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Biochemical and SSR marker based characterization of some Bengal landraces of rice suffixed with 'sail' in their name

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Abstract

Twenty five rice landraces of undivided Bengal (name of all these landraces have 'sail' or 'Shali' in their suffix) which were popular before popularization of semi-dwarf high yielding varieties, either for cooking, puffing, popping and flash flood tolerance have been studied using biochemical parameters and their linked SSR markers. Except Shali (blackish small bold), and Shali (golden long) all genotypes contain more than 24 % amylose. RM190 linked with amylose content generated a rare allele in Shali (golden long). Another linked marker RM251 could not explain the differential amylose content in studied rice genotypes. RM 236, a SSR marker linked with rice bran content shows a rare allele (160bp) in Shali (blakish small bold) and Netaisail. Rabansail, Kobirajsail, Mallicksail and Kakudsail have intermediate gelatinization temperature and remaining genotypes have high gelatinization temperature as indicated by alkali spreading value. Linked marker, RM 253 could not explain the difference in alkali spreading values. Screening the genotypes with RM 219, linked with submergence tolerance in rice, generated 4 alleles, *viz.*, 192bp, 204bp, 220bp and 240bp. Allele 1 (192bp) is present in Hardijhingasail and Sankarsail only, and may be considered as a rare allele. None of the genotypes contained 85% or more similarity between each other at molecular level that suggests the absence of duplication of the landraces. RM 206 generated six alleles although polymorphism information content (PIC) value was found to be higher in case of RM 253 (0.491). So both the markers could be used for diversity analysis in rice.

Key words: Rice landraces, grain quality, SSR

Introduction

West Bengal, has a long tradition of growing rice since ancient times. Several landraces of undivided Bengal having common suffix 'sail' in their name (like Latisail, Jhingasail, Rupsail etc.) had been popular before introduction of semi-dwarf high yielding rice cultivars. Superior grain quality, cooking, puffing, popping, flattening, bran content and several biotic and abiotic stress tolerance qualities exist within these landraces. Population explosion compelled people to opt high-yielding rice varieties for producing larger quantity of rice from a limited resource of land. As a result, erosion of several land races has already occurred within a very short time. So it is high time not only for collection but also for evaluation, bio-prospecting and proper documentation of our national treasure.

These tasks have become relatively easier after development of nearly saturated molecular (especially by simple sequence repeat) map in rice. The use of SSR to interpret population structure provides much greater resolution than other types of markers because of their high level of polymorphism and involvement of low cost [1, 2]. Several linked SSR markers have already been established with QTL of economic important quality parameters of rice like, amylose and starch content, seed coat colour and bran content, alkali spreading value etc., [3, 4]. These biochemical properties determine the nutritional, eating, cooking and puffing quality of rice. Similarly, QTL of submergence [5], drought [6], and salinity [7] tolerance have also been mapped by SSR and other PCR based markers.

In this study, 22 landraces of West Bengal and three land landraces from Assam (name of all these landraces have 'sail' in their suffix) which were popular for characteristics like cooking, puffing, popping and flash flood tolerance have been studied using the linked SSR markers. Purpose of this study is to characterize the genotypes by biochemical quality parameters as well by SSR markers linked with amylose, rice bran, alkali spreading value and submergence tolerance.

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Materials and methods

Materials

Twenty five rice landraces were collected from different districts of West Bengal and Assam (Table 1). Along with them, 2 high yielding popular varieties, *viz*., IR 36 and Swarna, were used as control.

Physical and biochemical parameters

Grain length, width and length/width ratio were calculated and the landraces were classified based on them according to the Standard Evaluation System of International Rice Research Institute, Philippines. Seed coat colour was scored visually and evaluated in a scale of 1 to 7, where, 1 = White, 2 = Light brown, 3 = Speckled brown, 4 = Brown, 5 = Red, 6 = Variable purple and 7 = Purple.

Amylose and starch content of the grains were calculated from average of three estimates by spectrophotometer following simplified method of Juliano [8]. Amylopectin content was calculated by subtracting the amylose content from the total starch content.

