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Molecular characterization of selected aromatic short grain genotypes of rice (*Oryza sativa* L.)

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Abstract

Fifty four selected genotype accessions of short grain aromatic rice (*Oryza sativa* L.) along with three checks were molecularly characterized by 10 selected markers identified for aroma and present on chromosome number 8. This was carried out in MAS Lab, Dept. of Genetics and Plant Breeding, RRH Lab, IGKV, Raipur. The data were recorded for all 10 SSR markers by Jaccard's similarity coefficient and Euclidean distance using NTSYS PC programme to study diversity among selected genotypes. Jaccard's similarity coefficient ranges from 0.40 to 0.99 with highest similarity coefficient between 5 groups at more than 0.99. Highest dissimilarity showed by Akbar badshah and Bassabhog from rest all of the genotypes. Total 57 rice genotypes taken under study were also divided into 5 main clusters, at 0.55 Jaccard's similarity coefficient.

Keywords: Aromatic rice, SSR markers, similarity coefficient, polymorphism, rice

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop that has been referred as "Global Grain" because of its use as prime staple food in about 100 countries of the world. Rice plays a pivotal role in Indian agriculture and it is the staple food for more than 70 % Indians. It is grown in all states and in all ecologies. India is very rich in rice genetic resources in general and aromatic rice in particular. Aromatic rice constitute small and special group of rice and highly priced due to their quality. It is estimated that India has over 85,000 germplasm accessions including wild form. These landraces are the reservoir of many useful genes. Chhattisgarh is having greatest diversity of rice including aromatic rice (Bisne and Sarawgi, 2008) [2]. Collection and characterization of the germplasm is not only important for utilizing the appropriate attribute based on donors in breeding programmes, but is also essential in the present era for protecting the unique rice. The amount of genetic variation in germplasm accessions and genetic relationships between genotypes are important considerations for designing effective breeding programs. Different types of DNA markers are available nowadays, each method differing in principle, application, type and amount of polymorphism detected, cost and requirement. SSRs (Simple Sequence Repeats) are an excellent molecular marker system for many types of genetic analyses, including linkage mapping, germplasm surveys and phylogenetic studies. SSRs are codominant, abundant and highly reproducible and exhibit a high degree of allelic variation (Temnykh *et al.*, 2000) [8]. Microsatellites [also known as simple sequence repeats (SSRs)] are simple, tandemly repeated, di- to tetranucleotide sequence motifs flanked by unique sequences (Hamada *et al.* 1982) [3].

Materials and Methods

Fifty seven lines mentioned in Table 1 along with three checks Basmati 370, Inidra Sugandhit Dhan -1 and TN-1 were used for molecular characterization which are aromatic rice genotypes. For assessing the genetic diversity of rice germplasm molecular study was performed, which included DNA isolation, quantification, dilution of DNA, PCR amplification using SSR primers, electrophoresis using polyacrylamide gel. Then they were subjected to PCR based Simple Sequence Repeat (SSR) technique to direct the polymorphism with 10 markers related to aroma present on chromosome no. 8. Then on the basis of presence and absence of band scores were given to individual bands of a marker as '1' for presence and '0' for absence. These all markers data for 57 genotypes were analysed using NTSYS PC 2.02e. Similarity coefficient analysis and clustering for 57 rice genotypes was performed by Jaccard's similarity coefficient and Euclidean distance. Cluster analysis was done using UPGMA (Unweighted Pair Group Method with Arithmetic averages) method based upon genotypic data.

