

Assessment of Genetic Diversity of Philippine Rice Cultivars Carrying Good Quality Traits using SSR Markers

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Microsatellite markers, also known as simple sequence repeat (SSR) markers have been useful for the detection of genetic diversity. Twenty-four rice cultivars carrying good quality traits were evaluated for genetic diversity using 164 SSR markers. A total of 890 alleles were detected by 151 polymorphic markers with an average of 5.89 per locus. Out of these markers, 89 generated a total of 147 rare alleles. Based on Shannon's diversity index, an overall genetic diversity of 0.71 was revealed indicating a high level of genetic variation among these cultivars. Polymorphism information content (PIC) values of the markers ranged from 0.18 (RM420) to 0.91 (RM473B) with an average of 0.68 per marker. Cluster analysis of these cultivars enabled to identify 3 groups at 40% level of similarity with additional sub-clusters within each group. Group 1 corresponded to the 8 *japonica* subspecies, whereas Groups 2 and 3 comprised the *indica*. Cultivars under groups 1 and 2 are known for their aroma and good cooking and eating quality traits. Between the 2 rice subspecies, *indica* gave more alleles than *japonica* and likewise displayed a higher genetic diversity. Genetic diversity of *indica* was high on chromosome 11, while that for *japonica* was high on chromosome 2. The study revealed that SSR markers facilitated the classification of these cultivars according to their subspecies. The results also indicated that these quality rice cultivars exhibited a higher genetic diversity and therefore very useful for rice breeding programs, especially for genetic mapping studies and eventually for application of marker-assisted selection (MAS) in the programs.

Key Words: *Oryza sativa* L., SSR markers, grain quality traits, *indica*, *japonica*.

Introduction

Rice quality is one of the most important characters, as it exerts large effects on the market value and consumer acceptance. The demand for high quality rice cultivars is increasing owing to recent changes in consumer preferences and strong market requirement. Grain quality of rice is determined by many factors such as grain appearance, nutritional value, cooking and eating qualities (Juliano *et al.* 1990). Aroma is an important quality characteristic of high quality rices and it plays a considerable role in price and marketing. Consumers purchase rice based on quality characters and attach higher implicit prices to these attributes. Development of cultivars with good grain qualities is an important objective of improvement programs today, and its relevance is much greater (Pingali *et al.* 1997). However, rice improvement for these traits, using conventional breeding methods is difficult for plant breeders. Most of the traits that determine the quality are quantitative in nature. Polygenic inheritance

and environmental interaction have compounded the difficulties in attempts at improving these complex traits through conventional breeding. Breeding for good quality traits requires selection of parents with a wider genetic diversity. A narrow genetic base in the breeding materials limits genetic gains in breeding. Sufficient knowledge about genetic diversity in the genepool is a prerequisite to adopt an efficient and valuable breeding approach.

The recent development of DNA markers has provided new opportunities for the genetic improvement of rice grain quality (Causse *et al.* 1994, Harushima *et al.* 1998). Molecular markers have been found to be powerful tools in the assessment of genetic variation. Microsatellite loci, also known as simple sequence repeats (SSRs) are among the most commonly used molecular markers. Microsatellites are PCR-based markers that are efficient and cost-effective to use. Compared with other markers, they are abundant, codominant and interspersed throughout the genome. These markers can detect a significantly higher degree of polymorphism in rice (Ni *et al.* 2002, Okoshi *et al.* 2004) which becomes ideal for studies on genetic diversity and intensive genetic mapping (Cho *et al.* 2000). SSR markers can estimate genetic diversity between cultivars e.g. between

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parents of a gene pool or between plants extracted from a population (Panaud *et al.* 1996, Akagi *et al.* 1997) or between populations. Olufowote *et al.* (1997) reported that microsatellites were more powerful for the identification of within-cultivar variation, the same way as Ravi *et al.* (2003), who recognize that SSR markers can detect finer levels of variation among closely related breeding lines than RAPD. Zhou *et al.* (2003) investigated the genetic diversity and genetic structure of natural populations of *O. rufipogon* in China using SSR markers and the information was found to be significant for their conservation. Traore *et al.* (2004) determined the genetic diversity of several West African rice accessions in terms of grain quality parameters and were able to identify genetic resources for novel quality traits using SSR markers.