Alkali spreading value

Six milled rice grains were kept in Petri dish with 10 ml 1.7% potassium hydroxide for 23 hours at 30°C in such a way that they do not touch each other. Disintegration of grains was estimated visually and scored in a 1-7 scale [9].

DNA Extraction, PCR amplification and allelic diversity analysis

Leaves from tender seedlings were washed thoroughly, dried with tissue paper and DNA was extracted using the method as described earlier [10]. All extracted DNA samples were quantified and further diluted to a uniform concentration of 10ng/µl.

The choices of linked SSR primers were mainly on the basis of their association with quality parameters [3] and submergence tolerance [5]. 25 µl reaction volume contained 2µl (20 ng) DNA, 2.5 µl 10XPCR buffer, 1µl 2.5 mM dNTPs, 1 µl (100 ng) each forward and reverse primer, 0.5U Taq DNA Polymerase enzyme and 17µl double distilled sterile water. Banding patterns were visualized in 2.5% agarose (‘Genei’, Bangalore) 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) values were calculated as follows:

$PIC = 1/n\ \Sigma\ 2f\ (1-f)$, where f, Proportion of a particular allele among the genotypes.

The genetic associations among landraces were evaluated from Jaccard’s similarity coefficient. Dendogram was generated with unweighted pair-group method arithmetic average (UPGMA) algorithm, using NTSYS-pc Version 2.1 software [11].

Result and discussion

In this study, 22 rice landraces, collected from different districts of West Bengal and 3 from Assam, were studied. All the landraces of West Bengal have the suffix ‘sail’ common in their names. Physical and biochemical grain quality parameters like length/breadth ratio, 100-grain weight, grain colour (brownness), amylose and amylopectin content and alkali spreading value (ASV) were studied (Table 1). Allelic diversity of these landraces were studied using SSR markers linked with the biochemical parameters (Fig. 1).

Among the landraces, Shali blackish small bold is a red grain genotype whereas Jhingasail (sl. 6), Ranisail (sl. 9) and Mihinagrasail (sl. 13) are with brown grains. A rare allele of 160bp is observed in Shali blackish small bold and Netaisail, for RM236, linked SSR marker with the rice bran content, QTL on chromosome 2 [3]. So RM 236 might be used in marker assisted breeding programme to improve the rice bran content, an important factor for nutritional value of rice and has a high demand in international market, particularly where Shali blackish small bold will be used as donor parent. Allelic profile of RM349, another SSR marker linked with rice bran content QTL on chromosome 4, does not distinguish the above-mentioned landraces from others.

Length and slenderness of grains are important factors for customer preference. 12 out of 25 landraces are slender grained, and rest of the genotypes has medium grains.

Amylose content of these genotypes varies from approximately 20% (Shaili golden long) to 32% (Durgasail). Except Shali (blackish small bold), and Shali (golden long), all genotypes contain more than 24% amylose. SSR marker RM 190, linked with the QTL for amylose content on chromosome 6, generated a rare allele of 135bp (Fig. 3) in Shali (golden long) that correlates with relatively low amylose content (less than 20%). Other two alleles generated by this marker correlates with amylose content 20% or more. Another SSR marker RM7, linked with the QTL for amylose content on chromosome 3, generated three reproducible

