table 2.*Salient features of amplification profiles of the rice genotypes for 3 SSR markers.

Marker	Linked with	Forward and Reverse Primer	Repeat Motif	Chromosome location	No of alleles	No of genotypes with multiple alleles	Highest frequency allele(%)	PIC –value
RM-464A	Submergence tolerance	F: AAC GGG CAC CTT CTG TCT TC						
		R:TGG AAG ACC TGA TCG TTT CC	(CT) 27	9	1	0	-	0
RM-265	Submergence tolerance	F : CGA GTT CGT CCA AGT GAG C						
		R:CAT CCA CCA TTC CAC CAA TC	(GA) 8	9	1	0	-	0
RM-219	Percent spikelet sterility, submergence	F: CGT CGG ATG ATG TAA AGC CT						
		R:CAT ATC GGC ATT CGC CTG	(CT) 17	9	4	28	100	0.3252

this trait. Using AFLP map of rice, a major gene for submergence tolerance [*Sub1*(t)] was located on chromosome no. 9 and QTL associated with submergence tolerance on chromosome no. 6,7,11 and 12 (Nandi et al., 1997). Although RM-464A is a new co-dominant marker and highly reliable for selection for *Sub1* because of the close linkage (only 0.7 cM apart), it did not show any polymorphism among the genotypes used in this study. This result also corroborates with the finding that RM–464A is a better marker for introgression of *Sub1* into *japonica* background than into *indica* background because of similar or identical size of the RM-464A fragment amplified from Dx 202-9 and those from most *indic*a rice cultivars (Xu et al., 2006). During the present study it was also found that RM-464A along with another SSR marker, RM-265, was unable to distinguish any of the 28 genotypes, implying that these are not suitable for marker assisted selection in *indica* genotypes.

From a breeding perspective, the amplification profile of RM-219 suggests that this SSR can be useful for monitoring introgression of the *Sub-1* locus. However, known submergence tolerant genotypes like 'Pankaj', and 'Jitendra' lack polymorphism for this marker with non-tolerant genotypes like IR-36, IR-20, IR-64, and 'Rasi', which would prevent the use of RM-219 for selection in segregating populations derived from parental combinations. Transfer of submergence tolerance gene from 'Dinesh', 'Panikalas', 'Bhasakalmi' into IR-64 or IR-36 genetic background into the segregating population could be monitored easily through this marker.

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