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Genotyping of Sarawak Rice Cultivars Using Microsatellite Markers

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ABSTRACT

Genetic diversity of 53 Sarawak rice cultivars, originating from Southern Sarawak, was assessed using 54 microsatellite markers. Initial polymorphism detection was conducted using 54 primer pairs distributed on 12 rice chromosomes. Polymorphic markers were chosen from the initial screening results in order to obtain microsatellite marker panels that can differentiate the rice cultivars undertaken in the study. The chosen microsatellite marker panel consisted of RM1, RM240, RM489, RM252, RM413, RM204, RM11, RM404, RM316, RM271, RM206, and RM19, with one representative from each chromosome. A total of 43 alleles were detected with an average of 3.58 alleles per locus. The polymorphism information content (PIC) values obtained from the microsatellite marker panels ranged from 0.306 to 0.730, with an average of 0.622. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram (r = 0.789) revealed 2 major groups with 6 sub-clusters and the wide range of similarity values (0.24-1.0) obtained showed a high degree of diversity among the cultivars. The results suggest microsatellite markers as a useful tool for the estimation of genetic diversity and cultivar differentiation and present invaluable genetic information for future breeding and association mapping efforts.

Keywords: Rice, microsatellite markers, polymorphism, genetic diversity, Sarawak

INTRODUCTION

Rice is a major food source of humans and the second major global calories contributor after wheat (FAO, 2008). It belongs to the genus *Oryza* that has modest size genomes with 20 wild species and 2 advanced cultigens, known as *Oryza sativa* and *Oryza glammerima* (Chang, 2003). *Oryza sativa*, the Asian cultivated rice, consists of two major subspecies, namely *Indica* and *Japonica*, which are believed to have been spread from China towards the Southeast Asia, particularly Malaysia (Morishima, 2000; Chang, 2003; Subudhi *et al.*, 2006).

Rice genetic resources are important for crop improvement and its sustainable production. Preservation and conservation of genetic resources are therefore useful since genetic diversity provides information to monitor germplasm and prediction of potential genetic gains (Chakravarthi and Naravaneni, 2006). As rice is a staple cereal crop consumed by humans, high degree of similarity between different cereal genomes in terms of gene content and gene order facilitates crop improvement and breeding for other cereal crops as well (Garris *et al*., 2005). Rice genetic resources can be utilized to improve crop performance through

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breeding with objectives of improving rice quality and yield (Bao *et al*., 2000; IRRI, 2007), disease resistance (Brar and Khush, 1997), insect resistance (Sharma *et al*., 2007), and lodging resistance (Yue *et al*., 2005).

Molecular marker technology provides a powerful tool in the assessment of genetic relationships within and among species, in which differences among accessions can be revealed at the DNA level (Ni *et al*., 2002; Chakravarthi and Naravaneni, 2006). Simple sequence repeats (SSRs) or microsatellites are tandemly arranged repeats of short DNA motifs ranging from 1 to 6 base pairs, which exhibit variations in the number of repeats at a locus (Tautz, 1989; McCouch *et al*., 1997; Mackill, 1999; Temnykh *et al*., 2000). The codominant characteristics of the microsatellite markers and their well known map positions on the rice genome reveal high polymorphisms among different plants (Chen *et al*., 1997; Temnykh *et al*., 2000; Garcia *et al*., 2004).

In particular, microsatellite markers have been widely applied in rice genetic studies as they are able to detect high levels of allelic diversity (McCouch *et al*., 1997). The applications of microsatellite markers in rice research include studies on genetic diversity of Yunnan rice germplasm by Tu *et al*. (2007), genetic diversity analysis of traditional and improved Indonesian rice germplasms by Thomson *et al*. (2007), and other general rice genetic diversity studies by Ni *et al*. (2002), Ravi *et al*. (2003) as well as Chakravarthi and Naravaneni (2006).

Sarawak is a rich biodiversity centre, with diverse types of rice that rang from commercial high yielding varieties to indigenous traditional cultivars. However, genetic studies on local rice cultivars in Sarawak were only initiated in 2006, in which three Bario varieties were studied using 50 microsatellite markers for the purpose of establishing Bario certification scheme (Tan *et al*., 2006). More recently, an unpublished report on genotyping of 25 Sarawak rice cultivars, using 12 microsatellite markers suggested microsatellite markers, can be useful in cultivar identification and differentiation among some Sarawak rice cultivars (Valentine, 2008).

