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Quality assessment and molecular characterization of rice genotypes using DNA markers

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ABSTRACT : Genetic relatedness among rice varieties were assessed using ten RAPD, two SSR and two STS markers. The 56 rice genotypes studied included improved varieties and landraces collected from different parts of India. The number of alleles per RAPD locus ranged from 3 to 7, averaging 4.7 alleles per locus. Polymorphism information content (PIC) values ranged from 0.474 to 0.811, with an average of 0.665. The number of alleles per SSR locus ranged from 2 to 4, averaging 2 alleles per locus and PIC values ranged from 0.069 to 0.278, with an average of 0.017. The band size for a given SSR locus varied between 170 bp and 180 bp and for STS markers the band size varied between 175bp to 180bp with an average pic value of 0.299. Characterization of the genotypes was also done for important quality parameters including amylose content, gel consistancyn and gelatinization temperature. Cluster analyses were used to group cultivars by constructing dendrograms based on DNA marker analysis and physico-chemical characterization of grains. The dendrogram based on molecular marker analysis grouped the 56 rice cultivars into different diverse groups. Clustering of these varieties according to their genotypes as well as phenotypes revealed the possible linkage or pleiotropic effects of the genomic regions associated with some grain quality traits. Information generated through cluster analysis based on phenotypic and genotypic data could be efficiently used in breeding rice varieties harboring grain quality traits. Pure-line selection can be made using farmer varieties that are characterized morphologically and phenotypically in the study.

KEY **W**ORDS : Rice genotypes, DNA markers, Molecular characterization,

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Rice (*Oryza sativa* L.) is one of the major staple food crops for more than half of the world population and being grown worldwide (Sasaki and Burr, 2000). Rice accounts for 21, 14 and 21 per cent of global energy, protein and fat supply, respectively (Kennedy and Burlingam, 2003). Therefore, improving the grain quality is very important for rice breeders. Starch is the major component of polished rice (~95 % of dry weight). Three physico-chemical characteristics of starch *i.e*. amylose content (AC) (Webb, 1980 and Juliano, 1985), gel consistency (GC) (Cagampang *et al.*, 1973) and gelatinization temperature (GT) (Little *et al.*, 1958) determine eating, cooking and processing quality of rice. Amylose content is important because firmness and stickiness are two properties of cooked rice that influence consumer preference and use of different classes of rice.

Molecular marker technologies can assist conventional breeding efforts and are valuable tools for the analysis of genetic relatedness and the identification and selection of desirable genotypes for crosses. DNA based molecular markers have proven to be powerful tool in the assessment of genetic variation and in the elucidation of genetic relationships within and among the species of rice (Ragunathanchari *et al.*, 2000). Among molecular marker systems used to identify and assess the genetic diversity and phylogenetic relationships in plant genetic resources, random amplified polymorphic DNA (RAPD) technique developed by Williams *et al.* (1990) is the fastest and simplest. In rice, RAPD analysis has been extensively used for diversity analysis. Simple sequence repeats (SSR) or microsatellites which are simple or tandemly repeated having di, tri, tetra- nucleotide sequence motifs flanked by unique sequences (Hamada *et al.*,1982). These are useful markers for genetic diversity analysis because they detect high level of allelic diversity, occur frequently throughout plant genomes and are easily assayed by PCR and they have been extensively used to identify genetic variation among rice species (Ren *et al.*, 2003). The sequence-tagged site (STS) is a relatively short, easily amplifiable sequence (200 to 500 bp) which can be specifically amplified by polymerase chain reaction and detected in the presence of all other genomic sequences and whose location in the genome is mapped. These markers recognized as single-copy DNA sequences of known map location which could serve as markers for genetic and physical mapping of genes along the chromosome.

RESEARCH PROCEDURE

Plant materials :

The experimental material for the present investigation consisted of 56 rice genotypes which were obtained from ARS, Gangavati and local farmers of Raichur district. The present investigation was carried out at Plant Molecular Biology laboratory, Department of Genetics and Plant Breeding College of Agriculture, UAS, Raichur. For preparation of leaf samples and DNA extraction, 10 seeds from each genotype were planted in pots and leaf samples of young seedlings were prepared after 25 days and kept at -80°C.

Genomic DNA extraction :

DNA was extracted from the rice seedlings according to the modified CTAB method (Saghai-Maroof *et al.*, 1984). DNA quality was checked by electrophoresis in an agaros gel and quantification was accomplished using a spectrophotometer.

DNA markers and PCR amplification :

A set of ten RAPD, two SSR and two STS (Table 2) were used in this study. DNA samples were amplified in 20 µl reaction volumes containing of 2 µl template DNA (15 ng), 13.55 µl ddH_2O, 2 µl PCR buffer (10x), 0.48 µl $MgCl_2$ (17.5 mM), 1.0 µl dNTPs (2.50 mM), 1.2 µl of primer and 0.25 µl of Taq DNA Polymerase (5 U/µl). PCR was carried out in a thermal cycler. The cycle profile for RAPD: Initial denaturation at 94°C for 5 min, 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 36 °C,1 min extension at 72°C, and then 5 min at 72°C for the final extension. The cycle profile for SSR: Initial denaturation at 94°C for 5 min, 35 cycles of 1 min denaturation at 94°C, 30 sec annealing at 55 °C, 1 min extension at 72°C and then 5 min at 72°C for the final extension and PCR profile for STS: Initial denaturation at 94°C for 2 min, 35 cycles of 45 sec denaturation at 94°C, 1 min annealing at 60 °C, 2 min extension at 72°C, and then 10 min at 72°C for the final extension.

