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ASSESSMENT OF GENETIC DIVERSITY FOR PHYSICAL AND COOKING QUALITY TRAITS IN RICE (*ORYZA SATIVA* L.) CULTIVARS USING SIMPLE SEQUENCE REPEATS MARKERS

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ABSTRACT
Considering the due importance of grain quality in commercial success of the variety, simple sequence repeats (SSRs) were used to find out genetic diversity for physical and cooking quality traits in 31 rice cultivars/ genotypes.Among 31 SSR markers used, 26 SSR marker loci generated polymorphic patterns and a total of 90 alleles were detected. The number of alleles per locus and PIC values ranged from 2-5 with a mean of 3.46 alleles per locus and 0.35(RM2125) to 0.89(RM333) with an average PIC of 0.62, respectively. In clustering pattern Ashoka 200F, AAUDR-1 and GR-9 uplandcoarse grain varieties were grouped together while irrigated varieties GR-11, GR-12, GAR-1, GAR-2, GR-3 and GR-6 exhibited similar clustering pattern.

INTRODUCTION

Rice *(Oryza sativa* L.) is one of the commonly consumed cereals and food staples for more than half of the world's population. Rice provides 20 percent of the world's dietary energy supply. It is also a good source of thiamine, riboflavin, niacin and dietary fibre. Kernel dimensions are primary quality factors in most phases of processing, drying, handling equipment, breeding and grading. Grain size and shape are the first quality characteristics considered in developing new varieties (Owens, G., 2001). The percentage of whole grain is the most important parameter for the rice processing industry.

Grain quality is an important consideration in rice production. The basic objective of most of the crop improvement programsis to realize a marked improvement in crop yield. But yield isa complex character which is controlled by association ofvarious characters. (Reddy *et al.*, 2013). One of the major concerns in rice production is grain quality (Nandan *et al.*, 2010). Grain quality is second only to yield as a major rice breeding objective (Singh *et al.*, 2000).Grain quality of rice is primarilycomposed of the appearance of the grain and its milling and cooking properties. These parameters primarily involve the physical and chemical characteristics of starch. As component traits, grain length, width and thickness are closely associated with grain weight and yield. Rice grain length and shape are important to consumers, because they determine the physical appearance and affect the cooking quality of the grain (Jiang *et al.,* 2005).

Developing molecular markers that are associated with agronomic and grain quality traits would help breeders to develop new cultivars having improved yield potential as well as cooking and sensory quality traits that are desired by consumers.

Knowledge regarding the amount of genetic variation in germplasm accessions and genetic relationships between genotypes are important considerations for designing effective breeding programs. In many cases, resolution power for revealing polymorphisms in genetic analyses and/or for differentiating between closely related genotypes remain uncomparable to the characterization of germplasm diversity was carried out by morphological and biochemical markers. (Herrera *et al.,* 2008). Advances in plant genetics and molecular biology have led to the development of many types of molecular markers which can be used to characterize germplasm. Different types of DNA markers are available nowadays, each differing in principle, application, type and amount of polymorphism detected, and cost and requirement (Panaud *et al.*, 1996). It is now feasible to analyze both the simple inheritedtraits and the quantitative traits and then identifying theindividual genes controlling for physical and cooking quality traitswhich couldfacilitate selection in rice.

The objectives of this study were to use SSR markers toestimate the genetic diversity within a 31 rice genotypes.

MATERIALS AND METHODS

Total 31 diverse rice genotypes obtained from the Main Rice Research Station, Anand Agricultural University, Nawagam, Gujarat, India were used for molecular analysis. Total DNA was extracted from three weeks old seedlings by CetylTrimethyl Ammonium Bromide (CTAB) method with minor modifications (Ahmadikhah, 2008). The qualitative analysis of the isolated DNA was performed spectrophotometrically using Nanodrop N.D. 1000 (Software V.3.7.1). Each polymerase chain reaction was carried out in 20μl reaction volume containing 30ng of DNA, 10X polymerase buffer (10x Tris with 20mM MgCl2), 25mM dNTPs, 0.5 μL of each primer (10pmol), 1 unit of Taq polymerase (Fermentas). Thermal cycler programme for PCR comprised 95ºC for 5 minutes for initial denaturation, followed by 35 cycles of 95ºC for 45 seconds, 55 to 65ºC for 45 seconds, 72ºC for 45 seconds and ending up with 10minutes at 72ºC for the final extension. The annealing temperature was adjusted based on the specific requirements of different primer combinations. The PCR products were resolved by electrophoresis in 2.5% agarose gel containing 0.5 μg/mL of Ethidium Bromide prepared in 1X TBE buffer at a constant voltage of 80v for period of 2h. The gel was visualized in UV transilluminator and documented using SYNGENE GENESNAP G-BOX gel documentation system. Coefficients of similarity were calculated as Jaccard's similarity coefficient by SIMQUAL subroutine in SIMILARITY routine. The matrix of similarity was clustered using UPGMA algorithm under Sequential Agglomerative Hierarchical Nesting module of the NTYSYS pc Version 2.02 (Rohlf, 1994). Identity software was also used for data analysis (Wagner and Sefc, 1999) which revealed information about expected heterozygosity, observed heterozygosity, number of alleles and allele frequency and standard deviation. Relationships among rice cultivars are graphically represented in the form of dendrogram.

