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Short Communication

Molecular profiling of rice (*Oryza sativa* L.) genotypes differing in micronutrients (iron and zinc) content

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Abstract

The present study was undertaken to study the genetic diversity among fourteen rice genotypes, micronutrient rich *indica*, Basmati, *japonica* and commercial cultivars using 50 microsatellite markers uniformly distributed across the rice genome. A total of 257 alleles were detected that ranged from 1 to 9 with an average of 5.14 alleles per locus. The overall size of amplified PCR products ranged from 73 to 585 bp. The Polymorphic Information Content (PIC) value ranged from 0 to 0.862 with an average of 0.66. The NTSYS-pc UPGMA tree cluster as well as WINBOOT analysis placed the Fe-rich genotypes with Zn-rich Jaya and high-yielding *indica* rice genotypes, Fe-rich Taraori Basmati in Basmati rice group with Pusa Basmati 1 and Basmati 370, zinc rich TNG67 in *japonica* group. The profiting of the genotypes would pave the way for genetic improvement for micronutrients in rice.

Key words: Genetic diversity, microsatellite marker, micronutrient, *Oryza sativa* L.

Rice (*Oryza sativa* L.), the world's most important food crop that feeds over half of the global population, is a model plant species for genomic research. Molecular markers have proven useful in both basic and applied research, such as DNA fingerprinting, varietal identification, diversity and phylogenetic analysis, marker assisted breeding, and map based cloning of genes in rice [1-3]. Microsatellites are tandemly arranged repeats of short DNA motifs (1-6 bp in length) that frequently exhibit variation in the number of repeats at a locus. Previous studies in rice have contributed to the development of several hundred microsatellite markers and a genetic map consisting of 320 SSRs [3, 4]. These markers have been used to analyze diversity[5] and to locate genes and QTLs on rice chromosomes [6]. In the present study, we evaluated the genetic diversity among 14 rice genotypes using 50 SSR markers.

The genotypes included micronutrient rich [7] *indica*, Basmati, *japonica* and commercial cultivars (Table 1). Genomic DNA was isolated using CTAB method [8] from young leaf samples of each rice genotype and checked for quanlity and quantity. The PCR reaction was conducted in a reaction volume of 20µl containing 1X PCR buffer, 100 µM dNTPs, 0.3 pmol of each primer, 1.5 unit *Taq* DNA polymerase and 50-75 ng template DNA. DNA bands were then visualized using the Silver Sequence DNA Sequencing System (Promega Technical Manual, Part # TM023). The polymorphism information content was determined for the markers according to Anderson *et al.* [10] based on allele pattern of all varieties analyzed. The frequency of polymorphism between different varieties of rice for each type of marker was calculated based on presence (taken as 1) or absence (taken as 0) of common bands [11]. The 0/1 matrix was used to calculate similarity genetic distance using 'simqual' sub-program of software NTSYS–PC [12]. Bootstrap analysis with 1000 replicates was carried out to find the statistical support for internal branches of the UPGMA tree [13].

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Table 1. Detail of fourteen selected rice genotypes differing in mineral content

Group	Genotypes	Micronutrient content (mg/kg)*		Detail and type of variety
		Fe	Zn	
Indica	PAU201	75.5	24.2	*Indica*
	HKR47	103.5	11.5	*Indica*
	IR36	47	26.5	*Indica* M-40-431-24-114 x Jaya Salt tolerant
	CSR10	83.5	28.5	Cross bred *indica* developed from crosses and backcrosses by CSRI/Jaya
	Jaya	175.5	29.5	*Indica*
	BR4-10	51.5	25	Aus
	HKR95-157	441.5	17.5	*Indica*
	Palman-579	409	28.5	Cross breed *indica* (IR 8/Tadukan). Tolerant to bacterial blight
	Taraori Basmati	207.5	39.5	Pure line selection from local Basmati
Basmati	Pusa Basmati 1	88.5	28.5	Developed from the crosses of Pusa 150/Karnal Local
	Basmati 370	81	28	Traditional Basmati
	TNG67	102.5	37.5	*Japonica*
Japonica	Azucena	78.5	20.5	Tropical *japonica*
	NPT II	79	21	*Japonica*

*Fe and Zn content as per Brar *et al*. [7]

