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Genetic Diversity of Malaysian Rice Landraces Based on Single Nucleotide Polymorphism (SNP) Markers

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ABSTRACT

A collection of 42 Malaysian rice cultivars which consists of 40 traditional rice varieties and two Malaysian elite varieties were genotyped using a set of 96 SNP markers to understand their diversity levels. Forty of the traditional rice cultivars originated from Peninsular Malaysia, Sabah and Sarawak. Out of 96 SNPs genotyped, only 67 SNPs were used for statistical analysis since the rest of the markers were either monomorphic or could not amplify most of the samples. The polymorphism information content (PIC) values ranged from 0.0232 to 0.3748 with an average of 0.1496. The gene diversity and heterozygosity values ranged from 0.0235 to 0.4997 and 0.0000 to 0.1429 with an average of 0.1703 and 0.0362, respectively. These low values of gene diversity and heterozygosity suggested low genetic diversity in the studied collections. The UPGMA dendogram showed the cultivar collections could be clustered into two major groups. The results were further supported by principal co-ordinate analysis (PCoA). The results from this study allow the characterization of germplasm collections which can aid in better genetic conservation management and selection of parental lines for breeding programs.

***Key words**: Oryza sativa L., SNP marker, Genetic diversity, Malaysian rice landraces*

INTRODUCTION

Rice which belongs to the Poaceae family is one of the most important food crops in the world. One third of the world population consumed rice as their main source of energy. Current statistics released by FAO showed stagnant rice production in Malaysia since 2010 with an average of 2.5 million tonnes per year[1]. Landrace rice has only a small contribution in the total production compared to elite cultivated varieties since their yield is low. Albeit of little contribution to the total rice production, landrace rice present can be a major food source in a certain region or area. Besides, landrace rice maintained by small farmers enriches the rice gene pool in terms of genetic diversity.

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Previous researchers believed that the landrace rice contains useful genes[2,3]. Beside, researchers also have identified a broad spectrum of blast resistant gene from japonica rice landraces[4].

Genetic diversity reflects the amount of genetic variability amongst individuals or populations within a variety or species. Most researchers believed *Oryza rupifogon* is the ancestor of Asian cultivated rice[5-7]. Genetic variability measures the tendency of individuals to vary from one another based on morphometrics or molecular markers. Molecular markers provide the best approach in estimating the genetic diversity in plant germplasms since genotypic data are more reliable and consistent compared to morphometric data which could be influenced by the environment. The advancement of molecular marker technologies has significantly revolutionized marker utilization. Nowadays, most researchers assessed genetic diversity using microsatellite markers as compared to a decade ago when the utilization of Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) markers was more prevalent. However, recently researchers assessed genetic diversity using SNP markers[8-10].

In this study, SNP markers were used to assess the genetic diversity of 40 Malaysian rice and two elite rice varieties. SNP are favourable markers since they are biallellic, codominant in nature, abundant throughout the genome and amenable to high-througput genotyping[11]. Besides, there is no null allele and stutter band incidence which occur in microsatellite data that may lead to inaccurate assessment of the genetic diversity. This is the first report on the diversity of Malaysian rice landraces using SNP markers. The findings of this research will greatly assist in managing the genetic resources of Malaysian landrace cultivars.

MATERIALS AND METHODS

Plant materials

The plant samples used in this study consisted of 40 traditional rice cultivars and two Malaysian elite cultivars namely MR219 and MR269. Nine of the traditional rice cultivars originated from Peninsular Malaysia while the rest were from Sabah and Sarawak. Details about the plant materials are summarized in Table 1. All the plant materials were obtained from the MARDI Rice Genebank, Seberang Perai.