As Sarawak is rich in rice biodiversity, the hidden potential of the local rice cultivars is yet to be discovered. Nonetheless, the characterization of genetic information on the rice cultivars in Sarawak is still lacking. Therefore, this study was aimed at identifying the microsatellite marker panels so as to differentiate the Sarawak rice cultivars, analyze genetic diversity, and enlarge their genetic base of these rice cultivars.

MATERIALS AND METHODS

Plant Material and DNA Extraction

A total of 53 rice cultivars listed in Table 1 were collected from the areas shown in *Fig. 1* and evaluated in the present study. Rice seeds were sterilized with 5% Clorox solution (v/v) for 10 minutes, rinsed with distilled water and germinated in Petri-dishes in the laboratory. The germinated seeds, with approximately 1 cm root length, were transplanted into pots and maintained for 8 months. The leaf samples, about 4-5 cm in length from individual rice plants of each cultivar, were collected and immediately frozen in –80 °C. Thirty milligrams samples of each cultivar were weighed, and the DNA extraction was conducted according to the protocol by GF-1 Plant DNA Extraction Kit (Catalog No. GF-PT-50; Vivantis Technologies).

Selection of the Microsatellite Markers

Fifty-four published microsatellite markers from the regional research papers were selected based on the degree of the polymorphism shown. The polymorphic information content (PIC) was compared in order to select potential markers to differentiate the cultivars studied. The details for these markers can be obtained from RiceGenes database (www.gramene.org). The selected primers were synthesized by First BASE Laboratories, Malaysia.

PCR Amplification and Band Detection

The PCR amplification was performed according to the recommended protocol provided by the DNA Amplification Kit (Catalog No. PL1202-

TABLE 1
Rice cultivars

No	Cultivars	Source	Divisions
1	Bajong Wangi	Lubok Nibong (LN)	Betong
2	Biris	DOA Semenggok (D)	Kuching
3	Biris	Stumbin (ST)	Sri Aman
4	Buntal Pulut	Roban (RO)	Betong
5	Kuduk	DOA Semenggok (D)	Kuching
6	Kuning	Betong (B)	Betong
7	MR219	Stumbin (ST)	Sri Aman
8	MR219	Kpg. Bunan, Serian (KBS)	Kuching
9	Rotan	DOA Semenggok (D)	Kuching
10	Rotan	Betong (B)	Betong
11	Sabak	Betong (B)	Betong
12	Suratani	DOA Semenggok (D)	Kuching
13	Tajol	Kpg. Bunan, Serian (KBS)	Kuching
14	Selasih	Tatau (T)	Bintulu
15	Serendah Kuning	UPMKB (U)	Bintulu
16	Bario Halus	UPMKB (U)	Bintulu
17	Adan Sederhana	UPMKB (U)	Bintulu
18	Bukit Wangi	Lubok Nibong (LN)	Betong
19	Buntar-B	DOA Semenggok (D)	Kuching
20	Empawah	Stumbin (ST)	Sri Aman
21	Empawah Merah	DOA Semenggok (D)	Kuching
22	Lasak	DOA Semenggok (D)	Kuching
23	Lemak	Meradong (M)	Sarikei
24	Lemak	Roban (RO)	Betong
25	Mamut	Stumbin (ST)	Sri Aman
26	Mamut-2	Stumbin (ST)	Sri Aman
27	Muyun	Tatau (T)	Bintulu
28	Rotan Wangi	Lubok Nibong (LN)	Betong
29	Sampangan B	DOA Semenggok (D)	Kuching
30	Sia	Lubok Nibong (LN)	Betong
31	Tulang	Tatau (T)	Bintulu
32	Wangi	Stumbin (ST)	Sri Aman
33	Bubuk Wangi	Stumbin (ST)	Sri Aman
34	Silah	Roban (RO)	Betong
35	Silah	Betong (B)	Betong
36	Raden	Meradong (M)	Sarikei
37	Secasan Putih	DOA Semenggok (D)	Kuching
38	Ukong	DOA Semenggok (D)	Kuching
39	ARC-II	DOA Semenggok (D)	Kuching

Table 1 (Continued)