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Table 1: List of 57 aromatic accessions of rice along with seven checks used in the present study

S. No.	Accession Number	Accession Name	S. No.	Accession Number	Accession Name
1.	A:468	Aama gohi	30.	R:295	Ram jira
2.	A:274	Atma shital	31.	B:1087	Bhanta phool
3.	A:625	Atma shital	32.	B:1091	Bhanta phool
4.	A:593	Akbar badshah	33.	D:1004	Durar phool
5.	B:1728	Aadshah	34.	J:339	Jau phool
6.	B:1358	Baj raj	35.	M:906	Mali phool
7.	B:1166	Barang	36.	S:980 II	Samund chini
8.	B:381	Badshah bhog	37.	S:672 I	Samundra fen
9.	B:405	Badshah bhog	38.	B:667	Badshah bhog
10.	B:1322	Badshah bhog	39.	G:335	Gobind bhog
11.	B:2355	Badshah bhog	40.	G:585	Govind bhog
12.	B:2461	Badshah bhog	41.	L:359	Laxmi bhog
13.	B:2469	Bassa bhog	42.	L:834	Laxmi bhog
14.	B:1094 II	Bisnu bhog	43.	B:675 I	Bishun bhog
15.	L:1238	Laxmi bhog	44.	E:15	Elaychi
16.	R:509	Raja bhog	45.	L:749	Lokti machhi
17.	U:69 II	Urai buta	46.	T:206	Til kasturi
18.	C:30 II	Chini Kapoor	47.	R:397	Ramjira
19.	D:1370	Dubraj	48.	S:663 I	Shri kamal
20.	D:116 II	Dudaga	49.	S:665	Shri kamal
21.	D:1205	Dudgi	50.	K:1859	Kadam phool
22.	G:115	Ganga baloo	51.	CH-1	Vishnu bhog Selection-1
23.	G:405	Ganga baroo	52.	CH-2	Badshah bhog Selection-1
24.	G:845	Ganga baru	53.	CH-3	Dubraj Selection-1
25.	G:397	Ganga baru	54.	CH-4	Tarun bhog Selection-1
26.	G:230	Ganga prasad	55.	CH-5	Basmati 370
27.	J:112	Jal woga	56.	CH-6	Indira Sugandhit Dhan-1
28.	K:1456	Kala jira	57.	CH-7	TN-1
29.	K:2424	Kala jira			

Results and Discussion

The DNA of 57 rice accessions were subjected to PCR based Simple Sequence Repeat (SSR) technique to detect the polymorphism with 10 markers related to aroma present on chromosome no. 8 listed in Table 2. Six of them are showed polymorphism which were RM 25, RM 230, RM 284, RM 310, RM 337 and RM 515.

Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers (McCouch *et al.*, 2002) [5]. Similarity coefficient ranged from 0.40 to 1.00. Dendrogram is presented in Fig. 1 reveals formation of five major clusters at 0.55 similarity coefficient. Name of the genotypes according to cluster is mentioned in Table 2, which shows that cluster A contains only two genotypes Akbar badshah and Bassa bhog. Cluster B contains 6 genotypes Atma shital (A:274), Durar phool, Bishun bhog, Elaychi, Jau phool and Ganga prasad. Cluster C contains only one genotype Urai buta. Cluster D contains maximum number of genotypes (34) viz. Ramjira (R:295), Kadam phool, Ganga baru (G:845), Laxmi bhog (L:834), Badshah bhog selection-1, ram jira (R:397), Badshah Bhog (B:667), bhanta phool (B:1087), Kala jira (K:2424), Kala jira (K:1456), ganga baloo, Shri kamal (S:663 I), Dubraj selection

1, Dudaga, dubraj (D:1370), Chini Kapoor, Raja bhog, Indira sugandhit dhan 1. TN1, tarun bhog selection 1, Bisnu bhog, Badshah, Badshah bhog (B:2461), Baran, Badshah bhog (B:405), Amagohi, Badshah bhog (B:381), Basmati (B:370), Vishnu bhog selection 1, Shri kamal (S:665), Badshah bhog (B:2355), Badshah bhog (B:1322), Bajraj and Atma shital (A:625). Cluster E contains 14 genotypes viz. Samundra fen, Til kasturi, Laxmi bhog (L:359), Smaund chini, Maliphool, Gobind bhog, Govind bhog, Ganga baru (G:397), Ganga baroo, Dudgi, Lokti machhi, Jal voga, Bhanta phool (B:1091) and Laxmi bhog (L:1238). Similar type of study was done by Balwant *et al.*, (2011) [1] in 50 rice genotypes and by Padmaja *et al.*, (2011) [6] in 72 rice genotypes.