Separation of amplified products by agarose gel :

The amplified products from each tube along with 2µl of loading dye (bromophenol blue) were separated on 1.4, 2.5 and 3 per cent RAPD, SSR and STS, respectively in agarose gel containing ethidium bromide at 70 volts (<5volts per cm of gel) using 1x TAE buffer of pH 8.0. Lambda DNA double digest was used as DNA molecular weight marker. The gel was photographed by using documentation system (Bio Rad).

Data analysis :

The amplified fragments were scored as '1' for presence of single band and '0' for the absence of a band. Pair wise genetic similarities between genotypes were estimated by Jaccard similarity co-efficient. Clustering was done using the symmetric matrix of similarity co-efficient and cluster obtained based on unweighted pair group arithmetic mean (UPGMA) using NTSYS-PC. Polymorphic information content values for primers were calculated following the formula (Nei, 1973):

$$PIC = 1 - \sum_{i=1}^{n} Pij^2$$

where,

Pi is the frequency for the j^{th} allele for marker i and summation extends over n alleles.

RESEARCH ANALYSIS AND REASONING

The findings of the present study as well as relevant discussion have been presented under following heads :

Molecular diversity analysis using RAPD markers :

Molecular diversity assessment among fifty six rice genotypes using RAPD markers revealed high variability among genotypes at molecular level. Jaccard's co-efficient revealed the maximum genetic similarity of 95.00 per cent between HMT and Chinna ponni and minimum genetic relatedness of 55.00 per cent between Mese bhatta and Sanna nellu and of 57.00 per cent between KH-4 (Varanasi) and Sanna nellu (Rajani *et al.*, 2013). Fifty six rice genotypes were grouped into eight clusters at 0.78 per cent Jaccard's similarity co-efficient. In these genotypes the genomic region may be similar or different depends upon genetic makeup of an individual and crossing among different groups of genotypes would be desirable for breeders. Cluster VIII was the largest comprising of forty two genotypes which was further subdivided into three clusters, VIII A, VIII B, with 5 and 37 genotypes respectively. Whereas the cluster V (Ratan choodi) and VI (Barma black) were solitary with single genotypes in it and indicating the uniqueness of this genotypes at the RAPD marker regions sampled (Pervaiz *et al.*, 2006).

Molecular diversity analysis using SSR markers :

The two SSR motifs used in present study produced four alleles with an average of two alleles per locus in RM-190 and RM-223, respectively. In RM-190 the allelic differences is at 125 bp and 130 bp based on the number of repeat motifs in $CT_{(11)}$ and the repeat motif $CT_{(11)}$ is less in 125 bp compare to 135bp. Similarly RM-223 having band size of 170 bp and 180 bp based on the number of repeat motifs in $GT_{(25)}$ and the repeat motif $GT_{(25)}$ is less in 170 bp compare to 180 bp. Present study was supported by Ying-Xiu *et al.* (2007) and this value was similar for Venezuelan rice cultivars and the average of 3 alleles per locus reported by Kibria *et al.* (2009) and Cluster analysis was found that four clusters were formed at genetic similarity level of 0.78-0.86 which contained eleven, three, forty and two rice genotypes, respectively. The results of SSR marker analysis revealed that genotypes with the same origins were clustered into the same classes and also genotypes with the same amylose classes were grouped into the same clusters with some exceptions because of allelic differences in primers.

Table 1a : List of RAPD markers used for characterization

Sr. No.	Primers name	Sequence (5'-3')
1.	OPA-2	TGCCGAGCTG
2.	OPA-3	AGTCAGCCAC
3.	OPA-12	TCGGCGATAG
4.	OPA-18	AGGTGACCGT
5.	OPB-11	GTAGACCCGT
6.	OPC-6	GAACGGACTC
7.	OPC-9	CTCACCGTCC
8.	OPC-13	AAGCCTCGTC
9.	OPC-15	GACGGATCAG
10.	OPAG-3	TGCGGGAGTG

Table 1b : List of SSR and STS markers used for characterization of rice

Marker name		Sequence (5'-3')	
		Forward	Reverse
SSR	RM-190	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG
	RM-223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
STS	Sbe3a	TCGGTCCTAATATTTTGCGCT	CCTTAACTTGACACCGAATCCG
	Sbe3b	TCGGTCCTAATATTTTGCGCT	CCTTAACTTAGCACCGAATCCG