40	Kanowit Merah	DOA Semenggok (D)	Kuching
41	Selimbau Merah	DOA Semenggok (D)	Kuching
42	Selambau Merah	DOA Semenggok (D)	Kuching
43	Lebat	Kpg. Bunan, Serian (KBS)	Kuching
44	Lebat	Lubok Nibong (LN)	Betong
45	Chelum	Betong (B)	Betong
46	Chelum Halus	Stumbin (ST)	Sri Aman
47	Boria	Betong (B)	Betong
48	Hitam	Stumbin (ST)	Sri Aman
49	Palang	Meradong (M)	Sarikei
50	Sebuti	Roban (RO)	Betong
51	Tembakau	Betong (B)	Betong
52	Selasih	Roban (RO)	Betong
53	Kanowit	Tatau (T)	Bintulu

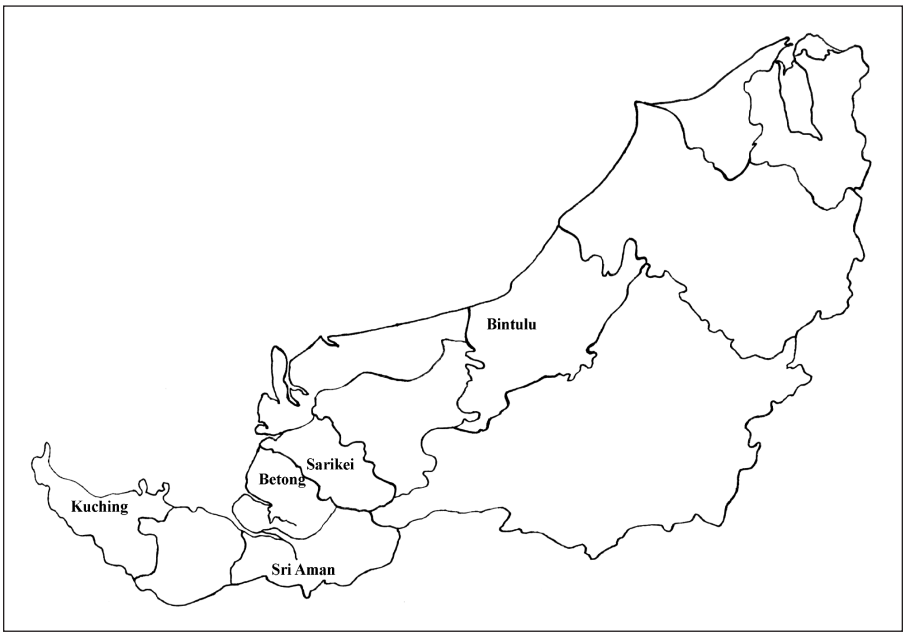

Fig. 1: Sarawak map showing the sampling areas

GF; Vivantis Technologies, Malaysia). An initial screening of the microsatellite markers was carried out in the electrophoresis systems (Model: FB300; Fisher Scientific) using 2.0% of agarose gel (Genetic Analysis Grade; Fisher Scientific) at 100 V for 90 minutes in 0.5x TBE buffer. Later, the PCR-products from the selected microsatellite markers were subjected to band detection using polyacrylamide gel electrophoresis (Mini-PROTEAN 3 Cell; Bio-

Rad Laboratories). Meanwhile, polyacrylamide gel (5%) was prepared according to the procedure described by Sambrook and Russell (2001) and run at 80 V, where the running time was dependent on the size of the PCR products, i.e. from 45 to 60 minutes, for larger products. The gel obtained was stained using ethidium bromide and visualized under UV light (302 nm) using FluorChem 5500 gel imaging system and analyzed by AlphaEaseFC Version 3.3.2 software.

Selection of the Microsatellite Marker Panels

The microsatellite marker panels, with one representative marker from each chromosome, were selected from the initial screening of 54 microsatellite primers. Banding pattern of microsatellite markers was compared to identify polymorphic primers. The microsatellite primers with the highest levels of polymorphism and unambiguous banding patterns were therefore chosen.

Data Analysis

The banding patterns obtained were scored in binary format as present (1) or absent (0) to analyze the genetic relationship between cultivars, as well as the number of alleles per loci to determine the polymorphism information content (PIC) values. The PIC value of each microsatellite marker was calculated using the formula $PIC = 1 - \sum_{j=1}^{n} P_{ij}^2$ where P_{ij} represents frequency of the *j*th allele for the *i*th marker, while n represents the total number of alleles to identify the polymorphic and monomorphic markers. The binary matrix was used to calculate the similarity as DICE coefficient using 'SIMQUAL' sub-routine in NTSYSpc version 2.20r N software package (Rohlf, 2005). The resultant similarity matrix was employed to construct dendrograms using the 'Sequential Agglomerative Hierarchical Nesting' (SAHN) based on the 'Unweighted Pair Group Method with Arithmetic Means' (UPGMA) to infer genetic relationships. Meanwhile, the Mantel matrix correspondence test was carried out using the MXCOMP procedure in NTSYSpc Version 2.20r N to define the degree of congruence in the estimation of genetic relationships by the SSR markers.