Table 1. List of the 42 Malaysian rice cultivars used in this study and their origins

No	Variety	Origin	Accession Number*
1	MR219	Malaysia Elite	MRGB11633
2	MR269	Malaysia Elite	MRGB12120
3	Chempa Padi Huma	Malay Peninsula	MRGB05080
4	Siong Pelandok	Malay Peninsula	5101
5	Putih Huma	Malay Peninsula	6049
6	Chendana Wangi	Malay Peninsula	Acc-6288
7	Bongkok	Malay Peninsula	Acc-5105
8	Santap Wangi	Malay Peninsula	Acc-6292
9	Mayang Lega	Malay Peninsula	Acc-6004
10	Kuku Belang	Malay Peninsula	Acc-6674
11	Rambut	Malay Peninsula	Acc-7129
12	Kambaring	Sabah (Borneo)	Acc-7529
13	Nangka	Sabah (Borneo)	Acc-7516
14	Putus Tunang	Sabah (Borneo)	Acc-7540
15	Beliong	Sabah (Borneo)	Acc-7508
16	Semilai	Sabah (Borneo)	Acc7543
17	Pagalan	Sabah (Borneo)	Acc-7560
18	Kungkuling	Sabah (Borneo)	Acc-7565
19	Kedinga	Sabah (Borneo)	Acc-7583
20	Turayan	Sabah (Borneo)	Acc-7571
21	Padi Kolomintuhon	Sabah (Borneo)	Acc-9866
22	Dihangkang Putih	Sabah (Borneo)	Acc-9894
23	Tahi Ayam	Sabah (Borneo)	Acc-9962

24	Padi Solung	Sabah (Borneo)	Acc-9930
25	Padi Emas	Sabah (Borneo)	Acc9963
26	Gebokong	Sabah (Borneo)	Acc-9959
27	Padi Porak	Sabah (Borneo)	Acc-9965
28	Padi Susiah	Sabah (Borneo)	Acc-9968
29	Padi Mansud	Sabah (Borneo)	Acc-9954
30	Padi Beruang	Sabah (Borneo)	Acc-9956
31	Padi Adong	Sabah (Borneo)	Acc-10001
32	Padi Tiga Bulan	Sabah (Borneo)	Acc-9958
33	Padi Tubowan	Sabah (Borneo)	Acc-10003
34	Padi Pengalaan	Sabah (Borneo)	Acc-9971
35	Padi Lasui	Sabah (Borneo)	Acc-10006
36	Liwagu Antap	Sabah (Borneo)	Acc-9993
37	Mansau	Sarawak (Borneo)	Acc-3369
38	Biris	Sarawak (Borneo)	Acc-6891
39	Padi Wangi	Sarawak (Borneo)	Acc-6893
40	Padi Cigarette	Sarawak (Borneo)	Acc-11816
41	Chelom	Sarawak (Borneo)	Acc-7155
42	Alek	Sarawak (Borneo)	Acc-9037

*MARDI accession number

Genomic DNA extraction and DNA quality control

Genomic DNA was extracted using a previous developed protocol[12] with some modification in term of lysis buffer ingredients. Approximately 1 gram of each cultivar's leaves was ground using TissueLyser (Qiagen, Netherlands).The ground samples were incubated at 65°C for 1 hour using 600 µL extraction buffer (2% PVP, 4 mM DIECA, 5mM Ascorbic acid, 1.4 M NaCL, 100 mM Tris-HCL(pH 8.0) and 20 mM EDTA). The DNA of the samples was precipitated using an equal volume of cold isopropanol before washing with 70% ethanol. The DNA pellet was air-dried and resuspended in 50 µl TE (Tris-EDTA) buffer. The DNA was quantified for DNA concentration using Thermo Labsystems Fluoroskan Ascent ™ (Thermo Scientific, USA) and DNA integrity was observed on 0.8% agarose gel.

SNP selection and genotyping

SNPs were mined from three rice genomes; i) Nipponbare, a japonica variety which was sequenced by the International Rice Genome Sequencing Program. ii) Variety '93-11' which was sequenced by the Beijing Genome Institute and iii) Genome of variety 'Indica 1' which was sequenced by the DNA Landmark Inc. A total 96 SNPs which distributed across all twelve chromosomes in rice were selected for this study.