Table 2 : Characteristics of studied rice varieties

Sr. No.	Genotypes	AC(%)	GC mm	GT scale	GT character
1.	Mugada sugandh	18.15	112.33	7	L
2.	Sanna nellu	20.70	112.67	5	I
3.	Chinna ponni	18.55	123.33	2	H
4.	NMS-2	16.40	99.67	5	I
5.	Coimbatore sanna	16.46	116.67	4	I
6.	Mysore sanna	20.55	96.33	3	H-I
7.	Madras sanna	19.93	113.00	5	I
8.	Gham sale	18.73	108.33	6	L
9.	Bangar sanna	20.57	97.00	3	H-I
10.	Surgeon	20.26	63.00	2	H
11.	Tuyi malli	22.63	99.67	2	H
12.	Kichidi samba	19.59	83.33	4	I
13.	Mysore mallige	21.48	117.00	2	H
14.	Kappu basumati	20.55	48.67	1	H
15.	HMT	19.69	100.33	4	I
16.	Parimala sanna	19.41	54.33	4	I
17.	Jeerige sanna	19.68	75.00	3	H-I
18.	Delhli sanna	19.73	93.67	7	L
19.	Uggi bhatta	21.33	53.00	4	I
20.	Kyasakki	20.58	94.67	2	H
21.	Kagi sale	24.75	100.33	3	H-I
22.	Gouri sanna	20.44	71.00	7	L
23.	Ratna choodi	19.46	110.33	2	H
24.	Raja bhoga	21.67	103.00	5	I
25.	Kari jiddu	21.19	62.67	2	H
26.	Meese bhatta	20.68	39.63	6	L
27.	ADT 43	15.88	99.67	2	H
28.	Gandha sale	20.22	101.33	3	H-I
29.	Navaara	19.80	120.67	5	I
30.	Selum sanna	25.56	107.67	1	H
31.	Ambe mohar	19.87	44.33	2	H
32.	Raj kamal	26.19	79.67	2	H
33.	Burma black	6.56	82.67	6	L
34.	Raj mudi	19.34	111.00	2	H
35.	KH- 4(Varanasi)	18.86	38.33	5	I
36.	BPT 5204	20.43	94.67	2	H
37.	Bale brodu mudlu	17.76	70.33	7	L
38.	Karasu	20.59	116.67	2	H
39.	Ra-2	24.46	109.67	7	L
40.	Ra-4	21.75	131.00	7	L
41.	Gangavati sona	18.68	96.67	1	H
42.	MTU-1010	17.55	132.33	3	H-I

Table 2 : Contd……..

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43.	Jaya	22.94	134.33	7	L
44.	Gangavati emergency	17.61	135.00	2	H
45.	Gidda emergency	20.72	105.67	1	H
46.	Gangavati sanna	21.83	134.67	4	I
47.	Ratan sagar	20.20	76.00	1	H
48.	Swarna	21.60	97.33	3	H-I
49.	WAB-450	21.61	105.67	2	H
50.	JGL-1798	20.24	115.00	2	H
51.	Sahabhagi dhan	21.79	113.00	7	L
52.	Tulasi	19.65	101.33	6	L
53.	Varalu	20.62	124.33	7	L
54.	Jaldhidhan	20.98	95.67	6	L
55.	CSR-22	21.05	107.00	2	H
56.	Tellahamsa	17.72	39.67	7	L

GT = Gelatinization temperature, AC = Amylose content, GC = Gel consistency; H = High; H-I = Intermediate to high; L = Low

Lapitan *et al.* (2007) reported that SSR markers can distinguish quality rice subspecies and classified cultivars with the same cooking and eating quality and Kibria *et al.* (2009) grouped 14 aromatic rice genotypes with SSR markers in two main clusters and the dendrogram revealed that the genotypes that are derivatives of genetically similar type clustered together.

Molecular diversity analysis using STS markers :

STS marker used in this study produces four alleles with an average of two alleles per locus in Sbe3-a and Sbe3-b, respectively. The product size of Sbe3-a and Sbe3-b lies between the range of 175 bp and 180 bp. Liu *et al.* (2004). Cluster analysis was found that five clusters were formed at genetic similarity level of 0.7 to 0.85 and which contained eight, fifteen, one, one and thirtyone rice genotypes, respectively. The differences in clustering of genotypes are because of existence of allelic variations among genotypes. If allelic differences are less among genotypes the amplified region showing more conserved sites for starch branching enzyme. There was low correlation between clustering patterns of physio-chemical and molecular data, which could be due to use of less number of primers in the evaluation which failed to generate enough polymorphism and small population size. Despite the low correlation between physio-chemical and molecular clustering pattern, there were some genotypes, which grouped in the same cluster at both the levels.

Conclusion :

Results of this research indicated that the use of RAPD, SSR and STS markers linked to grain quality traits distinguish rice varieties from each other for quality characteristics. Measuring quality traits of rice varieties (*i.e.* amylose content, gel consistency and gelatinization temperature) are time consuming and expensive. Furthermore, some quality traits like gelatinization temperature can be measured only in limited time (maximum 3 month after harvest). In rice breeding programs especially in initial generations, there are so many lines but only a few seeds are obtained from each line, so direct measurement of cooking and eating quality of lines is difficult. Therefore, it is necessary to suggest alternative methods. The results also showed that different markers linked to genes or QTLs controlling grain quality properties are suitable tools for marker assisted selection (MAS) to select rice lines with high quality.

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