RESULTS

SSR Analysis

Fifty-four SSR primer pairs on 12 rice chromosomes were used to genotype the rice cultivars. The results showed that 46 primer pairs provided a clear DNA amplification, while 8 others revealed a low yield PCR amplification which required further optimization of PCR conditions. In more specific, the primers with low yield amplification results were RM514, RM55, RM161, RM334, RM162, RM118, RM171, and RM144. Among the primers used in the initial screening, 36 primer pairs (66.7%) produced polymorphic banding patterns, while the rest of the primers (33.3%) showed monomorphic banding patterns. Examples of these polymorphic banding patterns are shown in *Fig. 2*.

The polymorphic primers identified from 54 primer pairs screened are shown in Table 2. The results obtained show that the majority (69.4%) of the polymorphic primers produced bands which consist of dinucleotide repeat region, while seven primers produced bands having trinucleotide repeats (namely RM452, RM489, RM125, RM152, RM144, RM519, and RM19) and one tetranucleotide (i.e. RM421).

The polymorphic primers, with compound repeat region, were also obtained as shown in RM316, RM228, and RM333. Meanwhile, the large number of the monomorphic amplification products obtained during the screening was unexpected as the PIC values reported in the previous literature were relatively high.

Microsatellite Marker Panel

A total of 43 alleles were amplified from 53 cultivars studied, ranging from 2 to 5 alleles per locus with average of 3.58 alleles per locus (Table 3). The microsatellite panel chosen

TABLE 2
Microsatellite markers for the initial screening of the genetic relationship of 53 Sarawak rice cultivars based on the agrarose gel electrophoresis

Primers	Chromosome	Position	Repeat motifs	Expected product size (bp)	Polymorphism
RM1	1	29.7	$(AG)_{26}$	113	Polymorphic
RM243	1	57.3	$(CT)_{18}$	116	Polymorphic
RM237	1	115.2	$(CT)_{18}$	130	Polymorphic
RM226	1	154.8	$(AT)_{38}$	274	Monomorphic
RM154	2	4.8	$(GA)_{21}$	183	Polymorphic
RM452	2	58.4	$(GTC)_{9}$	209	Polymorphic
RM240	2	158.0	$(CT)_{21}$	132	Polymorphic
RM207	2	191.2	$(CT)_{25}$	118	Polymorphic
RM22	3	13.0	$(GA)_{22}$	194	Polymorphic
RM338	3	108.4	$(CTT)_{6}$	183	Monomorphic
RM55	3	168.2	$(GA)_{17}$	226	Monomorphic
RM514	3	216.4	$(AC)_{12}$	259	Polymorphic
RM489	3	29.2	$(ATA)_{8}$	271	Polymorphic
RM81B	3	77.5	$(TCT)_{10}$	110	Monomorphic
RM307	4	0	$(AT)_{14}(GT)_{21}$	174	Monomorphic
RM335	4	21.5	$(CTT)_{25}$	104	Monomorphic
RM252	4	98.0	$(CT)_{19}$	216	Polymorphic
RM124	4	150.1	$(TC)_{10}$	271	Monomorphic
RM413	5	26.7	(AG)11	79	Polymorphic
RM161	5	96.9	(AG)20	187	Polymorphic
RM421	5	111.2	(AGAT)6	234	Polymorphic
RM334	5	141.8	(CTT)20	182	Monomorphic
RM133	6	0	(CT)8	230	Monomorphic
RM204	6	25.1	(CT)44	169	Polymorphic
RM541	6	75.5	(TC)16	158	Polymorphic
RM162	6	108.3	(AC)20	229	Polymorphic
RM125	7	24.8	(GCT)8	127	Polymorphic
RM11	7	47.0	$(GA)_{17}$	140	Polymorphic
RM118	7	96.9	$(GA)_{8}$	156	Monomorphic
RM138	7	196.8	$(GT)_{14}$	233	Polymorphic
RM152	8	9.4	$(GGC)_{10}$	151	Polymorphic
RM404	8	60.9	$(GA)_{33}$	236	Polymorphic
RM284	8	83.7	$(GA)_{8}$	241	Monomorphic
RM149	8	103.7	$(AT)_{10}$	253	Polymorphic
RM316	9	1.8	$(GT)_{8}$-$(TG)_{9}$ $(TTTG)_{4}$-$(TG)_{4}$	192	Polymorphic
RM105	9	32.1	$(CCT)_{6}$	134	Monomorphic