Out of 50 primers used in the study, two or more of the fourteen varieties showed polymorphism at 48 SSR loci. A total of 257 alleles were detected at 50 SSR loci (Table 2). The number of alleles per locus ranged from 1 (RM325, RM513) to 9 (RM21, RM144 and RM447) with an average of 5.14 alleles per locus. These results were in agreement with Cho *et al*. [14], who reported an average of 2.0-5.5 alleles per locus in different rice germplasm. Panaud *et al*. [4] found 2 to 9 alleles for microsatellite markers in a set of 22 *indica* and *japonica* cultivars. The allelic frequency observed in present study are quite comparable to those reported earlier. The overall size of amplified PCR products ranged from 73-585 bp. The molecular size difference between the smallest and largest allele at a SSR locus varied from 1 (RM6641) to 328 (*BAD2*). Multiple alleles were also detected at six SSR loci and the highest number of multiple alleles was observed for *Bad2* marker [14]. Polymorphism information content (PIC), which is an indicative level of polymorphism, varied from 0 (RM325 and RM513) to 0.862 (RM21) with an average of 0.66 per locus. The Polymorphism information content (PIC) value provides an estimate of discriminating power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. Two loci, RM 144 and RM447 amplified highest number of alleles (9 in each case) and the lowest number of alleles was observed is one in two loci, RM325 and RM 513. The highest number of unique allele was observed for three loci, RM144, RM324 and RM447 (6 in each case). The highest percentage frequency alleles was 100% observed for two loci (RM325 and Bad2) and 21.64 % is the lowest percentage frequency allele was observed for three loci, RM235, RM 310 and RM528 (Table 2).

Similarity coefficient data based on the proportion of shared alleles using 'simqual' sub-program of NTSYS-pc UPGMA software was used to calculate the coefficient values among the rice genotypes. Two rice varieties, Basmati 370 and Taraori Basmati had maximum similarity coefficient of 0.646. Minimum similarity coefficient (0.051) was observed between Taraori Basmati and IR36. Group wise mean similarity coefficient values were also calculated for the various rice groups including mineral rich, *indica, japonica* and Basmati rice (Table 3). Mineral rich/mineral rich, *japonica*/*japonica*, *indica*/*indica* and Basmati/Basmati showed mean similarity of 0.244, 0.296, 0.25 and 0.505, respectively. Mineral rich group had greater similarity with Basmati (0.233) compared to *indica* (0.214) and *japonica* (0.155) rice groups. *Japonica*/

Table 2. Molecular data of the 14 rice genotypes

S.No.	Marker name	Chromosome number	Number of alleles	Genotype (No) with		Size range (bp)	PIC
				unique alleles	multiple alleles		
1	RM 1	1	6	3	0	82-125	0.791
2	RM 17	12	6	3	0	150-184	0.791
3	RM 21	11	9	5	0	130-164	0.862
4	RM 23	1	6	3	0	120-146	0.694
5	RM 30	6	6	3	0	73-120	0.786
6	RM 34	1	2	0	0	160-165	0.505
7	RM 53	2	6	3	0	178-192	0.776
8	RM 81	3	3	0	0	118-130	0.643
9	RM 137	8	4	1	0	232-238	0.643
10	RM 144	11	9	6	1	160-280	0.822
11	RM 152	8	7	4	1	108-159	0.832
12	RM 162	6	6	2	0	217-255	0.786
13	RM 201	9	5	2	1	144-173	0.745
14	RM 205	9	4	1	1	123-156	0.597
15	RM 228	10	7	4	0	108-153	0.832
16	RM 234	7	5	2	0	124-156	0.704
17	RM 235	12	6	1	0	85-134	0.816
18	RM 237	1	5	1	0	128-142	0.745
19	RM 240	2	5	3	0	95-145	0.551
20	RM 242	9	6	2	0	193-225	0.623
21	RM 248	7	2	2	0	74-102	0.745
22	RM 256	8	6	0	0	107-137	0.245
23	RM 257	9	6	3	0	122-182	0.765
24	RM 270	12	5	1	0	105-115	0.623
25	RM 284	8	6	1	0	128-142	0.806
26	RM 296	9	5	0	0	100-130	0.765
27	RM 300	2	5	1	0	100-130	0.745
28	RM 301	2	3	1	0	140-153	0.357
29	RM 310	8	6	2	0	80-123	0.837
30	RM 315	1	4	2	0	133-155	0.582
31	RM 320	7	7	3	0	174-270	0.792
32	RM 324	2	8	6	0	133-180	0.796
33	RM 325	7	1	0	0	-	0
34	RM 333	10	6	2	0	146-250	0.714
35	RM 335	4	5	4	0	104-135	0.755
36	RM 339	8	6	2	1	115-176	0.683
37	RM 400	6	7	4	0	186-245	0.796
38	RM 440	5	7	3	0	120-210	0.816
39	RM 447	8	9	6	0	110-170	0.847
40	RM 513	1	1	4	0	-	0
41	RM 528	6	8	0	0	245-278	0.847
42	RM 547	8	4	0	0	226-256	0.704
43	RM 585	6	5	3	0	174-250	0.551
44	RM 1089	5	6	2	0	170-235	0.832
45	RM 3331	12	4	1	0	130-160	0.623
46	RM 3412	1	3	0	0	210-250	0.602
47	RM 6641	2	2	0	0	134-135	0.336
48	RM 10772	1	2	0	0	392-395	0.459
49	*BADEX 7-5*	-	2	0	0	95-103	0.418
50	*BAD 2*	-	3	0	14	257-585	0.418
	Average		5.14	2.08	0.38	73-585	0.660

