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Short Communication

Microsatellite diversity among aerobic and lowland *indica* rice genotypes with differential water requirements

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Abstract

Molecular polymorphism in a set of 15 rice genotypes comprising of aerobic and lowland Basmati, *indica* and *japonica* rice varieties was evaluated using *BAD2* aroma-specific and 50 mapped SSR markers. A total of 260 alleles were detected and number of alleles per locus and polymorphism information content values ranged from 2 to 9 (average 5.1) and 0.226 to 0.836 (average 0.672), respectively. Notably, two SSR markers (RM212 and RM302) amplified specific alleles in all the five aerobic rice genotypes, which were absent in non-aerobic rice genotypes. SSR diversity data analyzed using NTSYS-pc clustering algorithms showed greater divergence between the Basmati and aerobic rice varieties as compared to lowland *indica* and aerobic rice varieties.

Key words: Molecular diversity, basmati, *BAD2*, aerobic, *indica* rice

Increasing scarcity of water has threatened the traditional way of irrigated rice production. To address the problem of water scarcity, research has been directed towards the development of "aerobic rice" varieties that combine the drought-resistant characteristics of upland varieties with the high-yielding traits of lowland varieties to achieve high and sustainable yields in non-flooded soil [1]. Aerobic rice does not require puddling and submergence but require less quantity of seed and saves water up to 70%. In India, development of aerobic rice varieties was initiated at the University of Agricultural Sciences, Bangalore using the available upland paddy and high-yielding rice germplasm and several aerobic rice genotypes were developed using conventional breeding and marker-assisted selection techniques in combination [2-3]. However, aerobic rice varieties developed to-date fall short of yield potential and quality features of commercially cultivated *indica* and basmati rice varieties.

Historically, genetic diversity and intraspecific classification in Asian rice has been studied using morphological, serological and hybrid fertility parameters, morpho-geographical data, isozymatic profile and DNA marker analysis [4-6]. The evolution of reliable molecular marker technologies has provided us a reliable tool for diversity and pedigree analyses, linkage mapping of genes/QTLs for important traits and their introgression with impeccable precision through marker assisted selection without altering the genetic background in rice [7]. Molecular markers particularly the microsatellite markers (SSRs) are valuable as genetic markers because they detect high levels of allelic diversity, are co-dominant and economically assayed by PCR [8]. In this paper, we report the genetic diversity among aerobic and non-aerobic lowland *indica* rice varieties using 51 microsatellite DNA markers (SSRs) and describe its implications in the breeding for water-efficient low-land *indica* and basmati rice cultivars.

A total of 15 rice genotypes were used including two premium traditional Basmati (Taraori Basmati and Basmati 370), two cross-bred Basmati (Pusa Basmati 1460 and Pusa 1121), four *indica* (HKR47, PAU201, CSR10 and IR36), two *japonica* (Nipponbare and Azucena) and five aerobic (MAS25, MAS26, MAS109, MAS-ARB25 and MAS-ARB868) rice varieties. Pusa

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Basmati 1460 (bacterial leaf blight resistant, improved Pusa Basmati 1) and Pusa 1121 have been developed from various crosses between Basmati and *indica* rice varieties at IARI (New Delhi). While aerobic rice varieties, MAS25, MAS26, MAS109, have been developed from crosses between upland paddy and high-yielding rice germplasm at University of Agricultural Sciences, Bangalore [9], MAS-ARB25 (IET-20649) and MAS-ARB868 (IET-20653), have been developed from IR64/Azucena/xx IR64 crosses at IRRI, Manila, Philippines. Genomic DNA was isolated from 0.5 g leaf tissue collected from five one-month-old plants using CTAB method [10]. DNA quantity was estimated by ethidium bromide staining on 1% agarose gels using a standard containing 100 ng/µl genomic DNA. Microsatellite marker analysis was carried out using 50 mapped SSR and aroma gene specific *BAD2* markers (Table 1). The map position, original source and repeat motifs for these markers can be found in Temnykh *et al*. [11] and at http://www.gramene.org. PCR amplification, denaturing polyacrylamide gel electrophoresis and silver staining was essentially carried out as described earlier by Jain *et al*. [12]. Experiments were repeated at least twice to assign unique, null and multiple electromorphs [6]. The frequency of molecular marker polymorphism was calculated based on presence or absence of common bands. The polymorphism information content (PIC) value was calculated according to Anderson *et al.* [13] based on the allelic patterns of all the genotypes analyzed. Genetic similarities between the cultivars were measured by the similarity coefficient based on the proportion of shared electromorphs using 'Simqual' sub-program of NTSYS-PC (Version 2.02 Exeter Software, Setauket, NY, USA) software package [14]. The resultant distance matrix data was used for two-dimensional scaling of rice genotypes by Principal Component Analysis (PCA).