Table 2 (Continued)

RM278	9	77.5	$(GA)_{17}$	141	Polymorphic
RM215	9	99.4	$(CT)_{16}$	148	Polymorphic
RM474	10	0.0	$(AT)_{13}$	252	Monomorphic
RM271	10	59.4	$(GA)_{15}$	101	Polymorphic
RM171	10	92.8	$(GATG)_5$	328	Monomorphic
RM484	10	97.3	$(AT)_9$	299	Monomorphic
RM216	10	17.6	$(CT)_{18}$	146	Polymorphic
RM228	10	130.3	$(CA)_6(GA)_{36}$	130	Polymorphic
RM333	10	110.4	$(TAT)_{19}$-$(CTT)_{19}$	191	Polymorphic
RM202	11	54.0	$(CT)_{30}$	189	Polymorphic
RM287	11	68.6	$(GA)_{21}$	118	Polymorphic
RM206	11	102.9	$(CT)_{21}$	147	Polymorphic
RM144	11	123.2	$(ATT)_{11}$	237	Polymorphic
RM19	12	20.9	$(ATC)_{10}$	226	Polymorphic
RM277	12	57.2	$(GA)_{11}$	124	Monomorphic
RM463	12	75.5	$(TTAT)_5$	192	Monomorphic
RM519	12	62.6	$(AAG)_8$	122	Polymorphic
RM235	12	101.8	$(CT)_{24}$	124	Monomorphic

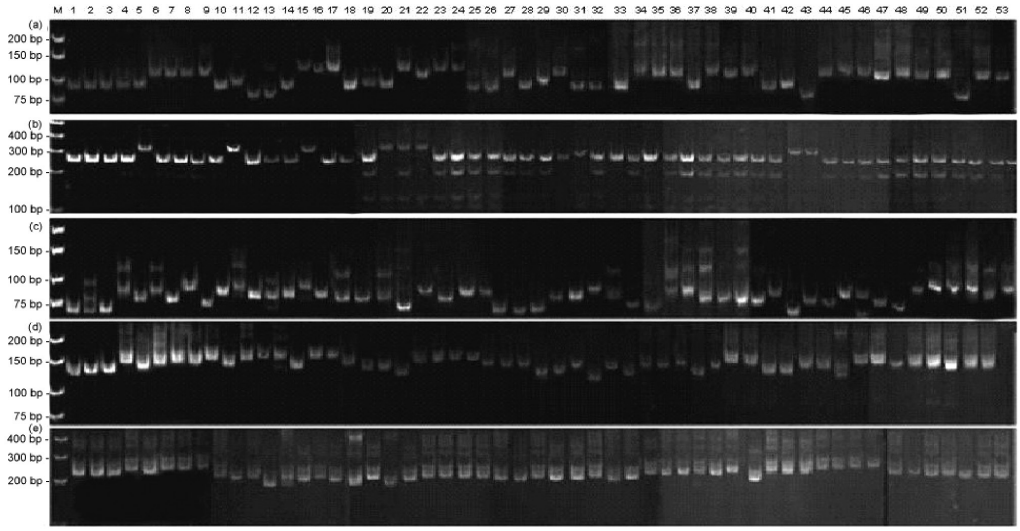

Fig. 2: The microsatellite banding patterns among 53 Sarawak rice cultivars shown by (a) RM1, (b) RM489, (c) RM413, (d) RM11 and (e) RM404 using polyacrylamide gel electrophoresis (PAGE)

showed the PIC values ranging from 0.306 (RM489) to 0.730 (RM413). The average PIC value obtained for the microsatellite panels in this study was 0.622. However, a combination of 12 primers, as the microsatellite marker panels described above, gave an overall PIC value of 0.969, indicating a high polymorphism for cultivar differentiation. Rare alleles were obtained from two markers, namely RM1 and RM404. A rare allele in RM1, as detected with an estimated size of 105 bp, was observed in Sabak and Sampangan B, while a rare allele in RM404 was detected with estimated size of 210 bp, as observed in Suratami and Tajol. Further details on the microsatellite marker panels chosen are shown in Table 3.