In the present study, Philippine traditional and other aromatic and quality rice cultivars were analyzed for genetic variation using SSR markers. Specifically, the objective was to determine the genetic diversity of these quality rice cultivars as the first step for proper identification of distinct cultivars and selection of suitable parents for future gene mapping studies in our rice breeding program.

Materials and Methods

Plant materials

A total of 24 rice cultivars, 8 *japonica* and 16 *indica*, were evaluated in the present study (Table 1). Classification of these cultivars into *japonica* and *indica* was based on the eco-geographic study/evaluation. These cultivars were obtained from the Rice Germplasm Center located at the International Rice Research Institute (IRRI) and also from the Philippine Rice Research Institute (PhilRice), Philippines.

Genomic DNA extraction and SSR assay

DNA was extracted from 21-day-old seedling leaves collected from at least 2-3 seedlings in each cultivar, according to the modified CTAB method (Murray and Thompson 1980). One hundred sixty four SSR markers were selected from the list of Cornell's rice microsatellite markers displayed at the Cornell University Rice Genes web site; <http://www.gramene.org/microsat/ssr.html>

The Polymerase Chain Reaction (PCR) was conducted in a reaction solution of 20 µl containing 50 ng of template DNA, 0.25 µM of each primer, 100 µM of each dNTPs, 1 X reaction buffer (20 mM Tris pH 8.3, 50 mM KCl, 1.5 mM

Table 1. Description of the 24 quality rice cultivars evaluated for genetic diversity

Cultivar Name	Cultivar Group*	Country of Origin	IRGC Acc. No.	Key Quality Traits
Azucena	<i>Trop. japonica</i>	Philippines	328	Aromatic, non-glutinous, intermediate amylose content, traditional
Basmati 370	<i>Indica</i>	India	3750	Aromatic, non-glutinous, low amylose content
Milagrosa	<i>Indica</i>	Philippines	5159	Aromatic, glutinous, low amylose content, traditional variety
Poloy tinawon	<i>Trop. japonica</i>	Philippines	8023	Aromatic, glutinous, low amylose content, traditional variety
Ifugao rice	<i>Trop. japonica</i>	Philippines	8037	Aromatic, glutinous, low amylose content, traditional variety
Nipponbare	<i>Japonica</i>	Japan	12731	Aromatic, non-glutinous, low amylose content
Pare baine pulut	<i>Trop. japonica</i>	Indonesia	27348	Aromatic, non-glutinous, low amylose content
Dinorado	<i>Trop. japonica</i>	Philippines	30333	Aromatic, non-glutinous, traditional variety
IR29	<i>Indica</i>	Philippines	30412	Glutinous, low amylose content
IR841-85-1-1-2	<i>Indica</i>	Philippines	32591	Non-glutinous, low amylose content
Azucena	<i>Trop. japonica</i>	Philippines	47125	Aromatic, non-glutinous, intermediate amylose content, traditional
Reket penjalin	<i>Trop. japonica</i>	Indonesia	54349	Aromatic, glutinous, low amylose content
IR65	<i>Indica</i>	Philippines	66971	Glutinous, low amylose content
IR72	<i>Indica</i>	Philippines	76330	Non-glutinous, high amylose content
IR74	<i>Indica</i>	Philippines	76331	Non-glutinous, low amylose content
Bordagol	<i>Indica</i>	Philippines	81726	Non-glutinous, intermediate amylose content
PSBRc10	<i>Indica</i>	Philippines	88278	Non-glutinous, high amylose content
PSBRc52	<i>Indica</i>	Philippines	99707	Non-glutinous, low amylose content
PSBRc60	<i>Indica</i>	Philippines	99709	Non-glutinous, high amylose content
Burdagol	<i>Indica</i>	Philippines	PhilRice collection	Aromatic, non-glutinous, intermediate amylose content
IR67406	<i>Indica</i>	Philippines	PhilRice collection	Aromatic, non-glutinous, intermediate amylose content
AR32-19-3-3	<i>Indica</i>	Philippines	PhilRice collection	Non-glutinous, intermediate amylose content
AR32-19-3-4	<i>Indica</i>	Philippines	PhilRice collection	Non-glutinous, intermediate amylose content
Membrano	<i>Indica</i>	Indonesia	PhilRice collection	Non-glutinous, intermediate amylose content

*Eco-geographic classification based on morphological traits

MgCl₂, 0.01% Gelatin) and 2.5 units of Taq DNA polymerase. The PCR amplification was performed using a Perkin-Elmer thermo-cycler, according to the cycle profile: initial denaturation at 94°C for 5 minutes and then 35 cycles of 1 min denaturation at 94°C, 1 min annealing at the temperature depending on the marker used (55°C, 61°C and 67°C), and 2 min extension at 72°C and 5 min at 72°C for the final product extension. Amplification products were stored at -20°C until further use.

