

Genetic divergence of Malaysian upland rices revealed by microsatellite markers

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Abstract

Molecular markers are useful tools for evaluating genetic diversity and determining cultivar identity. A total of 50 upland rice accessions, 24 from Peninsular Malaysia and 26 from West Malaysia (Sabah state) were investigated using 10 microsatellite (SSR) markers distributed across the rice genome to study the genetic diversity. A total of 49 alleles were detected across the 50 accessions. The number of alleles per locus ranged from 2 to 11 with an average of 4.9. The average Polymorphism Information Content (PIC) value was 0.710. A dendrogram was constructed using Jaccard's similarity coefficient, and accessions were clustered into 7 groups. The most of the accessions were clustered according to their geographical origin. Shannon's information index ranged from 0.5269 to 2.0050. Nei's gene diversity (h) ranged from 0.3432 to 0.8273. Overall gene flow was 0.0011. In order to develop suitable upland rice varieties, accessions 03838, 03835, 07537, 07538, 03826, 07574, 07588, 07585, 07540, 07575, 07541, 07543, 07544, 07576, 07571, 07539, 03825, 03830 could be used as parents for future hybridization breeding program in Malaysian environment.

Keywords: Molecular marker, microsatellite, genetic diversity, upland rice.

Abbreviations: PCA- Principal Component analysis; MARDI- Malaysian Agricultural Research and Development Institute; RFLP- Restriction Fragment Length Polymorphism; RAPD- Random Amplified Polymorphic DNA; AFLP- Amplified Fragment Length Polymorphism; UPGMA- Unweighted Pair Group Method with Arithmetic Mean.

Introduction

Rice (*Oryza sativa*) is one of the most important food crops for human compared to other cereals. Around 3 billion people in the world use rice as a basic food, which can provide 50 to 80 % of their daily calorie. Rice is also cultivated more than 150 million hectares and production is around 600 million tons in the world yearly (Delseny et al., 2001; Guimaraes, 2009; Tyagi et al., 2004).

Rice can grow in different geographical conditions include tropical and subtropical countries (Normile 1997; Ram et al., 2007). More than half of global rice is cultivated and consumed in Asia such as China, India, and Indonesia. These countries also produce high yield of rice (Chakravarthi and Naravaneni, 2009; Hossain, 2007).

There are different types of rice such as deep-water rice, irrigated rice, rain-fed lowland rice, and upland rice (Poehlman and Sleper, 1995). The 11% of global rice production belongs to upland rice, which is cultivated around 14 million hectares of land. As it is evident, upland rice has a small role in total production but it is major food in some tropical countries (Kondo et al., 2003). Three countries such as Bangladesh, Indonesia, and Philippines are the largest area for planting of upland rice; however, the yield is very low, with an average of about one t/ha, and highly variable (Thanh et al., 1999). In Malaysia upland rice is cultivated in Sabah and Sarawak. The yield average of upland rice in Malaysia ranges from 0.46 to 1.1 t/ha. The total national rice production is roughly 2.24 million metric tons in 2005 (Sohrabi et al., 2012).

Malaysian upland rice accessions were evaluated for 12 growth traits, yield and yield components. All of the studied traits were significantly differed among the accessions. High heritability along with high genetic advance was registered for yield of plant, days to flowering, and flag leaf length-to-width ratio, suggesting preponderance of additive gene action in the gene expression of these characters. According to UPGMA cluster analysis, all accessions were clustered into six groups. Twelve morphological traits provided around 77% of total variation among the accessions (Sohrabi et al., 2012).

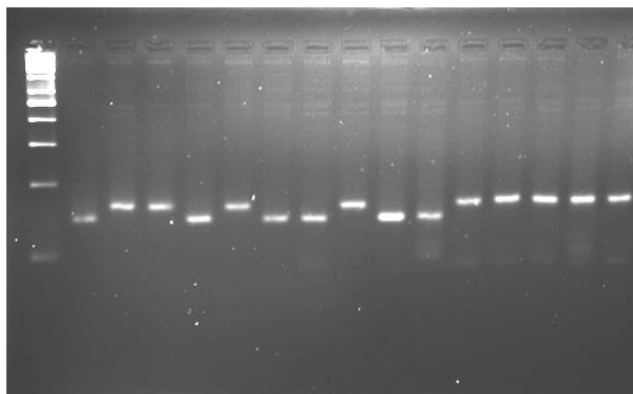
One step for successful breeding programs depends on genetic diversity of the genotypes. Using the information of genetic diversity can devise the best strategies for logical utilization of genetic resources within and among closely related crop varieties. The analysis of genetic diversity within and among varieties is important for breeders because it can help to assess the variation in the germplasm and also can predict potential genetic gains (Chakravarthi and Naravaneni, 2009).

