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Full Research Article

Molecular Characterization of Red Rice Germplasm Using SSR Markers

Kavita Gupta[1*], A. K. Sarawgi[1], V. Ravindra Babu[2]

[1]Dept. of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi KrishiVishwa Vidyalaya, Raipur (492 012), India

[2]Project Directorate of Indian Institute of Rice Research, College of Agriculture, Raipur, Hyderabad (492 012), India

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Correspondence to

E-mail: mayurikavi1989@gmail.com

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Abstract

Microsatellites are simple sequence repeats (SSR) of 1−6 nucleotides. Their potential for automation and their inheritance in a co-dominant manner are additional advantages when compared with other types of molecular markers. SSRs are highly polymorphic, genome specific, abundant and co-dominant, and have recently become important genetic markers in cereals including rice and wheat.In present study SSR markers was applied for molecular characterization of 32 genotypes including 30 red rice genotypes and 2 popular white pericarped varieties as checks. Molecular characterization showed considerable level of genetic diversity among the selected genotypes. All amplified products with primers had shown polymorphic and distinguishable banding patterns which indicate the genetic diversity of rice genotypes. A total of 124 reproducible and scorable polymorphic bands generated with 19 primers among the 32 genotypes. The similarity matrices were subjected to sequential agglomerative hierarchical nested (SHAN) clustering using UPGMA in NTSYS-pc version 1.70. Considerable amount of diversity is present between different genotypes of different clusters. This diversity is of great significance in new breeding programme. Genetic improvement mainly depends on the extent of genetic variability present in the population. The molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities.

1. Introduction

Rices with a red bran layer are called red rices. Rice is the staple food of over half the World's population. It is the predominant dietary energy source for 17 countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa. Rice provides 20% of the World's dietary energy supply. Molecularmarkers have proven to be powerful tools in the assessment of genetic variationand in the elucidation of genetic relationships within and among species.Several molecular markers viz. RFLP (Becker et al., 1995, Paran and Michelmore, 1993), RAPD (Tingey and Deltufo, 1993; Williams et al., 1990), SSRs (Levinsonand Gutman, 1987), ISSRs (Albani and Wilkinson, 1998), AFLP (Mackill et al., 1996, 1995; Vos et al., 1995; Zhu et al., 1999) and SNPs (Vieux et al., 2002) are presently available to assess the variability and diversity at molecular level (Joshi et al., 2006). A critical analysis of the genetic variability is a prerequisite for initiating any crop improvement programme and for adopting of appropriate selection techniques (Dhanwani et al., 2013).

Genetic diversity can be assessed by morphological/ molecular markers but forreliable and efficient analysis of genetic diversity, molecular marker technologyplays a significant role. Microsatellites (SSR) are highly informative polymerasechain reaction (PCR) based markers that detect length polymorphisms at loci withsimple sequence repeats (Powel et al., 1996). Microsatellites are co-dominantmarkers and their map positions on the rice genome are well known. Ricemicrosatellites have been found that they are ideal markers for characterizinggenetic diversity among closely related rice varieties (Chakravarthi and Naravaneni, 2006).

Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique germplasm that compliments existing cultivars. Among PCR based markers inrice, microsatellites are abundant and well distributed throughout the genome (Akagi et al., 1996; Mc Couch et al., 1998). They are valuable tools for assessing theallelic diversity and are assayed efficiently by the PCR (Mc Couch et al., 1998).

The present study has been conducted to estimate the pattern and level of genetic diversity and relatedness among the red

rice genotypes to reveal the genetic diversity among them for their potentialuse in the rice breeding programme.

2. Materials and Methods

The material for the present study consists of 32 genotypes including 30 red rice genotypes and 2 popular white pericarped varieties as checks (Table 1).

The methodology adopted is as under:

Table 1: Study material of present study

Sl. No.	Genotypes	Sl. No.	Genotypes
1.	Bamleshwari	17.	Baroundaofftype
2.	Durgeshwari	18.	Jai mukarjii
3.	Bhadra	19.	Kansari
4.	Aruna	20.	Jalkeshar
5.	Asha	21.	Khondharo
6.	Haradgudidahi	22.	Khuddi
7.	Makom	23.	Govindbhog
8.	Kanakom	24.	Baroundaofftype
9.	Kekai	25.	Medmalen
10.	Khondi	26.	Paltu
11.	Bhanthaluchai	27.	Kanthisafri
12.	Kudesara	28.	Shrikamal
13.	Jhal	29.	Bhataguda
14.	Kankadiya	30.	Haradgudi
15.	Bisunbhog	31.	Bhujani
16.	Laxmibhog	32.	Pana

2.1. DNA isolation

The total genomic DNA was isolated from each genotype by CTAB method with certain modifications. The DNA was then quantified by using agarose gel electrophoresis (0.8%) and nanodrop at 260 and 280 nm. The concentratedDNA samples were diluted to a working concentration of 25 ng μl^{-1}. An equalamount of DNA from 32 genotypes were taken for PCR amplification.