RESULTS AND DISCUSSION

In the present investigation, 30 rice varieties and one advanced breeding lineNWGR-7011 were used for genetic diversity analysis with 26 SSR markers. An overall comparison of the markers and genotypes involved in genetic diversity analysis revealed that the markers could distinguish between different genotypes (Table 2). All 26 SSR markers were found to be polymorphic amongthe 31 rice genotypes. These markers amplified a total of 90 alleles (Table 3); the average number of alleles per locus was 3.46, ranging from 2 to 5. The overall size of PCR products amplified using 26 primer pairs ranged from 72-285 bp. SSR markers with (GA)17, (AG)16, (CT)11, (CT)20 and(TAT)19(CTT)19 motifs showed the maximum variation in allele size. There was a considerable range (20.8-80.65%) in allele frequency (Table 2). The alleles with frequencies less than 5% are defined as rare fragments. The analysis of allele frequency and distribution of polymorphic fragments revealed rare fragments among different rice genotypes

The maximum number of alleles, which was recorded for locus RM 431 and RM 333 which produced five alleles each. Whereas, nine primers *viz.*, RM-11, RM-201, RM-209, RM-242, RM-6881, RM- 170, RM-190, RM-217 and RM-247produced four alleles each. Lowest allele number was three which were recorded in RM-211, RM-212, RM-232, RM-257, RM-282, RM-336, RM-2125, RM-513, RM-503, RM-3, RM-314, RM-223, RM-225 and RM-234. (Table 2)

The highest allele length (285bp) was amplified for RM 223 in fourvarieties*viz.*, GR-6, GR-101, Pankhali 203 and IR64. The highest allele frequency (80.65%) was registered by the marker RM 2125, where RM2125showed lowest PIC of 0.35 in several elite varieties of rice, that RM 2125 least useful for assessment for molecular characterization.

Expected heterozygosity was observed in the range of 0.32-0.78, wherein the marker RM431 revealed the highest value of 0.78 for grain length. The average expected heterozygosity was 0.63. The present result corroborates with the results of Amarawathi *et al.* (2008), where marker assisted selection by several SSR markers including RM 431 on basmati ricewas performed. It was observed that the primers used in this study could unravel for detection long grain variety NWGR-7011 followed by Gurjari, GR-3 and AAUDR-1.

The allele frequency produced by 15 markers for physical quality traits grain length and width were observed in the range of 29.03-80.65%. The highest allele frequency (80.65%) was

Table 1: List of 31 genotypes of rice used in the study

Sr. No.	Name of Genotypes	Sr. No.	Name of Genotypes
1	GR-3Nawagam 19 x IR 9-60	17	JayaTN-1 x T-141
2	GR-4Zinnia-31 x IR-8-246	18	DandiPNL-2 x IET 8320
3	GR-6GR-3 X Pusa-33	19	NarmadaTN-1 X Basmati.370
4	GR-7GR-3 X Basmati. 370	20	MasuriM-80 x T-65/ M-80
5	GR-11Zinnia 31 x IR-8-246	21	IR 28IR-833-6-2-1-1/IR-1561-149-1//IR-24*4/O.nivara
6	GR-12GR-4 X IR-64	22	IR 64IR-5857-33-2-1 x IR-2061-465-1-5-5
7	GAR- 13GR-11 x IET-14726/22-1-8-1-1-1	23	NAUR-1GR-4 x IET 1705/ Pusa 2-48-24
8	GAR -1Narmada x IET-14708	24	SK -20Selection from local cultivar Sukhvel
9	GAR-2Gurjari x IET-14714	25	GNR -3IR-28 x GR-4
10	GR-101IR-8 x Pankhali 203	26	ASHOKA 200 FKalinga III x IR-64
11	GR-102IR-8 X P-203	27	AAUDR -1Sathi-34-36 x DadriKolam
12	GR-103GR-11 x Mahsuri	28	GR-9Sathi-34-36 x C. R 544-1-2
13	GR-104GR-101X Basmati 370	29	GR-5Selection from local variety
14	GurjariAsha x Kranti	30	Mahi sugandhaBK 79 x Basmati 370
15	Pankhali 203Selection from local cultivar Pankhali	31	NWGR-7011GR-4 x IET 1750
16	Krishna KamodSelection from local cultivar Kamod		