PCR-amplified products were subjected to electrophoresis in 8% polyacrylamide gel in 1 X TBE buffer at 100 volts where the running time depended on the size of the PCR products, from 1 hour to 3 hours for larger products. The gels were stained either with ethidium bromide for 10 minutes or SYBR Safe™ (Invitrogen) for 30 minutes. DNA bands were visualized under UV light using the Gel Documentation System.

Data analyses

Only clear and unambiguous SSR markers were scored. All the genotypes were scored for the presence (score '1') and absence (score '0') of the SSR bands. Polymorphism information content (PIC) was calculated, according to the method of Anderson *et al.* (1993):

$$PIC_i = 1 - \sum_{j=1}^n P_{ij}^2$$

where P_{ij} is the frequency of the j th allele for the i th marker, and is summed over n alleles. The calculation was based on the number of alleles/locus. Genetic similarities were estimated from the matrix of binary data using Jaccard coefficient. The similarity coefficients were used for cluster analysis of the rice cultivars utilizing the Unweighted Pair Group Method with Arithmetic Averages (UPGMA). The analysis and dendrogram construction were performed using the NTSYS-pc version 2.02 (Rohlf 1999). For further confirmation, the genetic diversity was also analyzed using Shannon diversity index (Hutchenson 1970).

Results and Discussions

SSR polymorphisms

Table 2 summarizes the results obtained based on the analysis of the 24 rice cultivars using the polymorphic SSR loci. The number of alleles varied widely among these loci. Among the 164 markers used in the analysis, 151 (92%) showed polymorphism with a total of 890 alleles identified across cultivars. The number of alleles ranged from 2 (RM114, RM312, RM408, RM420, RM451, and RM556) to 17 (RM473B) with an average value of 5.89 per locus. On a per locus basis, these numbers were much higher than the average of 2.0–5.5 alleles per locus for various classes of microsatellites reported by Cho *et al.* (2000) and were comparable to the average of 6.3 alleles per locus (range 2 to 11) reported by Yu *et al.* (2003), using different parental lines for an international molecular breeding program. The average number of alleles per locus obtained in the study was also

larger than that reported in previous studies using other types of markers such as isozymes (Glaszmann 1987) and RFLPs (candidate defense genes) (Moumeni *et al.* 2003). However, the numbers obtained in the present study were smaller than those reported by Yang *et al.* (1994) who observed an average of 9.3 alleles per locus over 100 SSR loci, and as many as 25 alleles at one locus and by Jain *et al.* (2004) who recorded 7.8 alleles/locus with a range of 3–22, using different Indian aromatic and quality rice germplasm accessions.

The PIC values, a reflection of allele diversity and frequency among the cultivars, also varied from one locus to another. The PIC value was 0.68 per marker and it ranged from 0.18 (RM420) to 0.91 (RM473B). The genetic diversity of each SSR locus appeared to be associated with the number of alleles detected per locus. The higher the PIC value of a locus, the higher the number of alleles detected. This observed pattern was consistent with the report of Yu *et al.* (2003) based on much larger samples but not with the results obtained by Yang *et al.* (1994) that used only 10 SSR loci in the analysis. Using Shannon's diversity index, an overall genetic diversity of 0.71 was obtained from the analysis, indicating a high level of genetic variation among these cultivars. Values for gene diversity explained the probability that two randomly chosen alleles originated from different ancestors and mirrored those of the PIC. Examples of SSR alleles as resolved with the PCR assay for RM245 and RM405 are illustrated in Fig. 1.

Loci amplifying di-nucleotide repeat motifs were found to be more polymorphic, with an average value of 6.1 alleles, than those with tri-nucleotide and tetra-nucleotide repeat motifs which both gave an average value of 5.4 alleles. Among the loci with perfect and compound di-nucleotide repeat motifs, markers with a GA repeat motif showed the largest variability. These results, suggest that the total repeat count of SSR loci is associated with the number of alleles. The larger the repeat number in the microsatellite DNA, the larger the number of identified alleles. These results were consistent with those reported by Cho *et al.* (2000) and Ni *et al.* (2002).