2.2. PCR

Amplification reactions were carried out in 0.2 ml PCR tubes containing 25 µl ofreaction mixture. The components used for the PCR reaction are 2 µl of DNA template (50 ng μ^{-1}), 2.5 µl of 10x PCR buffer with $MgCl_2$, 0.2 mM of each dNTPs (dGTPs, dATPs, dTTPs, dCTPs), primer (0.1 mM), 0.5mM taq polymerase.

2.3. Gel Electrophresis

3 µl loading dye (10x) was added to PCR products. Finally, 10 µl of each sample is loaded into the wells for facilitating the sizing of the various alleles. Step UpTM 50 bp DNA Ladder GeNeiTM, Merck Specialities Private Limited was loaded in the first well (4 µl).Gel was run at 180 volts till the dye reached bottom of the gel for about 40−50 minutes.

2.4. Visualization of bands

After completion of gel electrophoresis, gels were subjected to documentation with UV image analyzer and subsequently gel picture having DNA as a band was saved to the desktop connected with UV image analyzer.

2.5. Detection of polymorphism using simple sequence repeat (SSR) primers

Total 19 primers were used for detection of polymorphism, all SSR primers showed polymorphism (Table 2).

Table 2: Microsatellite markers used for molecular characterization among 32 genotypes

Chromosome numbers	SSR Primers	Primer sequences	
		Forward 5’ → 3’	Reverse 5’ →3’
1	JGT 01-15.9	GGGTGCCACTTGACGGTTTG	GGCGCTCCAACAAATGCTG
1	HRM 10936	CAGACGCAGTGTGTGTATGAAGTTCC	GGACCGATTTAAGTGAACGTTGATGG
2	HRM 13154	GCGGGACATTCATGTACACATTGC	CTAGGTCTGTACACGCACTGAAGG
3	JGT 03-0.01	CCGATGCACCAACACCCTCAC	GACGCATGGTGAGTTTGGAGTGAT
3	HRM 16006	ACGCTTCACATTGTAACACACAGG	TCTTTGAAGGTCATTCCTGGAACC
4	JGT 04-28.5	TTGAATAGATCGCACCGTTGAC	AAAAGGG CAGCTACGTCTGAGC
4	HRM 16801	CGTTCAAGGAGCTTGTGTTGATCC	GGACCGATTTAAGTGAACGTTGATGG
5	HRM 17950	GGAAATGTGCATAGGTAGTTCAGG	GAGTTGGG AACTGCTACAAACG
6	JGT 06-18.1	GAAGAACGTGGTTTGCATCGT	TGTACCGTCGGCGAAGAACGCACC

Continue...

Chromo-some numbers	SSR Primers	Primer sequences	
		Forward 5' → 3'	Reverse 5' →3'
6	HRM 20096	CGGTAAGC-CATAAATAG-ATCCCAAGG	TTTGAA-CAGCGACAC-GGTTTCC
7	JGT 07-22.8	TGGCGATC-TAGGAGC-GTCTGT	TGTAAA-CATTTCAA AAGGGCAC
7	HRM 21258	TATCATTCC GGTC-CAAAGTGTCG	TCCGGTCC AAAGTCT-CATTTGC
8	SSR-8.2	CATAATTC GATC-CAAGGGGACC	AGGAGCCCC AGCCTCCGT-CAG
8	JGT 08-19.5	TTCTGAAAAA GCTCTGAC-CAAGC	ACTAGCTA-CATGCTG-CAGTGCAT
8	HRM 22622	GATCGATCTT-TAGTCCCG-GTTGG	AACA-CAATTCACC-GGTCCTTAGC
9	HRM 24654	TTGCTAGGT-TAGCATCCG-GTACG	TGGTCTTT-GCGAATCT-GAATCC
10	HRM 24941	CGAATCTTG-GAACACAT-CAACG	GGGAG-GAGTGCTGT-GAGAGG
11	JGT 11-19.9	TCTGGAATGT-GTCCTTGGGG-GA	CTAGGTT-TAGGTGAC-CAACAGTC
12	ESSR 112-0.2	GGTGTTGCAG-GCGTCCTACT	TCATG-GAATGGAAA-CAACCA