Table 2: Summary of genetic variation in 31 rice genotypes including fragment size (bp), number of alleles, polymorphism information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He) and polymorphism percentage (Pp).

Sr. No.	Primer Name	Size range in bp	Number of alleles	He	PIC value	Highest frequency %
Grain Length						
1	RM 11	137-161	4	0.7425	0.73	29.03
2	RM 201	149-171	4	0.5732	0.56	54.84
3	RM 209	129-161	4	0.6118	0.62	46.77
4	RM 211	125-171	3	0.6515	0.64	51.61
5	RM 212	106-129	3	0.5986	0.59	54.84
6	RM 232	136-158	3	0.588	0.58	54.84
7	RM 242	159-221	4	0.7292	0.72	37.1
8	RM 257	125-179	3	0.6742	0.66	37.1
9	RM 282	148-168	3	0.6642	0.65	41.94
10	RM 336	131-209	3	0.6346	0.62	48.39
11	RM 431	211-284	5	0.7869	0.78	25.81
12	RM 2125	72-123	3	0.3231	0.35	80.65
13	RM 6881	78-105	4	0.6536	0.62	48.39
Grain Width						
1	RM 513	198-287	3	0.5838	0.57	51.61
2	RM 530	161-201	3	0.6261	0.62	58.06
Amylose Content						
1	RM 3	121-162	3	0.6769	0.56	35.48
2	RM 170	136-158	4	0.7356	0.73	38.71
3	RM 190	120-150	4	0.7277	0.73	38.71
4	RM 217	118-166	4	0.7573	0.74	29.03
5	RM 314	105-132	3	0.5986	0.59	54.84
Kernel length after cooing						
1	RM 44	95-112	2	0.4188	0.40	70.97
2	RM 223	125-285	3	0.6134	0.60	51.61
Gel Consistency						
1	RM 225	122-158	3	0.6542	0.65	45.16
2	RM 247	121-158	4	0.7324	0.73	38.71
Protein Content						
1	RM 234	121-161	3	0.4146	0.41	74.19
2	RM 333	164-512	5	0.6277	0.89	32.26

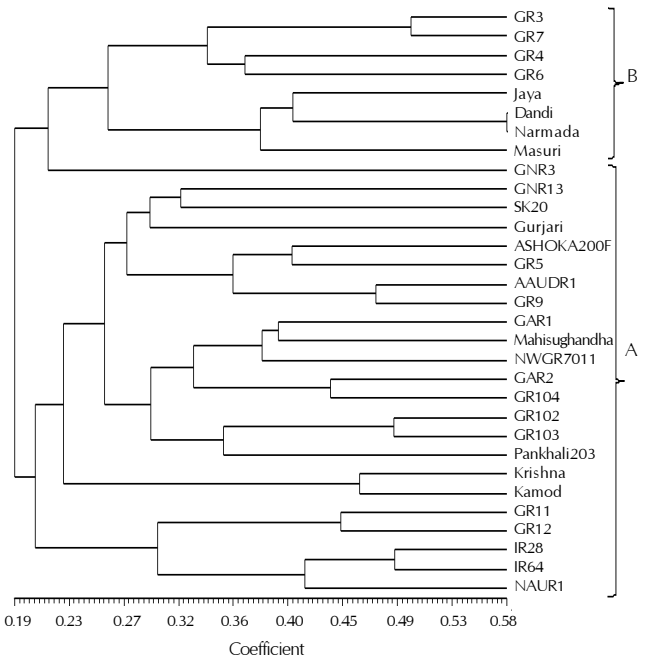

Figure 1: Dendrogram of genetic relationship among 31 Rice genotypesbased on SSR Markers

observed in RM2125 for varieties Dandi and GR11. Expected heterozygosity for 31 rice genotypes varied from 0.32-0.78, wherein the marker RM2121 revealed highest value (0.32).