Genetic Relationship among Sarawak Rice Cultivars

The UPGMA cluster analysis, based on the genetic similarity values for the SSR alleles, was constructed. The correlation (r) between the similarity index and the cophenetic value was estimated at 0.789, indicating a high level of congruence. The UPGMA dendrogram constructed showed two main groups among the cultivars studied, where 39.62% of the cultivars studied were grouped in cluster A and the remaining 60.38 % were grouped in cluster B (*Fig. 3*).

Cluster A, with the similarity values ranging from 0.3356 to 1.000, consisted of two sub-clusters and one individual cultivar. The cultivar Chelum (B) diverged individually from the other cultivars at 0.3356, and this was followed by the branching into two sub-clusters at 0.4261. The two sub-clusters formed include eight cultivars, which were clustered together in sub-cluster I, while another twelve cultivars fall within sub-cluster II. Bajong Wangi (Lubok Nibong) and Biris (Stumbin) were closely related with a similarity coefficient of 1.00. Two Biris cultivars from different sources (DOA Sarawak and Stumbin) and Mamut and Mamut 2 cultivars were found to be slightly different with a similarity coefficient of 0.750.

TABLE 3
A summary of the information on the microsatellite marker panel chosen based on the polyacrylamide gel electrophoresis (PAGE)

Chromosome	Primers	Annealing temperature (°C)	Estimated alleles range (bp)	No. of multiple allele genotype	No. of rare allele	No. of alleles amplified	PIC
1	RM1	55	85-120	0	1	5	0.689
2	RM240	55	115-135	1	0	2	0.456
3	RM489	55	190-300	0	0	2	0.306
4	RM252	55	120-260	0	0	4	0.639
5	RM413	55	65-85	1	0	4	0.730
6	RM204	55	110-150	2	0	4	0.717
7	RM11	55	130-150	1	0	3	0.645
8	RM404	55	210-230	0	1	5	0.692
9	RM316	55	160-200	3	0	3	0.612
10	RM271	55	90-110	0	0	4	0.726
11	RM206	55	130-170	2	0	4	0.674
12	RM19	55	220-255	2	0	3	0.579
					Total:	43	7.465
					Average:	3.58	0.622