Rare alleles were also observed in the present study. An allele is considered to be rare when it is revealed in less than 5% of the genotypes under analysis (Jain *et al.* 2004). Out of the polymorphic loci, 89 (59%) generated a total of 147 rare alleles. RM583 revealed the highest number of rare alleles (5), followed by RM259, RM266, RM280, RM429 and RM570 which produced 4 rare alleles each. Among the cultivars, on the other hand, Basmati 370 showed largest number of rare alleles (22) across polymorphic loci. The presence of rare alleles in this set of cultivars may indicate that these materials are useful for plant breeders and geneticists as a rich source of genetic diversity for rice breeding. This finding, however, requires further studies for accurate estimate of allele frequencies.

Occurrence of null alleles was also noted in a cultivar for a particular locus whenever an amplification product

Table 2. Data on the number of alleles, number of rare alleles, polymorphism information content (PIC) value, and genetic diversity of the SSR markers across 24 rice cultivars carrying good quality traits

SSR locus	No. of alleles	No. of rare alleles	PIC value	Genetic diversity*	Repeat type
RM10	8	0	0.83	0.86	(GA)15
RM103	4	0	0.68	0.71	(GAA)5
RM104	7	0	0.82	0.86	(GA)9
RM111	4	2	0.54	0.57	(GA)9
RM114	2	0	0.28	0.29	(GA)7
RM115	4	0	0.66	0.69	(AG)7
RM127	6	1	0.78	0.81	(AGG)8
RM130	3	1	0.52	0.54	(GA)10
RM134	3	1	0.43	0.45	(CCA)7
RM138	9	2	0.86	0.9	(GT)14
RM143	3	0	0.34	0.35	(CGG)7
RM153	5	2	0.61	0.63	(GAA)9
RM155	4	2	0.44	0.46	(CTT)7
RM167	12	3	0.84	0.88	(GA)16
RM17	6	0	0.65	0.68	(GA)21
RM170	6	0	0.74	0.77	(CCT)7
RM174	12	1	0.88	0.92	(AGG)7(GA)10
RM176	4	2	0.27	0.28	(CCG)8
RM178	4	1	0.67	0.7	(GA)5(AG)8
RM189	3	1	0.54	0.57	(AG)11
RM190	8	1	0.79	0.82	(GA)11
RM202	11	0	0.89	0.93	(GA)30
RM204	10	3	0.83	0.87	(GA)44
RM205	5	1	0.48	0.5	(GA)25
RM206	10	1	0.87	0.91	(GA)21
RM207	12	2	0.89	0.93	(GA)25
RM208	5	0	0.75	0.78	(GA)17
RM209	10	0	0.83	0.87	(GA)18
RM210	8	0	0.85	0.88	(GA)23
RM212	5	0	0.75	0.78	(GA)24
RM213	7	0	0.85	0.88	(GA)17
RM215	4	1	0.62	0.65	(GA)16
RM216	6	2	0.73	0.76	(GA)18
RM217	3	1	0.35	0.37	(GA)20
RM22	10	1	0.85	0.89	(GA)22
RM220	3	0	0.54	0.56	(GA)17
RM222	7	1	0.78	0.82	(GA)18
RM223	10	2	0.8	0.84	(GA)25
RM225	4	0	0.72	0.75	(GA)18
RM227	3	0	0.43	0.45	(GA)10
RM229	9	2	0.87	0.9	(GA)11
RM231	7	1	0.81	0.85	(GA)16
RM233B	6	1	0.73	0.76	(GA)20
RM234	6	1	0.8	0.83	(GA)25
RM235	4	1	0.54	0.56	(GA)24
RM237	6	1	0.76	0.8	(GA)18
RM240	4	0	0.75	0.78	(GA)21
RM241	5	0	0.66	0.69	(GA)31
RM243	11	1	0.88	0.92	(GA)18
RM244	8	1	0.86	0.89	(CT)4(CG)3C(CT)6
RM245	4	0	0.69	0.72	(GA)14
RM246	5	0	0.63	0.66	(GA)20
RM248	5	2	0.74	0.77	(GA)25
RM252	13	1	0.9	0.94	(GA)19
RM253	6	0	0.82	0.85	(GA)25
RM255	4	2	0.48	0.5	(GA)16
RM256	4	0	0.74	0.77	(GA)21