Among 11 specific primers utilized for detecting cooking quality traits genes/QTLs. The lowest allele length was recorded for RM 44 (95bp) in varieties GAR-2, Jaya, Dandi and NWGR-7011. The highest allele frequency (74.19%) was recorded by the marker RM 234 for protein, where PIC ranged from 0.40-0.89. The highest per cent of polymorphism was revealed for RM 333. Similar results were recorded by Fan *et al.* (2006).

The dendrogram (Fig. 2) divided rice genotypes into two clusters A and B, where the cluster 'A' was divided into sub clusters 'a1' and 'a2'. The sub cluster 'a1' comprised of five genotypes *viz.*, GR-11, GR-12, NAUR-1, IR-64 and IR-28 both IRRI cultures grouped together. The sub cluster 'a2' consisted of 17 solitary genotypes. The sub cluster 'b1' consisted of solitary genotype GNR-3. The sub cluster 'b2' consisted of eight genotypes *viz.*, Masuri, Dandi, Narmada, Jaya, GR-6, GR-4, GR-7 and GR-3 (Fig. 2).

For germplasm characterization and conservation, the assessment of genetic diversity is an essential component in crop. The results derived from analyses of genetic diversity at the DNA level could be used for designing effective breeding programs aiming to broaden the genetic bases of commercially

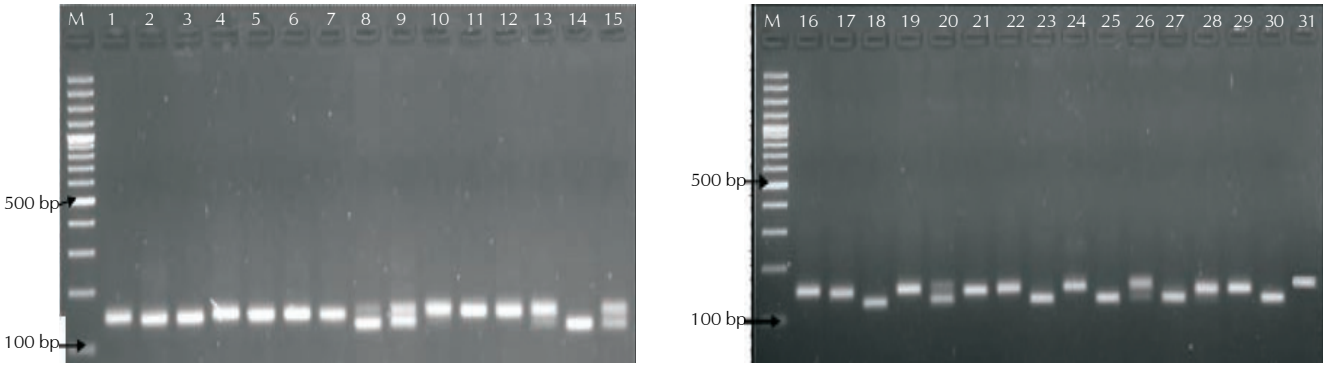

Figure 2: SSR Profile of RM315

grown varieties. The major factors affecting microsatellite variability are structure and length of simple sequence repeats are considered (Cho *et al.*, 2000). Grouping genotypes together based on length and breadth (physical traits) and kernel elongation, amylose content and volume expansion ratio (cooking quality traits). Consequently, Ashoka 200F, AAUDR-1 and GR-9 upland, the coarse grain varieties were grouped together, and irrigated varieties GR-11, GR-12, GAR-1, GAR-2, GR-3, and GR-6 exhibited similar clustering pattern. In general, SSR loci contains more repeats tend to be high polymorphic and have larger amplitude of variation among genotypes (Panaud *et al.*, 1996; Innan *et al.*, 1997 and Davla *et al.*, 2013). SSR used in the present study are specific to genes/ QTLs govering quality traits. The results of the genetic diversity could be useful for the selection of the parents for developing improved rice variety. The SSR markers with many repeat units exhibited higher *H* values, higher number of alleles and larger size differences among alleles.

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