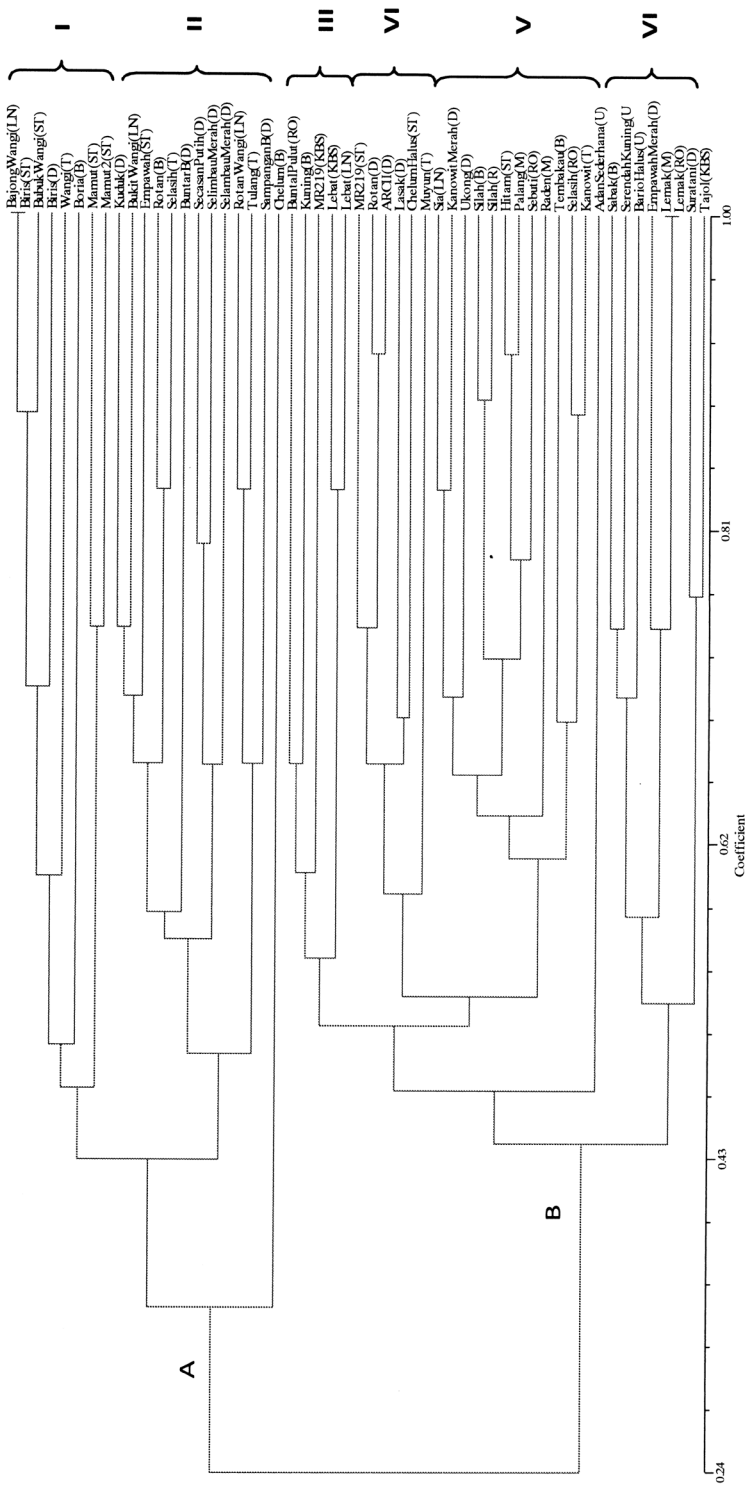

Fig. 3: Dendrogram showing genetic relationships among 53 Sarawak rice cultivars revealed by the cluster analysis based on 12 representative microsatellite markers (UPGMA, NTSYS-PC)

Cluster B contained four main clusters and one individual cultivar. Five cultivars were clustered in sub-cluster III, six cultivars in sub-cluster IV, twelve cultivars in sub-cluster V, followed by one individual cultivar (i.e. *Adan Sederhana*), and finally sub-cluster VI which consisted of eight cultivars. The cultivars with similar names that were found to be closely related were *Lebat, Silah* and *Lemak*, while MR219 and *Rotan* from the two different sources were shown to be different in terms of their genotypes.

DISCUSSION

SSR Analysis

The polymorphic markers obtained did not show any equivalent polymorphism information content as revealed in the previous literature. As for marker RM1, the present study showed a higher PIC value of 0.702 compared to 0.620 reported by Garland *et al.* (1999). Meanwhile, RM19 was reported to be polymorphic with the PIC value of 0.820 (Thomson *et al.*, 2007) but the PIC value of 0.678 was recorded in the present study. Meanwhile, the results for the monomorphic primer pairs were unexpected when compared to the published literature, which showed a relatively high polymorphism. For example, RM226, RM338, RM421, and RM 235 appeared to be monomorphic compared to 5, 6, 6, and 7 amplified alleles, as reported by Sundaram *et al.* (2008). Besides, RM335, RM215, and RM474 have been used by different groups of researchers and appeared to be monomorphic as opposed to studies by Ravi *et al.* (2003), Saini *et al*. (2004), Yuan *et al.* (2007), Sundaram *et al.* (2008), Thomson *et al.* (2007), Tu *et al.* (2007), and Lapitan *et al.* (2007). Generally, the PIC values reported in the literature were above 0.70, indicating high polymorphisms.

The difference in polymorphism shown above could probably be due to the different rice cultivars, as well as the number of cultivars screened. The large numbers of cultivars screened would increase the number of the alleles amplified and contribute to the PIC values. Furthermore, the diversity of the cultivars studied might affect the PIC value that serves as the scale of the polymorphism content. In the study conducted by Tu *et al.* (2007), their sample size covered 60 rice varieties from Yunnan province, with diverse geographical regions. Meanwhile, Coburn *et al.* (2002) reported the samples that covered two subspecies of rice, namely *Oryza sativa* ssp. *Indica* and *Japonica,* which might contribute to the PIC values of the markers as there were obvious variations in the varieties. Therefore, the relatively low PIC values in individual markers could be explained by the Sarawak cultivars studied being closely related and the small sample size.