Polymorphism Information Content provides an estimate of determining power of a marker based on the number of alleles at a locus and relative frequencies of these alleles. Maximum number of alleles (5) was amplified by marker RM 310 marker. The PIC value across markers ranged from 0.102 to 0.645 with an average of 0.374. Maximum PIC on chromosome 8 was 0.645 at marker RM 25 followed by RM 284 (0.593) and RM 310 (0.577) as depicted in Fig:2. So, further these markers could be Similar type of study done by Rehman *et al.* (2012).

Table 2: Name of the 57 genotyped according to clustering pattern

Cluster	No. of genotypes	Names of genotypes
A	2	Akbar badshah and Bassa bhog
B	6	Atma shital (A:274), Durar phool, Bishun bhog, Elaychi, Jau phool and Ganga Prasad
C	1	Urai buta.
D	34	Ramjira (R:295), Kadam phool, Ganga baru (G:845), Laxmi bhog (L:834), Badshah bhog selection-1, ram jira (R:397), Badshah Bhog (B:667), bhanta phool (B:1087), Kala jira (K:2424), Kala jira (K:1456), ganga baloo, Shri kamal (S:663 I), Dubraj selection 1, Dudaga, dubraj (D:1370), Chini Kapoor, Raja bhog, Indira sugandhit dhan 1. TN1, tarun bhog selection 1, Bisnu bhog, Badshah, Badshah bhog (B:2461), Baran, Badshah bhog (B:405), Amagohi, Badshah bhog (B:381), Basmati (B:370), Vishnu bhog selection 1, Shri kamal (S:665), Badshah bhog (B:2355), Badshah bhog (B:1322), Bajraj, Atma shital (A:625)

E	14	Samundra fen, Til kasturi, Laxmi bhog (L:359), Smaund chini, Maliphool, Gobind bhog, Govind bhog, Ganga baru (G:397), Ganga baroo, Dudgi, Lokti machhi, Jal voga, Bhanta phool (B:1091), Laxmi bhog (L:1238)
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Similarity coefficient analysis and clustering for 57 rice genotypes was performed by Jaccard's similarity coefficient and Euclidean distance. Jaccard's similarity coefficient analysis of 57 rice genotypes ranged from 0.40 to 0.99 with highest similarity coefficient between 5 groups at more than 0.99 viz. first between Atma Shital (A:625), Baj raj, Badshahbhog (B:1322), Badshahbhog (B:2355), Shri kamal

(S:665), Vishnubhog selection-1 and Basmati 370. Second between Badshahbhog (B:381), Aama gohi and Badshahbhog (B:405). Third group contains Dubraj (D:1370) and Dudaga. Fourth group contains Kala jira (K:1456), Kala jira (K:2424), Bhanta phool (B:1087), Badshahbhog (B:667), Ramjira (R:397) and Badshahbhog selection-1. Fifth group.