Table 2. (continued)

SSR locus	No. of alleles	No. of rare alleles	PIC value	Genetic diversity*	Repeat type
RM258	5	1	0.6	0.63	(GA)21(GGA)3
RM259	7	4	0.66	0.68	(GA)17
RM260	6	1	0.72	0.76	(GA)34
RM261	7	3	0.79	0.83	(GA)6
RM265	4	0	0.72	0.75	(GA)8
RM266	10	4	0.85	0.89	(GA)19
RM269	4	0	0.63	0.66	(GA)17
RM270	4	2	0.61	0.64	(GA)17
RM274	3	0	0.66	0.69	(GA)15-7-(CGG)5
RM280	9	4	0.8	0.84	(GA)16
RM282	3	1	0.54	0.57	(GA)15
RM284	10	2	0.79	0.82	(GA)8
RM286	9	1	0.78	0.82	(GA)16
RM291	4	2	0.34	0.35	(GAT)4GA(GT)4-(GT)4
RM293	5	1	0.74	0.78	(GT)20
RM297	6	1	0.78	0.82	(GA)13
RM304	4	1	0.58	0.6	(GT)2(AT)10(GT)33
RM311	4	1	0.62	0.65	(GT)3(GTAT)8(GT)5
RM312	2	0	0.47	0.49	(ATTT)4(GT)9
RM313	3	1	0.51	0.54	(GT)6CA(CG)5-6(GT)8
RM314	4	0	0.66	0.69	(GT)8(CG)3(GT)5
RM325A	5	0	0.7	0.73	(CAT)4TAG(CAT)5
RM331	5	1	0.7	0.73	(CTT)4GTT2(CTT)11
RM333	8	3	0.79	0.83	(TAT)19(CTT)19
RM339	6	1	0.63	0.66	(CTT)8CCT(CTT)5
RM342A	14	0	0.9	0.94	(CAT)12
RM345	5	2	0.44	0.46	(CTT)9
RM348	3	1	0.49	0.52	(CAG)7
RM349	6	3	0.65	0.68	(GA)16
RM35	10	2	0.85	0.88	(GA)19
RM350	4	0	0.61	0.64	(GA)10
RM351	5	0	0.77	0.81	(CCG)9(CGAAAG)4
RM39	3	1	0.51	0.54	(CT)17CCA(TC)3
RM404	9	1	0.85	0.88	(GA)33
RM405	5	0	0.53	0.55	(AC)14
RM406	8	3	0.58	0.61	(GA)17
RM408	2	0	0.5	0.52	(GA)13
RM42	6	2	0.76	0.79	(AG)6-(AG)2T(GA)5
RM420	2	1	0.18	0.19	(AAAT)7
RM429	8	4	0.69	0.72	(TG)10
RM435	7	1	0.8	0.83	(ATG)7
RM441	3	0	0.62	0.65	(AG)13
RM448	4	1	0.71	0.75	(GA)23
RM450	5	1	0.65	0.68	(AG)17
RM451	2	0	0.47	0.49	(GAT)8
RM455	3	0	0.65	0.68	(TTCT)5
RM457	5	1	0.63	0.67	(TTAA)5
RM463	5	1	0.75	0.79	(TTAT)5
RM470	6	2	0.51	0.54	(CTT)14
RM473B	17	3	0.91	0.95	(TCTA)14
RM48	8	2	0.81	0.84	(GA)17
RM482	7	1	0.82	0.85	(AT)2
RM486	6	0	0.77	0.8	(GA)14
RM490	5	1	0.71	0.74	(GA)13
RM494	4	0	0.54	0.57	(AGA)16
RM498	6	0	0.78	0.81	(CA)10
RM4A	4	0	0.67	0.71	(AG)n
RM506	4	0	0.56	0.58	(GA)13
RM508	4	0	0.68	0.71	(AG)17
RM510	4	2	0.57	0.6	(GA)15

Table 2. (continued)