The Microsatellite Marker Panels

The 12 selected marker panels in the present study showed an average of 3.58 alleles per locus and the average PIC value of 0.622. As compared to the previous studies (i.e. with the average number of alleles ranging from 4.86 to 13 alleles), the average number of the alleles obtained in this study was relatively low (Ni *et al.*, 2002; Saker *et al.*, 2005; Thomson *et al.*, 2007; Jayamani *et al.*, 2007; Sundaram *et al.*, 2008). The average PIC values obtained was in agreement to the average PIC values ranging from 0.62 to 0.68 by Ni *et al.* (2002), Akkaya and Buyukunal-Bal (2004), Thomson *et al.* (2007) and Jayamani *et al.* (2007), but was lower compared to those by Sundaram *et al.* (2008) who reported an average PIC value of 0.707.

The differences observed were expected as different loci and different rice varieties were used in the study. Meanwhile, the different primers used for the different loci screening contributed to the variation of the average number of alleles, as shown by Sundaram *et al.* (2008) who studied 35 rice genotypes with 25 microsatellite markers, Thomson *et al.* (2007) who studied 330 rice accessions with 30 microsatellite markers, and Ni *et al.* (2002) who investigated 38 cultivars with 111 microsatellite markers. As previously reported, a relatively higher number of loci screened and different primers used did contribute to the variation.

Although a relatively smaller sample size and fewer primers were used in the present study as compared to the previous studies, the selected markers were able to show an equivalent polymorphism content as reported in the earlier studies.

The microsatellite panel chosen consisted of perfect repeat motifs of dinucleotides and trinucleotides, except for RM316 which possessed a compound repeat motif. As a direct correlation was often observed between the number of perfect repeats and the level of polymorphism exhibited by PCR amplification, the marker panels chosen were observed to possess higher number of repeats compared to the other primers at the initial screening. This was also observed by Saghai-Maroof *et al.* (1994) and Russell *et al.* (1997) in their studies on barley genetics. For example, RM204 with the repeat motif of $(CT)_{44}$ having the highest number of perfect repeats, was more polymorphic compared to RM133 with the repeat motif of $(CT)_8$, RM541 with the repeat motif of $(TC)_{16}$ or RM162 with the repeat motif of $(AC)_{20}$ on chromosome 6. However, not all the loci in the selected microsatellite marker panel possessed the highest number of perfect repeats, while some primers required further optimization of PCR conditions.

The polymorphic primers producing clear amplification bands were selected to construct a microsatellite marker panel for use in further analysis. The number of alleles amplified was indirectly related to the polymorphism level of the marker. Luan *et al.* (2007) suggested that an effective allele number be assessed to estimate the level of genetic variation and select the markers with a relatively high number of alleles amplified. Besides, large allele size differences, which facilitate detection of alleles on gel, are necessary to obtain reproducible polymorphic banding patterns (Akkaya and Buyukunal-Bal, 2004). On the contrary, the primers that gave low intensities of amplification products and complicated banding patterns with stutter or contaminating bands were avoided. Optimization is required for these primers but it is rather laborious as alterations in the PCR conditions may only reduce but not necessarily eliminate the problems (Dograr and Akkaya, 2001; Akkaya and Buyukunal-Bal, 2004; Olejniczak and Krzyzosiak, 2006).

Genetic Relationships among the Sarawak Rice Cultivars

The clustering of the cultivars showed Mantel correspondence coefficient of 0.79 compared to Ravi *et al.* (2003) and Jayamani *et al.* (2007) who reported the values of 0.53 and 0.87, respectively. The findings of the present study showed that the information from the SSR analysis with the selected microsatellite panels was well conserved at 79% in this cluster analysis. Meanwhile, some cultivars with similar names but from different sources were found to be genetically different as shown by the cultivars *Rotan* and *MR219*. This might be attributed to geographic conditions or local traditional practices. As noted by Bajracharya *et al.* (2006), landraces displayed variability for the trait they gained their name from, but farmers did not maintain strict standards of landrace purity. Meanwhile, according to Brondani *et al.* (2006), any variety could, after years of successive cultivation, generate populations with a genetic constitution different from the original genotype due to adaptations to different environments. Variations in the geographic and climate conditions, diverse farming practices and diversified utilization of rice may also result in the loss of identity of the varieties grown (Tu *et al.*, 2007).

CONCLUSIONS

In the present study, a microsatellite marker panel with 12 polymorphic markers was identified, producing an overall PIC value of 0.969. The allelic diversity, determined by the microsatellite marker panel, had enabled the detection of differences among the Sarawak rice cultivars studied. However, the microsatellite marker panel could still be improved upon through further screening of polymorphic markers to enhance its discrimination power. The results

obtained in this study have provided information for the future development of a genetic database of the Sarawak rice cultivars.

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