A microsatellite fingerprint database has been generated using 51 markers (50 SSRs and *BAD2*) for 15 rice genotypes and used for determination of genetic relationships. A total of 260 alleles at 50 SSR and *BAD2* loci were detected in 15 rice varieties (Table 1). The number of alleles per locus ranged between 2 (RM433, RM556 and RM10916) and 9 (RM335) with an average of 5.1 alleles per locus. The overall size of PCR products amplified using 51 primer pairs ranged between 74 and 585 bp. The molecular size difference between the smallest and largest allele at a SSR locus varied from 2 (RM433) to 328 (*BAD2*). No null alleles were detected at any of the 51 loci. Maximum variation in allele size was observed for *BAD2* and SSR markers with GT repeat motifs (RM320, 96 bp), ATT repeat motifs (RM144, 60 bp) and CTT repeat motifs (RM440, 27 bp). Minimum variation in allele size was observed for markers with AG repeat motifs (RM433, 02 bp), GA repeat motifs (RM404, 04 bp) and CT repeat motifs (RM 215, 04 bp and RM237, 06 bp). Polymorphism information content (PIC), which is an indicative of level of polymorphism, varied from 0.226 (RM556) to 0.836 (RM144) with an average of 0.672 per locus. A number of SSRs have been identified, which can be used to differentiate among the Basmati and aerobic (31 SSRs; RM12, RM21, RM30, RM144, RM162, RM178, RM205, RM206, RM210, RM212, RM222, RM228, RM229, RM234, RM240, RM247, RM258, RM259, RM302, RM315, RM320, RM333, RM335, RM339, RM400, RM440, RM518, RM528, RM547, RM10916, *BAD2*) and *indica* and aerobic (8 SSRs; RM21, RM162, RM206, RM212, RM222, RM237, RM256, RM302) rice varieties. Remarkably, two of 50 SSR markers (RM212 and RM302) were among the most useful markers which amplified specific alleles (112 and 190 bp, respectively) only in all the five aerobic rice genotypes; these alleles were absent in other ten non-aerobic rice genotypes (Fig. 1). These two microsatellite markers have also been shown earlier to be associated with drought resistance in rice [15]. Such polymorphic markers stand greater chances of having a linkage with the genomic regions, which may have significant contributions towards the development of water efficient rice varieties.

Genetic relationships among rice genotypes as determined by UPGMA cluster analysis and two-dimensional PCA scaling showed that the 15 rice genotypes were divided into two major distinct groups (Fig. 2 a & b). The group I had four Basmati rice varieties,

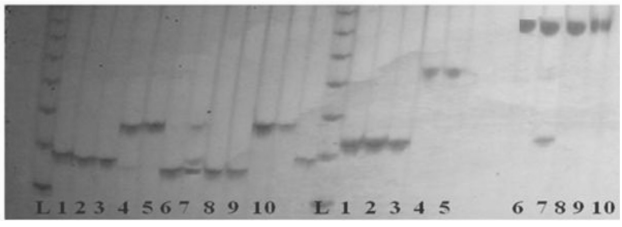


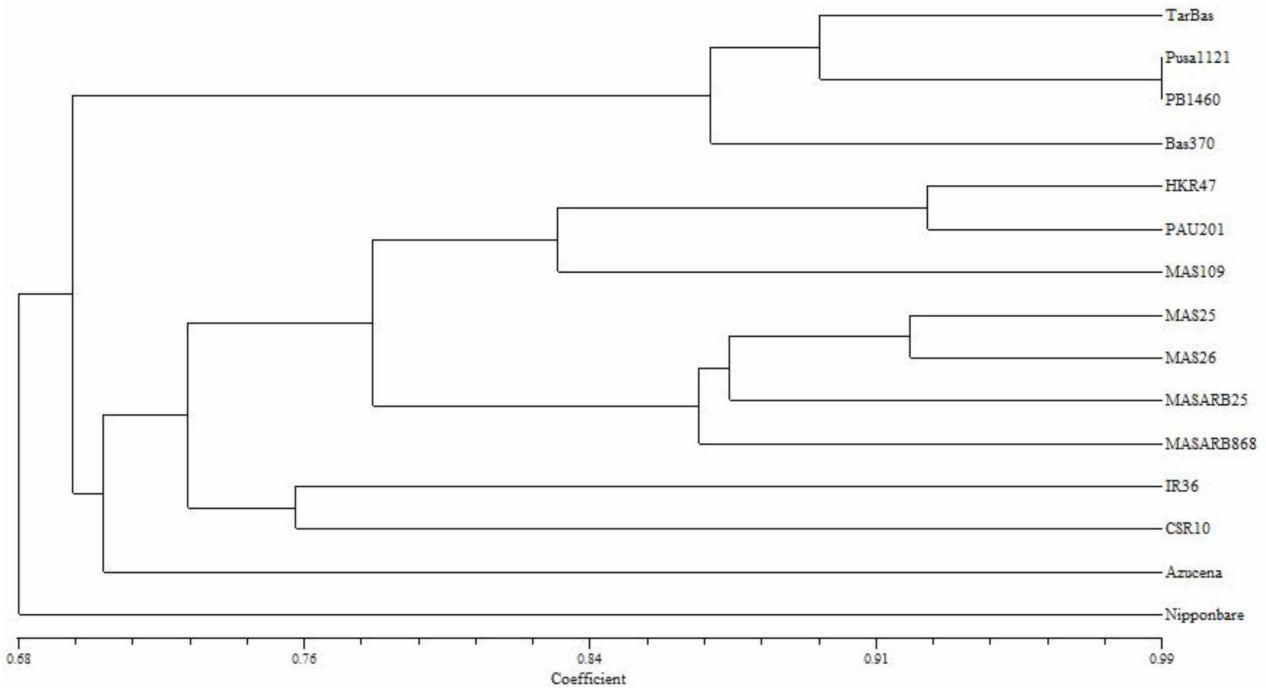
Fig. 1. A silver stained gel showing allelic polymorphism among parental rice varieties at RM212 and RM302 locus (from left side) respectively. Lane L -20 bp ladder, lanes 1 to 10 represents Taraori Basmati, Pusa1121, Pusa Basmati1460, HKR47, PAU201, MAS25, MAS26, MAS109, MASARB25, MASARB868 varieties for each RM locus respectively