The samples were genotyped using the Illumina Golden Gate Assay followed the standard manufacture's protocol. The assay used high-density BeadArray technology with the combination of extension and adapter ligation of allele specific primer, amplification using universal primer (tagged with allele-specific fluorescent label in their 5'end) and hybridisation of amplified products on a custom bead chip (Illumina). The fluorescence signal from the hybridised products was captured using the Illumina iScan (Illumina). Genome Studio software was used to score the genotype calling of the SNPs.

Statistical data analysis

The major allele frequency, gene diversity, heterozygosity and polymorphic information content (PIC) for each locus were calculated with the assistance of the Power Marker 3.5 software[13]. In addition, genetic distances across the genotypes were calculated using the Power Marker 3.5. The NTSYS software[14] was used to generate a dendogram using the UPGMA (Unweighted pair group method) method based on simple matching (SM) coefficients. Genetic structure of the germplasm collection was analysed by performing principal component analysis (PCoA) implemented in the GenAlex 6.41[15], based on the distance-standardized of the genetic distance calculated for codominant markers.

RESULTS AND DISCUSSIONS

SNP markers characterization

A total of 96 SNPs were used to evaluate the genetic diversity of the Malaysian rice landraces. Out of the 96 SNPs genotyped, only 67 SNPs showed successful amplifications for at least 95% of the total samples and showed polymorphisms across the cultivars. Eleven SNPs (OsSNP2990, OsSNP2854, OsSNP0612, OsSNP0355, OsSNP2270, OsSNP2270, OsSNP0645, OsSNP0378, OsSNP0231, OsSNP1066 and OsSNP2914) were monomorphic and 18 SNPs either failed to amplify or produced inconsistent result. The data analyses based on 67 polymorphic SNPs are presented in Table 2. The polymorphism information content (PIC) values ranged from 0.0232 (OsSNP0935, OsSNP1456 and OsSNP2771) to 0.3748 (OsSNP1803) with an average of 0.1496. Low PIC values were detected because the bi-allelic character of SNPs allowed only 0.5 as the maximum PIC value. The average value of PIC was considered low compared to previous studies by using SNP markers[16,17].The PIC value could be influenced by many factor such as breeding practices and activities, sensitivity of genotyping protocols, diversity in selected germplasms and the location of the markers (coding vs non-coding regions). Major allele frequencies ranged from 0.5125 (OsSNP1803) to 0.9881 (OsSNP0935, OsSNP1456 and OsSNP2771). A total of 70% of SNP markers showed major allele frequencies of above 0.9000. The gene diversity and heterozygosity values ranged from 0.0235 to 0.4997 and 0.0000 to 0.1429 with averages of 0.1703 and 0.0362 respectively. The low value of gene diversity and heterozygosities showed the low genetic diversity in the landraces rice studied.

Table 2. Summary of Characterization of 67 polymorphic SNP markers

No	SNP ID	Chr	Major Allele Frequency	Gene Diversity	Heterozygosity	PIC
1	OsSNP0143	1	0.9500	0.0950	0.0500	0.0905
2	OsSNP0158	1	0.9634	0.0705	0.0244	0.0680
3	OsSNP0181	1	0.9762	0.0465	0.0000	0.0454
4	OsSNP0283	1	0.9146	0.1562	0.0244	0.1440
5	OsSNP0556	2	0.9167	0.1528	0.0238	0.1411
6	OsSNP0561	2	0.9250	0.1388	0.0000	0.1291
7	OsSNP0571	2	0.9146	0.1562	0.0244	0.1440
8	OsSNP0670	2	0.9286	0.1327	0.0476	0.1239
9	OsSNP0681	2	0.9286	0.1327	0.0476	0.1239
10	OsSNP0698	2	0.9048	0.1723	0.0476	0.1575
11	OsSNP0700	2	0.9167	0.1528	0.0238	0.1411
12	OsSNP0715	3	0.9405	0.1120	0.0238	0.1057
13	OsSNP0935	3	0.9881	0.0235	0.0238	0.0232
14	OsSNP0974	3	0.9146	0.1562	0.0244	0.1440
15	OsSNP1002	3	0.9167	0.1528	0.0238	0.1411
16	OsSNP1014	3	0.9405	0.1120	0.0238	0.1057
17	OsSNP1043	4	0.6071	0.4770	0.0714	0.3633
18	OsSNP1105	4	0.8625	0.2372	0.0250	0.2091
19	OsSNP1161	4	0.8929	0.1913	0.0714	0.1730
20	OsSNP1163	4	0.5625	0.4922	0.1250	0.3711
21	OsSNP1191	4	0.9405	0.1120	0.0238	0.1057
22	OsSNP1401	5	0.9286	0.1327	0.0000	0.1239
23	OsSNP1456	5	0.9881	0.0235	0.0238	0.0232
24	OsSNP1487	5	0.9390	0.1145	0.0244	0.1080
25	OsSNP1507	5	0.9167	0.1528	0.0238	0.1411
26	OsSNP1555	6	0.9405	0.1120	0.0238	0.1057
27	OsSNP1571	6	0.9512	0.0928	0.0000	0.0885
28	OsSNP1714	6	0.9405	0.1120	0.0238	0.1057