Table 3. Mean Jaccard's similarity coefficient between various rice types based on SSR analysis

Groups	Jaccard's similarity coefficient
Mineral rich-Mineral rich	0.244
Mineral rich-*Japonica*	0.155
Mineral rich-*Indica*	0.214
Mineral rich-Basmati	0.233
Indica-Indica	0.250
Indica-Basmati	0.149
Indica-Japonica	0.136
Basmati-Basmati	0.505
Basmati-*Japonica*	0.130
Japonica-Japonica	0.296

indica and *indica*/Basmati groups had a mean similarity of 0.136 and 0.149, respectively.

Genetic relationship as determined by cluster analysis of SSR datasets are shown in Fig. 1. The SSR-dataset based dendrogram shows formation of distinct clusters at 0.15 similarity coefficient level (Fig. 1). The NTSYS-pc UPGMA tree cluster analysis led to the distribution of 14 rice varieties, into two major groups, I and II. The major group I is further divided into two subgroups with subgroup IA having four micronutrient rich varieties (HKR95-157, Palman 579, BR4-10 and Jaya) and two high-yielding *indica* rice varieties (HKR47 and PAU201). The subgroup IB had

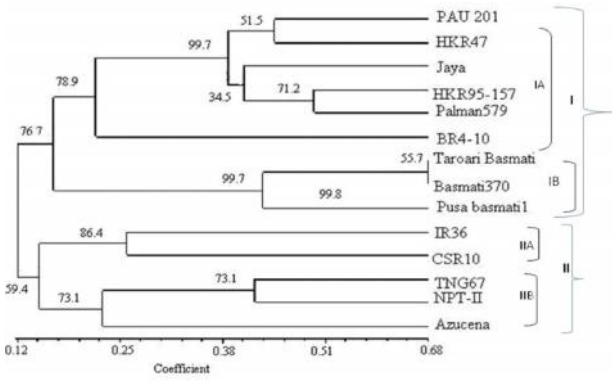

Fig. 1. Dendrogram showing genetic relationship among fourteen rice varieties based on the alleles amplified at 50 SSR loci

all the Basmati rice varieties (Taraori Basmati, Basmati 370 and Pusa Basmati 1). The major group II was also divided into two subgroups with subgroup IIA having *indica* rice varieties (IR36 and CSR10) and subgroup IIB having the entire *japonica* rice varieties (TNG67, NPTII and Azucena). The bootstrap values ranged between 34.5 % to 99.8%. These values can be considered to be statistical tests (confidence limits) for the validity of various groups. The higher the percentage, the greater will be the confidence that a particular group is true, rather than being an artifact of the clustering process.

Genetic relationships and diversity data generated using SSR marker systems was in agreement with the Glaszmann [21] classification of Asian rice germplasm. NTSYS-pc UPGMA tree cluster analysis showed the clustering of 14 rice genotypes into two major distinct groups that correspond to four subgroups. The group one further divided into two subgroups: subgroup-IA with micronutrient rich varieties (HKR95-157, Palman 579, BR4-10 and Jaya) and two high yielding *indica* rice varieties (HKR47 and PAU201) and subgroup-IB with all the three Basmati rice varieties (Taraori Basmati, Basmati 370 and Pusa Basmati 1). The major group II was further divided in to two subgroups: subgroup-IIA with *indica* rice varieties (IR36 and CSR10) and subgroup-IIB with all the *japonica* rice varieties (TNG67, NPTII and Azucena). Higher levels of genetic diversity between Basmati and non-Basmati (*indica*, *japonica* and wild genotypes) suggest that former may have a long history of independent evolution and diverged from non-Basmati rices a long time ago through conscious selection and patronage as reported earlier [16, 17].

The present study was also supported by isozyme based classification of Asian rice germplasm [18], where most of the Basmati and *japonica* rice varieties were placed in a distinct varietal groups V and VI, respectively. The present study clearly indicated that microsatellite markers are useful in assessing genetic diversity among micronutrient rich and commercially important rice genotypes. A basic molecular data set was created for the micronutrient rich and commercially important rice genotypes which can be used for varietal identification and characterization and further it will be of greater help in background selections during backcross breeding programs to improve the micronutrient content in rice. This study will also help in the breeding for

micronutrient rich and high yielding rice genotypes that may benefit both farmers and consumers.

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