Table 1. Data on the number of alleles, no. of genotypes with multiple alleles, null alleles, size range, PIC values among fifteen rice varieties obtained using *BAD2* and 50 SSR markers

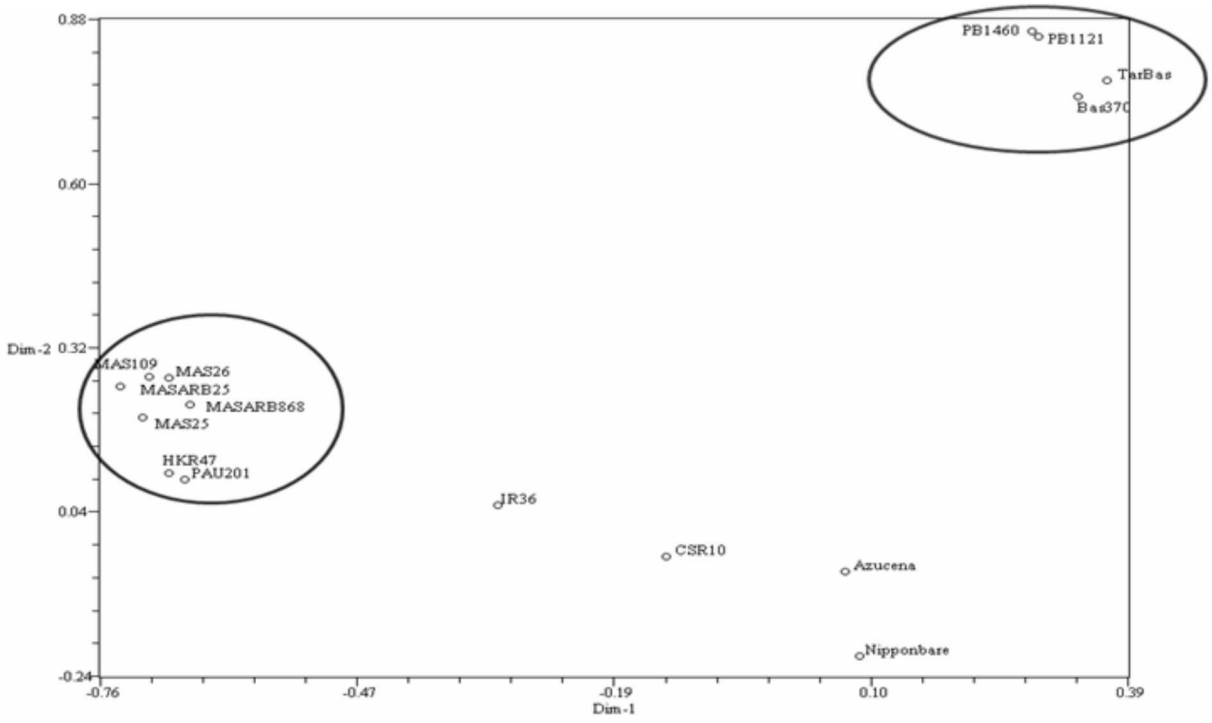
Marker	No. of alleles	Varieties with null alleles	Varieties with null alleles	Allele size		PIC value
				Range (bp)	Difference	
RM12	6	0	0	247-269	22	0.764
RM21	7	0	0	135-164	29	0.807
RM30	6	0	0	80-106	26	0.710
RM144	8	0	0	220-280	60	0.836
RM162	5	0	0	215-245	30	0.758
RM178	4	0	0	108-122	14	0.683
RM201	6	0	0	112-157	45	0.661
RM205	6	0	0	120-165	45	0.505
RM206	7	0	0	124-164	40	0.827
RM210	6	0	0	132-165	33	0.750
RM212	4	0	0	112-136	24	0.710
RM213	3	0	0	127-138	11	0.234
RM215	3	0	0	150-154	04	0.494
RM221	4	0	0	187-210	23	0.683
RM222	8	0	0	199-222	23	0.784
RM228	7	0	0	108-153	45	0.807
RM229	4	0	0	106-124	18	0.552
RM230	5	0	0	246-257	11	0.756
RM232	6	0	0	146-165	19	0.737
RM234	5	0	0	135-162	27	0.718
RM237	4	0	0	134-140	06	0.663
RM240	7	0	0	111-145	34	0.817
RM241	5	0	0	103-145	42	0.756
RM242	4	0	0	194-232	38	0.600
RM246	6	0	0	108-119	11	0.764
RM247	6	0	0	135-175	40	0.764
RM248	5	0	0	74-104	30	0.704
RM256	7	0	1	107-143	36	0.667
RM257	5	0	0	149-183	34	0.653
RM258	4	0	0	134-152	18	0.686
RM259	6	0	0	158-180	22	0.768
RM278	5	0	0	133-148	15	0.681
RM286	3	0	0	112-122	10	0.426
RM302	3	0	0	126-190	64	0.658
RM315	3	0	0	133-160	27	0.606
RM320	7	0	0	174-270	96	0.613
RM333	7	0	0	163-210	47	0.817
RM335	9	0	1	105-155	50	0.806
RM339	5	0	0	118-176	58	0.681
RM400	7	0	0	184-321	137	0.782
RM404	3	0	0	236-240	04	0.474
RM433	2	0	0	222-224	02	0.442
RM440	6	0	0	193-220	27	0.764
RM447	5	0	0	105-138	33	0.742
RM458	3	0	0	178-189	11	0.784
RM518	6	0	0	159-173	14	0.809
RM528	6	0	0	248-278	30	0.737
RM547	4	0	0	226-256	30	0.737
RM556	2	0	0	87-93	06	0.226
RM10916	2	0	0	215-220	05	0.480
BAD2	3	0	15	257-585	328	0.397
Total	260	0	17			34.28
Average	5.1	0	0.33			0.672