29	OsSNP1715	6	0.9512	0.0928	0.0000	0.0885
30	OsSNP1728	6	0.9524	0.0907	0.0000	0.0866
31	OsSNP1729	6	0.9524	0.0907	0.0000	0.0866
32	OsSNP1736	6	0.6190	0.4717	0.0952	0.3604
33	OsSNP1803	7	0.5125	0.4997	0.0750	0.3748
34	OsSNP1811	7	0.7381	0.3866	0.1429	0.3119
35	OsSNP1829	7	0.9512	0.0928	0.0000	0.0885
36	OsSNP1831	7	0.9048	0.1723	0.0476	0.1575
37	OsSNP1843	7	0.8929	0.1913	0.0714	0.1730
38	OsSNP1844	7	0.9048	0.1723	0.0476	0.1575
39	OsSNP1957	7	0.9167	0.1528	0.0238	0.1411
40	OsSNP2006	7	0.9762	0.0465	0.0000	0.0454
41	OsSNP2032	8	0.8929	0.1913	0.0714	0.1730
42	OsSNP2063	8	0.9524	0.0907	0.0000	0.0866
43	OsSNP2066	8	0.9405	0.1120	0.0238	0.1057
44	OsSNP2081	8	0.7976	0.3228	0.0714	0.2707
45	OsSNP2096	8	0.8095	0.3084	0.0476	0.2608
46	OsSNP2207	8	0.8780	0.2142	0.0488	0.1912
47	OsSNP2245	8	0.9524	0.0907	0.0000	0.0866
48	OsSNP2268	8	0.8810	0.2098	0.0476	0.1878
49	OsSNP2326	9	0.7500	0.3750	0.0714	0.3047
50	OsSNP2328	9	0.8929	0.1913	0.0714	0.1730
51	OsSNP2365	9	0.8929	0.1913	0.0714	0.1730
52	OsSNP2410	9	0.9634	0.0705	0.0244	0.0680
53	OsSNP2414	9	0.9500	0.0950	0.0000	0.0905
54	OsSNP2416	9	0.9024	0.1761	0.0488	0.1606
55	OsSNP2438	9	0.9762	0.0465	0.0000	0.0454
56	OsSNP2485	10	0.9167	0.1528	0.0238	0.1411
57	OsSNP2494	10	0.7857	0.3367	0.1429	0.2800
58	OsSNP2549	10	0.8929	0.1913	0.0238	0.1730
59	OsSNP2658	10	0.9268	0.1356	0.0000	0.1264
60	OsSNP2694	11	0.9286	0.1327	0.0000	0.1239
61	OsSNP2704	11	0.8929	0.1913	0.0238	0.1730
62	OsSNP2717	11	0.9524	0.0907	0.0000	0.0866
63	OsSNP2771	11	0.9881	0.0235	0.0238	0.0232
64	OsSNP2789	11	0.7738	0.3501	0.1190	0.2888
65	OsSNP2837	12	0.9167	0.1528	0.0238	0.1411
66	OsSNP2843	12	0.9405	0.1120	0.0238	0.1057
67	OsSNP2978	12	0.9000	0.1800	0.0500	0.1638
	Mean		**0.8945**	**0.1703**	**0.0362**	**0.1496**