Table 1. Physical and biochemical properties of grains of the rice genotypes

Landrace	Grain colour	100 grain weight	L/W ratio class	Starch %	Amylose %	Amylo-pectin %	ASV score
Shall (Golden, small, bold)	1	2.16	Medium	86.4	28.53	57.87	3
Shali (Blackish, small, bold)	5	1.86	Medium	62.6	20.69	41.91	3
Beharsail	2	2.78	Slender	75.0	29.07	45.93	2
Vimsail	3	2.27	Slender	60.1	27.65	32.45	1
Punjabsail	2	2.65	Slender	67.4	29.68	37.72	2
Jhingasail	4	2.01	Slender	75.5	24.10	51.4	3
Balaramsail	1	1.84	Slender	83.8	27.50	56.3	3
Nagrasail	2	1.87	Slender	74.5	29.05	45.45	2
Ranisail	4	1.88	Slender	76.9	28.07	48.83	2
Hardijhingasail	2	1.61	Slender	82.7	28.65	54.05	3
Sitasail	1	1.30	Slender	66.4	24.14	42.26	2
Nijersail	3	3.26	Medium	80.8	26.38	54.42	1
Mihinagrasail	4	1.99	Medium	88.8	28.38	60.42	2
Rabansail	3	2.42	Medium	93.3*	28.36	65.54	4
Sankarsail	1	2.39	Slender	91.6	27.17	64.43	3
Kobirajsail	2	2.37	Medium	90.7	24.62	67.08	4
Durgasail	1	2.48	Medium	74.6	30.21	44.39	2
Latisail	1	2.47	Medium	78.2	25.69	52.51	3
Mallicksail	1	2.01	Slender	72.1	24.50	47.6	4
Nakrasail	3	1.56	Medium	67.7	28.16	39.54	2
Netaisail	1	1.33	Medium	68.3	27.83	40.47	1
Agnisail	2	2.46	Medium	66.0	28.52	37.48	2
IR36	3	2.02	Slender	87.3	24.65	62.65	3
Swanja	1	1.85	Medium	84.9	25.13	59.77	3
Kakudsail	3	1.45	Slender	78.5	27.24	51.26	4
Shali (Golden, long)	1	1.15	Medium	75.6	19.98	48.36	2
Rupsail	1	2.10	Medium	80.6	28.10	52.5	2

alleles (153bp, 172bp and 192bp). One of them (192bp) correlates with more than 30% amylose content except Shali golden long. Amplification profile of another amylose linked marker, RM251 of chromosome 3 reveals the presence of 3 alleles *viz.*, 118bp, 147bp and 136 bp. None of them could explain the differential amylose content of the genotypes.

Amylose and amylopectin ratio could not explain simply the popularity of Jhingasail and Latisail as a source of puffed rice in West Bengal. However, their puffing quality might be explained through difference between Swarna (poor puffing quality), at the alleles (144 bp) of RM253. This polymorphic profile demands further exploration to ascertain their link with puffing ability in rice.

Results of alkali spreading value indicates that except Rabansail, Kobirajsail, Mallicksail and Kakudsail, all other genotypes have low or low to intermediate alkali spreading value. Alkali spreading value gives the indication of gelatinization temperature; the temperature at which rice grains become gelatinized during cooking. Except the above-mentioned four landraces, remaining landraces have high or high to intermediate gelatinization temperature. Rabansail, Kobirajsail, Mallicksail and Kakudsail have intermediate gelatinization temperature. Linked SSR marker RM253

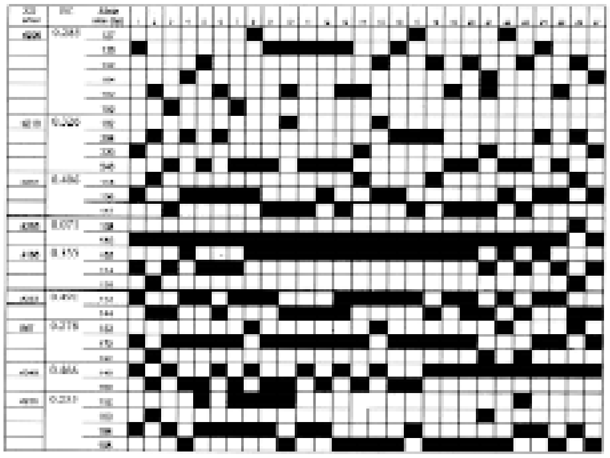

Fig. 1. Figerprinting pattern of the genotypes with 9 SSR markers and their PIC values; 1) Shali (Golden, small, bold), 2) Shali (Blackish, small, bold), 3) Beharsail, 4) Vimsail, 5) Punjabsail, 6) Jhingasail, 7) Balaramsail, 8) Nagrasail, 9) Ranisail, 10) Hardijhingasail, Il)Sitasail, 12) Nijersaii, 13) Mihinagrasail, 14) Rabansail, 15) Sankarsail, 16) Kobirajsail, 17) Durgasail, 18) Latisail, 19) Mallicksail, 20) Nakrasail, 21) Netaisail, 22) Agnisail, 23) IR36,24) Swarna, 25) Kakudsail, 26) Shali (Golden, long) and 27) Rupsail