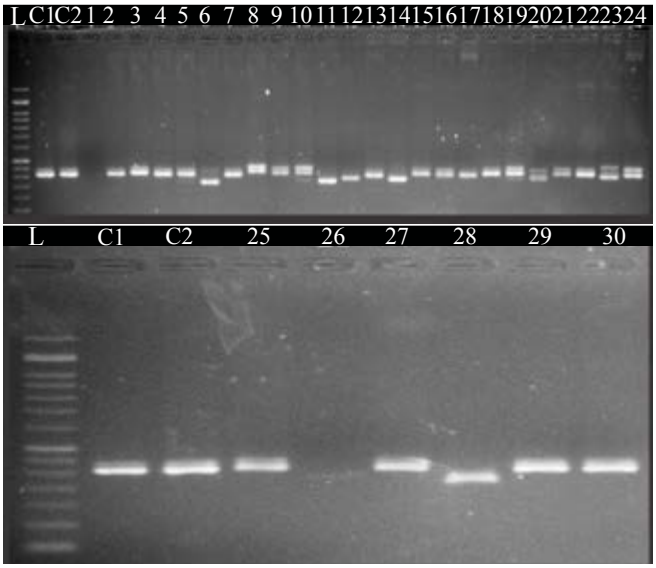

Figure 1: Banding pattern of HRM 04-28.5
Note: L-Ladder; C1: Check-1(IGKV R2); C2: Check-2 (IGKV RI244); 1: Mo-4; 2: mo-5; 3: Mo-8; 4: Mo-9; 5: mo-11; 6: Kekai; 7: Lohandi; 8: Bhataluchai; 9: Kudesara; 10: Haradgudi; 11: Jhal; 12: kankadiya; 13: Bisunbhog; 14: Govindbhog; 15: Laxmibhog; 16: Barounda-offtype; 17: Jai-Mukarjii; 18: kansari; 19: jalkeshar; 20: khondharo; 21: khuddi; 22: Kondi; 23: Luchai; 24: Paltu; 25: Kanthi-Safri; 26: Shrikamal; 27: Bhataguda; 28: Haradguhidahi; 29: Bhujani; 30: Pana

3. Results and Discussion

The results of present study indicated a considerable level of genetic diversity among the cultivars selected. 24 (HRM and JGT) primers were used in this study, results of 19amplified primers were taken into consideration since they had given reproducible bands. Banding pattern of two primers JGT 04-28.5 were given in (Figure 1). All amplified products with primers had shown polymorphic and distinguishable banding patterns which indicate the genetic diversity of rice genotypes. A total of 124 reproducible and scorable polymorphic bands generated with 19 primers among the 32 genotypes. The similarity matrices were subjected to sequential agglomerative hierarchical nested (SHAN) clustering using UPGMA in NTSYS-pc version 1.70. Scoring of gel pictures is presented in. Dendogram showing clustering pattern of 32 rice cultivars based on 20 SSR primers using UPGMA method is presented in (Figure 2).

Based on the results obtained all the 32 genotypes grouped into two main clusters A and B. The similarity coefficient ranged from 0.57 to 0.89. The major cluster A and minor cluster B consisted of 31 and 1 genotypes respectively and shared 0.57 similarity coefficient.

Cluster A showed two sub cluster A1 and A2 consisted of 29 genotypes Bamleshwari, Asha, Makom, IGKV R2 (Durgeshwari), Kanakom, Kekai, Kondi, Kudesara, Haradgudi, Jaimukarji, Laxmibhog, Kansari, Baronda-offtype, Jalkeshar, Khondharo, Khuddi, Kondi, Med-malen, Kanthi-safri, Paltu, Shrikamal, Bhataguda, Bhujani, Haradguhudahi, Pana, Bhataluchai, Jhal, Kankadiya, Bisunbhog) and 2 genotypes (Aruna and Bhadra) repectively. The cluster A1 further showed sub-clustering at nearly 65% similarity levels and which again sub-cluster into A1a and A1b consisted of 25 genotypes (Bamleshwari, Asha, Makom, Durgeshwari, Kanakom, Kekai, Kondi, Kudesara, Haradgudi, Jaimukarji, Laxmibhog, Kansari, Baronda-offtype, Jalkeshar, Khondharo, Khuddi, Kondi, Med-malen, Kanthi-safri, Paltu, Shrikamal, Bhataguda, Bhujani, Haradguhudahi, Pana) and 4 genotypes (Bhataluchai, Jhal, Kankadiya, Bisunbhog) respectively. The cluster A1a showed