Genetic diversity analysis

The genotypic data of 67 SNPs across 42 rice cultivars were analyzed to obtain the genetic distance used to generate dendogram using the UPGMA (Unweighted pair group method) method. The clustering was done using simple matching (SM) coefficients. In the unrooted dendogram (Figure 1), the cultivars were clustered into two major groups. Group 1 consisted of Malaysia elite varieties, MR219, MR269 and one traditional cultivar namely Chendana Wangi which originated from Peninsular Malaysia while, the remaining cultivars were clustered in group 2. The PCoA analysis divided each major group into two subgroups namely 1A, 1B, 2A and 2B. Subgroup 1A consisted of Elite varieties, MR219 and MR269 whereas subgroup 1B consisted of Chendana Wangi. Subgroup 2A consisted of traditional rice cultivars which originated from Peninsular Malaysia (excluding Chendana Wangi) while subgroup 2B consists of traditional rice cultivars that originated from Sabah and Sarawak.

Fig. 1: Unrooted dendogram of 42 Malaysian rice cultivars using UPGMA (Unweighted pair group method) method based on simple matching (SM) coefficient

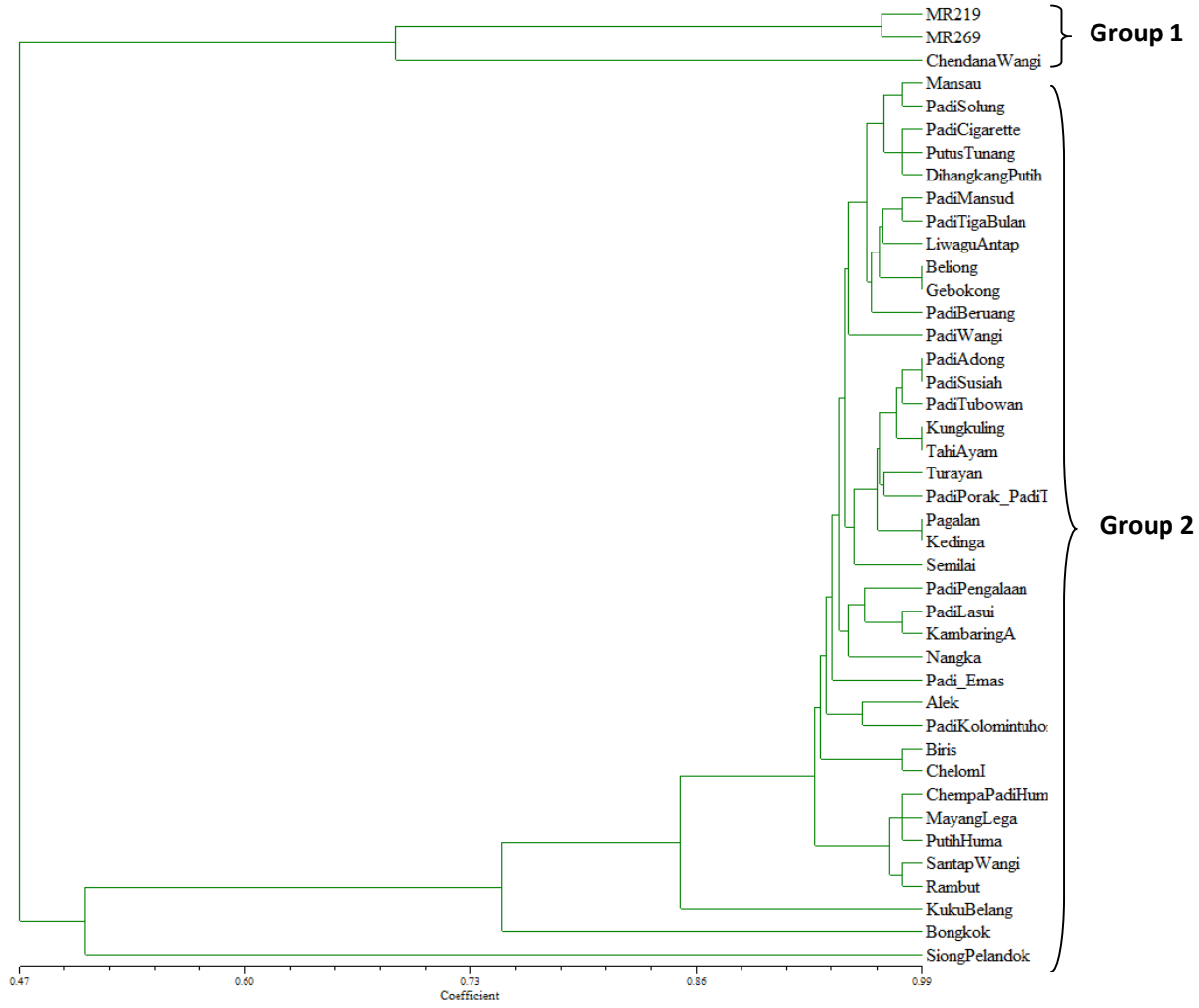
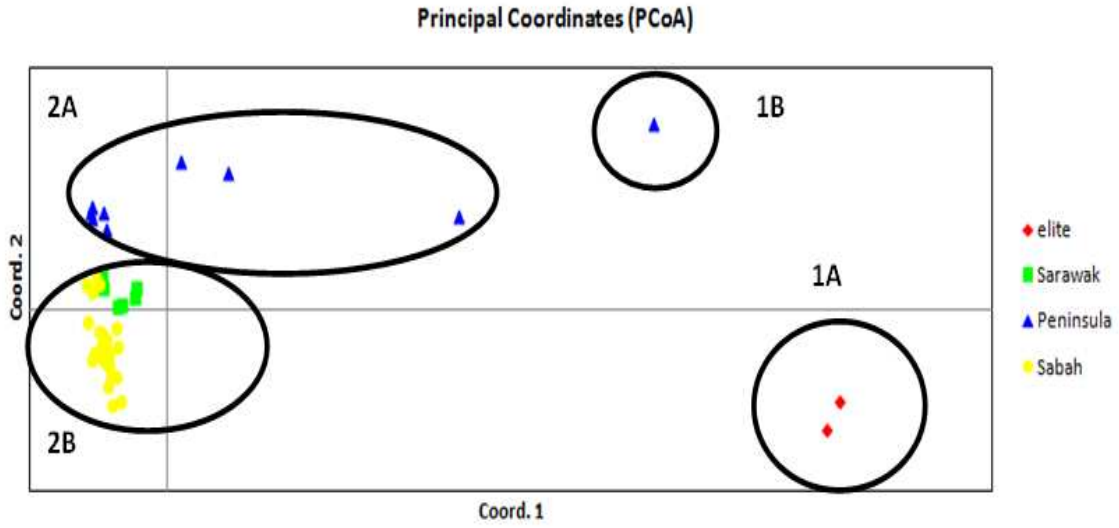


Fig. 2: Principal coordinates analysis (PCoA) of 42 Malaysian rice cultivars using 67 polymorphic SNP markers



CONCLUSION

Assessing the genetic diversity present in a crop of interest is a prerequisite step for understanding the unfavourable evolutionary patterns and for developing conservation strategies. The present study based on molecular analysis of selected Malaysian rice landrace cultivars showed low genetic diversity levels within the traditional rice cultivars. However, high genetic diversity was shown between Malaysian traditional rice cultivars and Malaysian elite cultivars. The findings of this research will be very useful for monitoring the diversity loss over time, thus leading to a better genetic resource management of rice germplasms in Malaysia.

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