SSR locus	No. of alleles	No. of rare alleles	PIC value	Genetic diversity*	Repeat type
RM515	6	3	0.67	0.71	(GA)11
RM520	5	0	0.67	0.7	(AG)10
RM53	9	0	0.7	0.73	(GA)14
RM530	4	0	0.7	0.73	(GA)23
RM531	4	0	0.56	0.58	(AT)15
RM535	3	0	0.52	0.54	(AG)11
RM536	6	1	0.75	0.79	(GA)16
RM541	5	1	0.7	0.74	(TC)16
RM543	4	0	0.72	0.75	(GCG)10
RM556	2	0	0.44	0.46	(CCAG)6
RM559	5	1	0.72	0.75	(ACA)6
RM568	3	0	0.55	0.58	(GA)10
RM569	9	2	0.81	0.85	(GA)16
RM570	7	4	0.74	0.77	(AG)15
RM571	6	2	0.74	0.78	(GT)11(AG)13
RM575	4	0	0.66	0.69	(AG)24
RM576	3	0	0.6	0.62	(AT)10(GT)14
RM577	4	1	0.66	0.69	(TA)9(CA)8
RM579	6	0	0.79	0.83	(GA)25
RM582	4	0	0.74	0.77	(TC)20
RM583	9	5	0.73	0.76	(CTT)20
RM584	7	1	0.82	0.85	(GA)14
RM585	8	0	0.85	0.89	(TC)45
RM586	7	0	0.83	0.87	(GA)23
RM588	7	0	0.77	0.8	(TGC)9
RM589	9	1	0.87	0.91	(GT)24
RM6	6	0	0.78	0.83	(AG)16
RM60	4	1	0.46	0.48	(AATT)5(ATCT)(AATT)
RM72	10	1	0.88	0.92	(TAT)5(CATT)15
RM81B	8	0	0.81	0.85	
RM85	7	2	0.71	0.74	(TGG)5(TCT)12
RM86	3	1	0.35	0.37	(CTT)16
RM87	8	0	0.85	0.89	(CTT)3T(CTT)11
RM88	5	0	0.75	0.78	(TCT)11
TOTAL	890	147			
MEAN	5.89	1.7	0.68	0.71	

*Computed based on Shannon's diversity index

could not be detected in their combination. Fifty-six SSR loci showed null alleles in one to seven of the 24 cultivars. The frequencies of null alleles were not included in the genetic diversity calculation for each SSR locus. Null alleles might decrease the apparent heterozygosity in a population and may result in the deviation of cultivars in a sample from Hardy-Weinberg expectations (Kalinowski and Taper 2006).

Clustering of rice cultivars

Genetic similarity values among the rice cultivars used led to the construction of a dendrogram presented in Figure 2. At a 40% level of similarity, the UPGMA cluster diagram showed 3 groups with additional sub-clusters within each group. This dendrogram revealed that the cultivars derived from a genetically similar type clustered together. Group 1 corresponded to the *japonica* cluster, whereas Groups 2 and 3 comprised the *indica* cluster.

Group 1 contained the 8 cultivars in the tropical *japonica* clusters which were classified into aromatic and with good cooking and eating qualities. All the cultivars in this cluster exhibited a waxy to low amylose content except for Azucena which showed an intermediate amylose content. The similarity coefficients between any two rice cultivars in this cluster ranged from 48 to 75%. This cluster could be divided into two groups. One group at a similarity coefficient of about 50% consisted of 7 cultivars in the tropical *japonica* cultivars (Poloy tinawon, Ifugao rice, Dinorado, Azucena 328 and 47125, Pare baine pulut and Reket penjalin). Cultivars in this group are of interest to rice breeders because some of them are used as donors for breeding for grain quality traits and disease resistance. Azucena and Dinorado, the Philippine fancy cultivars, which belong to this group, were selected by farmers in China due to their good grain qualities including aroma. They also command a higher price in the Philippine rice market because of these attributes. The two accessions of Azucena (328 and 47125) were clustered in this group despite their discrepancy in terms of amylose content within the group. Inclusion of these accessions into the group could be due to their key quality attributes such as aroma and good cooking and eating qualities and not due to the amylose content. The other group consisted only of Nipponbare, a temperate *japonica* cultivar from Japan with a slightly low amylose content, presumably because Nipponbare was the only cultivar in the temperate *japonica* cluster.

Group 2 was comprised of two *indica* cultivars, Basmati 370 and Milagrosa, which clustered at similarity coefficients of 58%. Although generally they are classified into *indica*, they did not cluster with the other *indica* cultivars (Group 3). Among the *indica* cultivars used in the present study, these two were considered to be fancy rice because of their pleasant and distinct aroma, and the soft and fluffy texture of cooked rice. Since these cultivars exhibit a low amylose content, rice is moist and tender and does not become hard upon cooking.