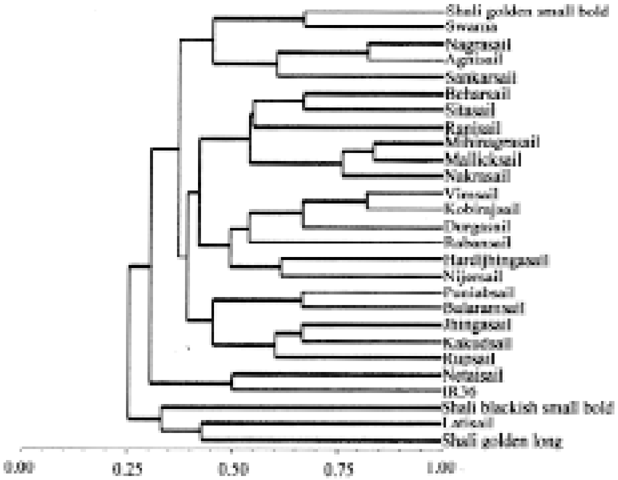

Fig. 2. Dendogram based on Jaccard's similarity coefficient

generated 2 alleles (112bp and 144bp), which could not explain the differences in alkali spreading value among the genotypes. So RM253 is not a suitable marker for MAS, particularly, when any of these land races will be used as a parent for improving gelatinization temperature.

Screening the genotypes with RM 219 generated 4 alleles, ranging from 192bp to 240bp. This marker was previously found to be linked to *Sub1* locus of Chromosome 9 by 3.4 cM, and was observed to be more suitable to study the concerned trait in a wide range of backgrounds [5]. Allele 2 (204bp) is present in 8 genotypes, as Shali blackish small bold, Vimsail, Jhingasail, Kobirajsail, Durgasail, Latisail, Swarna, and Shali golden long. Like Latisail, these genotypes are flash flood tolerant and might possess the locus Sub 1. Alkie 3 (220bp) is present on Shali golden small bold, Rabansail, Netaisail and Rupsail. Allele 1 (192bp) is present in Hardijhingasail and Sankarsail, only and may be regarded as a rare allele. Allele 4 (240bp) is present in other 13 landraces and IR36. These landraces are nontolerant to flash flood. Another SSR marker, RM265, linked to the flood tolerance QTL, was found to be monomorphic. It reveals RM 219 to be more suitable for screening . submergence tolerance in rice, than RM 265.

The dendogram (Fig. 2) based on Jaccard's similarity coefficient shows that the rice genotypes are quite different from each other and none of them contains 85% or more similarity between each other, at molecular level. It suggests the absence of duplication of same landrace in different locations with different local name. As revealed from molecular fingerprinting, Mihinagrasail and Mallicksail are most closely related (similarity coefficient 0.833) on the other hand, Shali golden long, Hardijhingasail, Netaisail and Kobirajsail are most distantly related (similarity coefficient 0.105). SSR marker RM 206, located on chromosome 11 generated maximum no of alleles (6) although polymorphism Information Content (PIC) value was found to be maximum in case of RM 253, located on chromosome 6 (PIC value 0.491). So, along with RM206, which was suggested earlier [12], RM 253 could also be used for diversity analysis in rice.

The study reveals that the Bengal landraces of rice may serve as an important source of various unexplored desirable alleles for quality improvement programme of rice. High amylose containing landraces, like, Durgasail, Beharsail, can be used to develop high amylose containing varieties of rice. Brown rice has a great demand in the international market. So, the landraces like, Shali blackish small bold, Netaisail can be exploited in future. Polymorphism identified at individual SSR loci and resultant diversity analysis may serve in selecting parental combination as well as in marker assisted breeding programme.