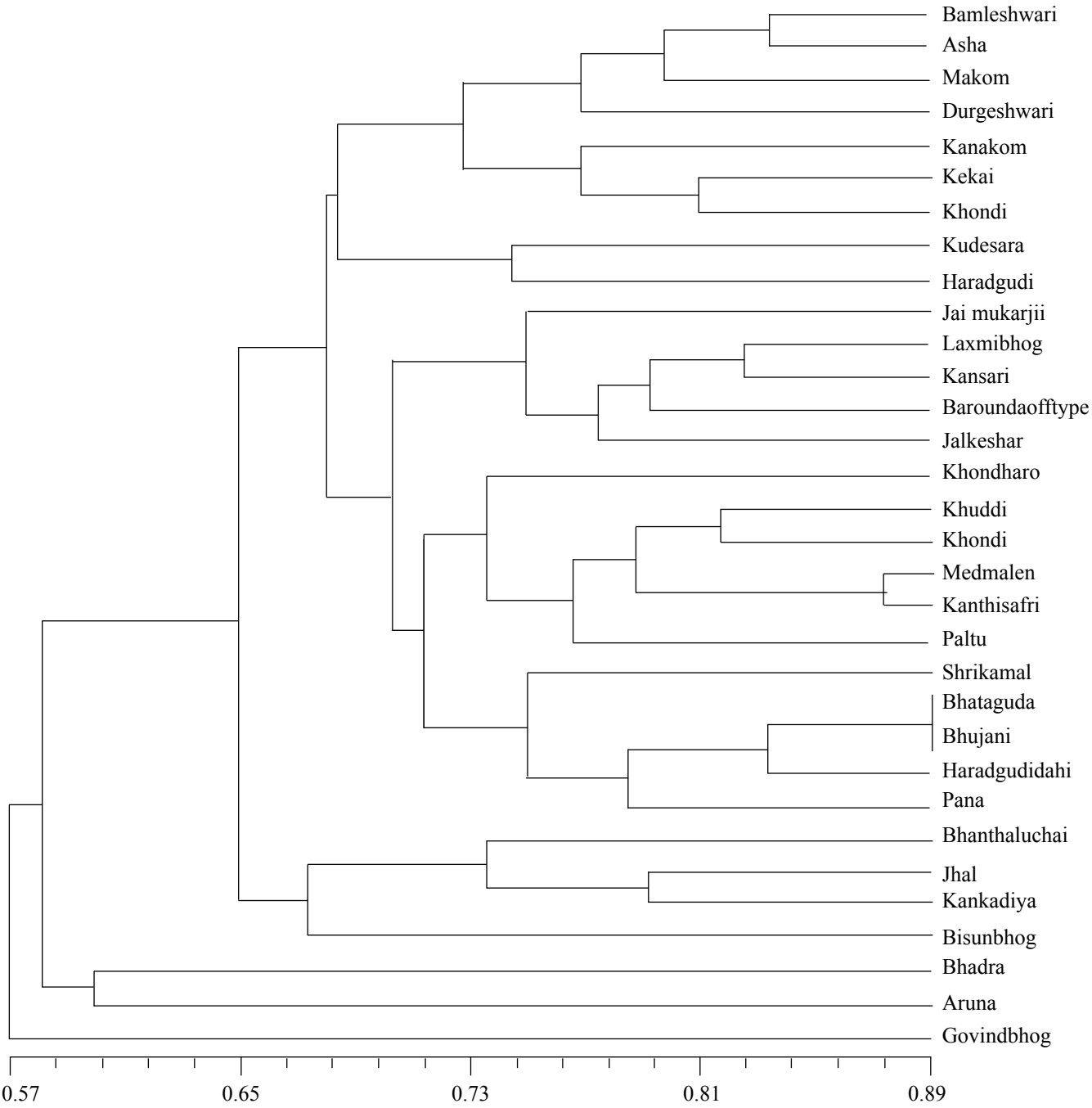

Figure 2 : Dendogram showing clustering pattern of 32 rice genotypes based on 19 SSR primers using UPGMA method

a sub-cluster A1a1 and A1a2 and consisted of 16 genotypes (Jaimukarji, Laxmibhog, Kansari, Baronda-offtype, Jalkeshar, Khondharo, Khuddi, Kondi, Med-malen, Kanthi-safri, Paltu, Shrikamal, Bhataguda, Bhujani, Haradguhudahi, Pana) and 9 genotypes (Bamleshwari, Asha, Makom, Durgeshwari, Kanakom, Kekai, Kondi, Kudesara, Haradgudi) respectively. In cluster, A1a1 genotypes Bhujani and Bhataguda Shared maximum similarity of (89%). Cluster B was the minor cluster consisted of only one genotype Govinbhog.

4. Conclusion

Molecular characterization showed considerable level of genetic diversity among the selected genotypes. All amplified products with primers had shown polymorphic and distinguishable banding patterns which indicate the genetic diversity of rice genotypes. A total of 124 reproducible and scorable polymorphic bands generated with 20 primers among the 32 genotypes. There is considerable amount of diversity between the two genotypes of the different clusters which is

of great significance. The genotypes which kept on the same clusters have also shown some minute diversity.

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