Group 3 was more diverse than the other two groups. This group clustered 14 out of the 16 *indica* cultivars used in the present study at a similarity level of 45%. The cluster could be further subdivided into 5 sub-groups with varying levels of similarity. The major sub-group at a similarity coefficient of about 55% consisted of 7 cultivars including the 3 cultivars (PSBRc 52, PSBRc 60 and PSBRc 10) released in the Philippines, 3 lines developed from IRRI (IR72, IR74 and IR67406) and Burdagol (an accession from PhilRice). The 3 PSBRc cultivars released in the Philippines together with IR74 clustered at a similarity coefficient of 60% while Burdagol and IR67406 clustered at a coefficient of about 63%. The latter cultivars are both aromatic and high-yielding. Burdagol is being used in the Philippine National Cooperative Test (NCT) as check cultivar for aroma. On the other hand, IR72 did not cluster very closely with any other cultivars in this group. IR72 shows a high amylose content. The second sub-group at a similarity coefficient of about 55% was comprised of two sister lines (AR32-19-3-3 and

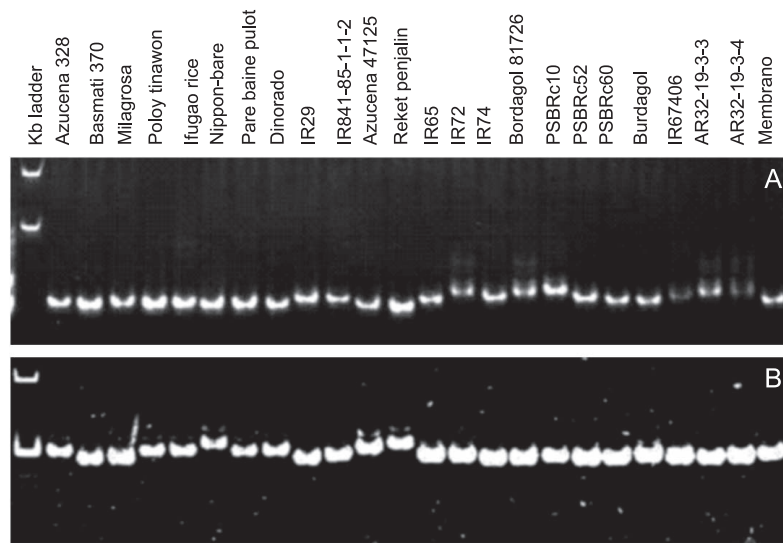


Fig. 1. Polymorphism observed using RM245 (A) and RM405 (B) in the 24 rice cultivars carrying good quality traits.

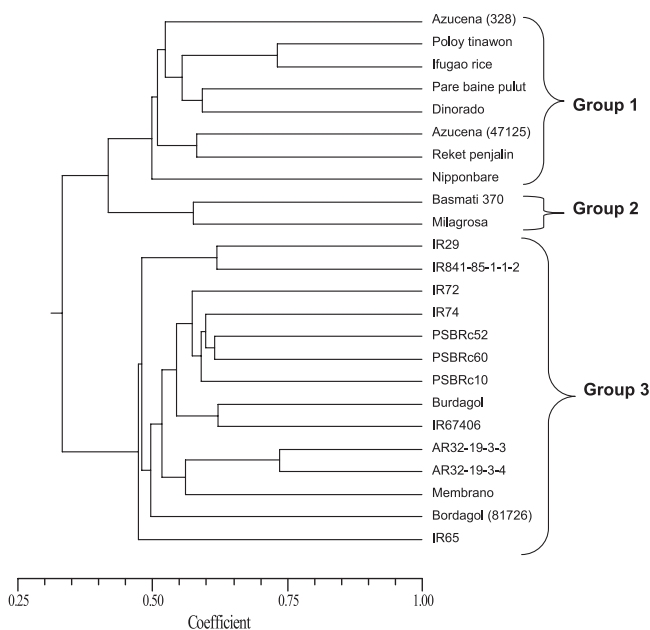


Fig. 2. Dendrogram of 24 quality rice cultivars derived from UPGMA cluster analysis using Jaccard coefficient based on 151 polymorphic SSR markers

AR32-19-3-4) and an accession from Indonesia (Membrano). AR32-19-3-3 is a pyramided line for bacterial leaf blight (BLB) resistance harboring the *Xa21* and *Xa5* genes. Membrano is being used at PhilRice as one of the parent cultivars for grain quality improvement because of its good grain appearance and high head rice recovery. The third subgroup consisted of IR29 and IR841-85-1-1-2 at a similarity coefficient of 63%. These cultivars showed a low amylose content. The fourth and fifth sub-groups in this cluster consisted only of one accession each, Bordagol 81726 and IR65, which clustered at similarity coefficients of 50% and 46%,

respectively. These two cultivars did not cluster with any cultivar in Group 3.