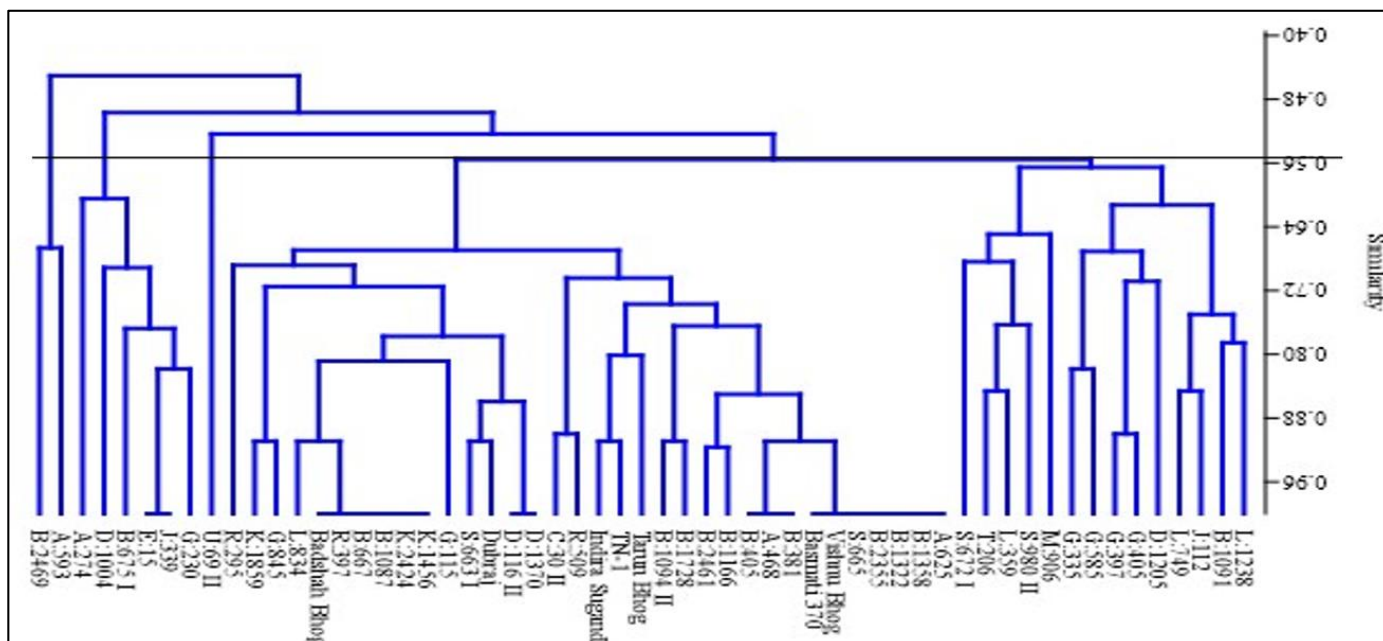


Fig 1: UPGMA based molecular dendrogram of SSR markers showing 57 rice genotypes

contains Jau phool and Elaychi. Highest dissimilarity showed by Akbar Badshah and Bassabhog from rest all of the

genotypes. This result is in accordance to Vohra *et al.* (2013) and Kanusoth *et al.* (2015).

Table 3: List of 10 markers and their PIC value

Marker	PIC Value
RM 25	0.645
RM 126	0.102
RM 152	0.135
RM 210	0.261
RM 230	0.564
RM 256	0.199
RM 284	0.593
RM 310	0.577
RM 337	0.133
RM 515	0.507

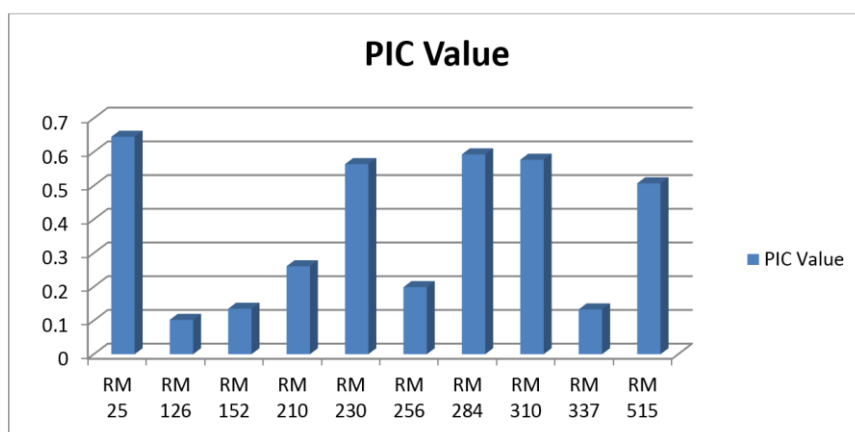


Fig 2: Graphical representation of PIC value of SSR markers

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