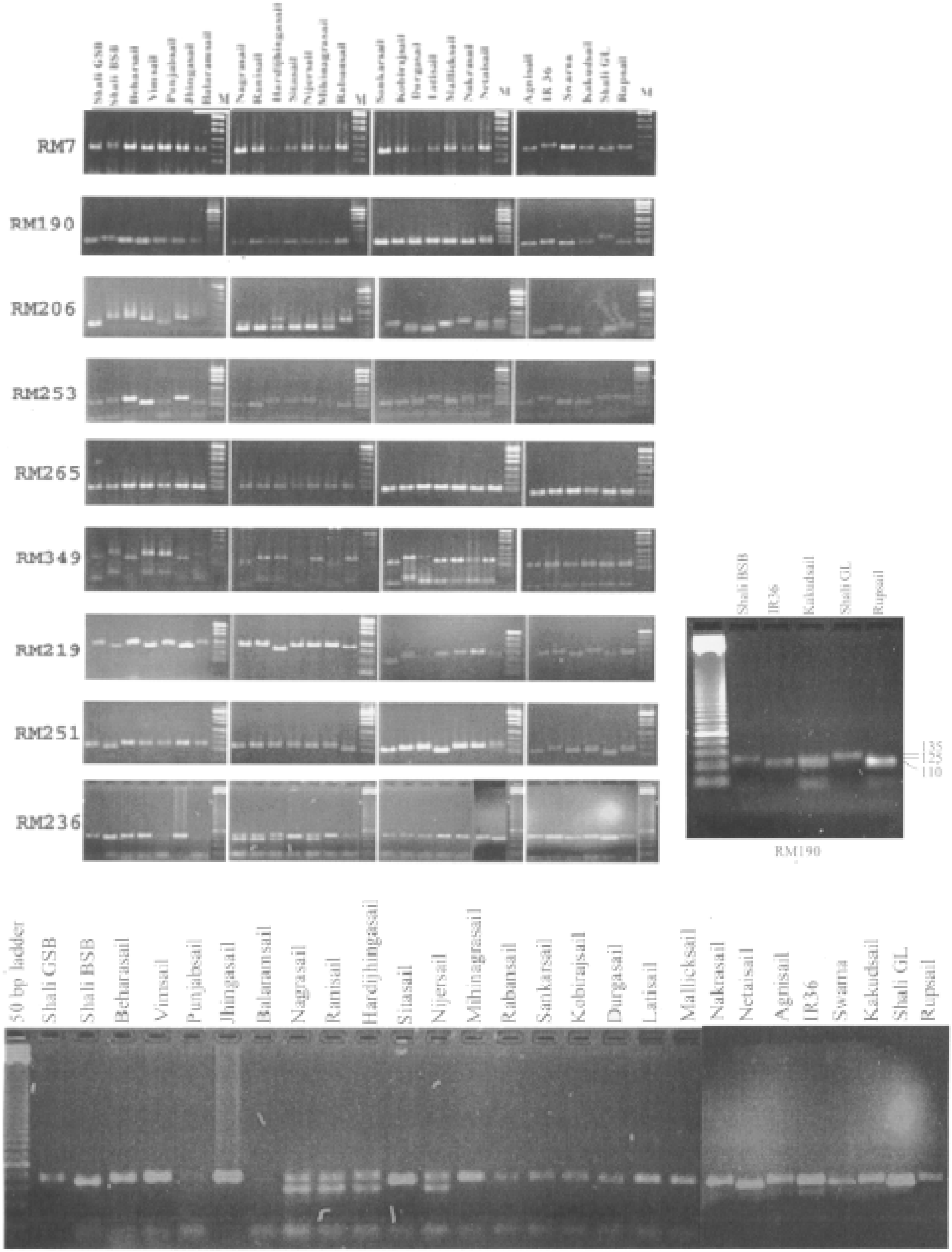

Fig. 3. SSR profile of rice genotypes in 2.5% agarose gel. M, standard marker (pUC/MSPI)

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