Table 3 shows the number of alleles and the PIC values of the microsatellite markers on 12 rice chromosomes in two subspecies, *indica* and *japonica*. It was observed that the number of alleles varied between the two subspecies. On the average, *indica* resolved more alleles (5.1) and displayed a higher genetic diversity (0.61) than *japonica* which gave respective values of 3.9 and 0.52. Consistently, *indica* revealed a larger number of alleles per locus than *japonica*. These findings are consistent with the following published reports: Zhang *et al.* (1992) reported that on the average, the diversity value of *indica* was 3 to 4 times higher than that of *japonica* using RFLP analysis; Second (1982) observed large differences in the allele frequency between *indica* and *japonica* types at a number of isozyme loci; and Morishima and Oka (1981) stated that based on a number of morphological characters and isozyme loci, 50% of the total variation between these two groups could be ascribed to genetic differentiation. Distinct geographic distribution of these two subspecies may contribute to their genetic differentiation. *Indica* is predominant in tropical and subtropical areas, while *japonica* is more adapted to temperate regions.

SSR loci on each of the 12 chromosomes for both subspecies also showed different levels of genetic diversity. *Indica* exhibited a high level of genetic diversity on chromosome 11 (0.73) while *japonica* on chromosome 2 (0.72). The number of alleles for *indica* was high on chromosomes 2 and 11 (6.3) while for *japonica*, it was high only on chromosome 11 (5.8). Respectively, *indica* and *japonica* displayed low levels of genetic diversity (0.51 and 0.13) and an average number of alleles (3.9 and 1.5) on chromosome 9. As mentioned in the report by Ni *et al.* (2002), the information about the genetic diversity of rice subspecies for specific chromosomes will be very useful for rice breeding programs,

Table 3. Number of alleles and PIC values of SSR loci on 12 rice chromosomes in *indica* and *japonica* subspecies

Chr. number	No. of loci	Number of alleles			PIC values		
		<i>Japonica</i>	<i>Indica</i>	Total	<i>Japonica</i>	<i>Indica</i>	Total
1	22	4.2	4.8	5.3	0.54	0.64	0.69
2	16	5.5	6.3	6.6	0.72	0.69	0.76
3	16	3.2	4.8	5.4	0.44	0.59	0.64
4	11	3.5	5.4	6.0	0.49	0.58	0.66
5	9	3.9	5.3	6.1	0.48	0.57	0.64
6	22	4.4	5.2	5.6	0.61	0.61	0.68
7	8	3.9	4.0	5.0	0.57	0.53	0.63
8	18	5.2	5.6	6.3	0.62	0.60	0.70
9	4	1.5	3.9	4.0	0.13	0.51	0.58
10	8	4.1	5.5	5.8	0.60	0.68	0.70
11	10	5.8	6.3	8.1	0.66	0.73	0.78
12	7	2.6	3.9	4.3	0.40	0.54	0.60
Mean		3.9	5.1	5.7	0.52	0.61	0.67

especially for gene mapping and eventually for the application of the marker-assisted selection (MAS) in the programs. In their study, Tanaka *et al.* (2006), were able to detect quantitative trait loci (QTLs) for stickiness and appearance based on eating quality test using *japonica* cultivars. QTLs for stickiness were identified on chromosomes 2 and 6 while on chromosome 2 for the amylose content and glossiness of cooked rice.

In summary, the present study provided an overview of the genetic diversity of the rice cultivars carrying good quality traits. The use of SSR markers in genetic diversity analysis enabled to group the cultivars according to their subspecies level. The results further indicated that since the SSR markers are neutral and co-dominant, they are powerful tools to assess the genetic variability of the cultivars under study. The information about the genetic diversity of these quality rice subspecies, *indica* and *japonica*, will be very useful for proper identification and selection of appropriate parents for use in breeding programs, including gene mapping, and ultimately for emphasizing the importance of MAS in rice improvement not only in the Philippines but also in other countries.

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