Taraori Basmati, Pusa 1121, Basmati 370 and Pusa Basmati 1460. The second group was further divided into subgroups having *indica* (HKR47 and PAU201), aerobic (MAS25, MAS26, MAS109, MASARB25 and MASARB868) and *japonica* (Azucena) rice varieties.

The polymorphism among different genotypes obtained by using SSR markers has been a strong and authentic tool for genetic analysis in rice [6, 8]. SSR diversity data analyzed using clustering algorithms showed greater divergence between the Basmati



(a)



(b)

Fig. 2. Dendrogram (a) and two dimensional PCA scaling (b) of fifteen rice varieties using SSR allelic diversity data at 51 loci

(Taraori Basmati, Pusa 1121, Basmati 370 and Pusa Basmati 1460) and aerobic (MAS25, MAS26, MAS109, MASARB25 and MASARB868) rice varieties as compared to lowland (HKR47 and PAU201) *indica* and aerobic rice varieties. This inference is in conformity with the earlier isoenzyme [5] as well as microsatellite polymorphism [6-8, 12] based classifications, where most of Basmati rice genotypes were placed into a separate distinct group than that of '*indica*' and 'aus' rice genotypes. Based on isoenzymatic polymorphism, Glaszmann [5] placed the early maturing and drought tolerant upland rice varieties (known as 'aus' varieties) in a distinct varietal group II different from that for typical *indica* (varietal group I) and *japonica* (varietal group VI) rice varieties. Basmati and aromatic rices of Indian subcontinent were classified in group V. Group II, III. IV and V rices were atypical but conventionally classified as *indica* [16]. It must be noted that aerobic rice genotypes were placed closer to *indica* and *japonica* than the Basmati rice group which can be explained by the fact aerobic rice varieties used in this study were developed from crosses involving *indica* and *japonica* rice genotypes [2]. As reported earlier in Basmati x *indica* crosses, hybrid sterility and low seed setting were also observed in aerobic x Basmati crosses (data not shown), which can be attributed to higher degree of genetic divergence between the two rice types [17].

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