Genetic diversity is mainly evaluated by measurements of physiological and morphological difference of quantitative and economically important traits but these methods have some disadvantages such as cost of time and labor during the measurement. This methods cannot define the exact levels of genetic diversity in germplasm because the traits normally appear by interaction of genes and environment (Schulman, 2007; Zeng et al., 2004). Always the gene expression is

Table 1. The SSR polymorphisms of ten used markers in 50 upland rice accessions in two states of Malaysia.

SSR markers	Size range (bp)	Number of alleles	Expected heterozygosity	Observed heterozygosity	PIC
RM108	74 – 90	4	0.7412	0.0000	0.7017
RM 164	244 - 294	5	0.7903	0.0000	0.7669
RM 166	305 - 441	4	0.7182	0.0200	0.6961
RM 224	123 - 175	6	0.8090	0.0000	0.8074
RM 229	105 - 131	4	0.6956	0.0400	0.7011
RM 249	123- 132	2	0.3467	0.0000	0.3747
RM 250	155 - 190	5	0.7628	0.0000	0.7667
RM 257	105 - 246	11	0.8360	0.0000	0.8848
RM 317	146 - 166	4	0.6980	0.0000	0.7025
RM 437	258 - 322	4	0.6594	0.0000	0.7012

PIC- Polymorphic information contents.

**Fig 1.** SSR banding pattern in 50 accessions of Malaysian upland rice accessions amplified by primer RM108.

affected by environment, so, selection based on morphological traits is selective (Asif and Zafar, 2005; Astarini et al., 2004; Kumar et al., 1998).

Molecular markers are powerful tools for analyzing genetic diversity within and among varieties. They are important tools for basic and applied research. There are different molecular markers which are based on polymorphism of protein or DNA (Schnable et al., 2009). Molecular markers that can show differences between accessions at DNA level are more direct, reliable, and also are the benefit tools for germplasm protection and management.

Several types of molecular markers are available for evaluation of genetic diversity such as RFLP, RAPD, AFLP, SSRs. In rice, molecular marker have been used to identify accessions (Olufowote et al., 1997; Virk et al., 1995), to determine the genetic structure and pattern of diversity for cultivars of interest (Akagi et al., 1997; Mackill, 1995; Yang et al., 1994; Zhang et al., 1992), to optimize the assembly of core collections and discovery of QTLs (Kishima et al., 2005).

Among various PCR-based markers, microsatellites (SSRs) are more suitable and effectively used for assessing genetic diversity among closely-related rice cultivars compared to other molecular markers; because it can be easily amplified by PCR reaction, highly informative, mono locus, co-dominant, easily analyzed, and it can detect higher degree polymorphism in rice. Therefore, the present study was undertaken to know the genetic diversity among the accessions of upland rice using SSR markers. Based on our knowledge, the studies on genetic diversity of upland rice using molecular markers are inadequate in Malaysia.

In this study we are trying to evaluate the genetic diversity of Malaysian upland rice cultivars using SSR markers.

Results

Polymorphism of SSR markers

Out of 23 tested microsatellite markers, 10 were successfully used to assess the extent of genetic diversity across the 50 upland rice genotypes. The successful primers exhibited strong polymorphism among the genotypes. A total of 49 alleles were recorded and number of alleles per locus ranged from 2 for RM249 to 11 for RM257 with an average of 4.9. The smallest size of allele, belonged to RM108, ranged from 74 to 90 bp and the largest size was observed in RM166, which ranged from 305 to 441 bp (Table 1). The polymorphism are shown among the accessions in primer RM108 (Fig 1) and RM224 (Fig 2).

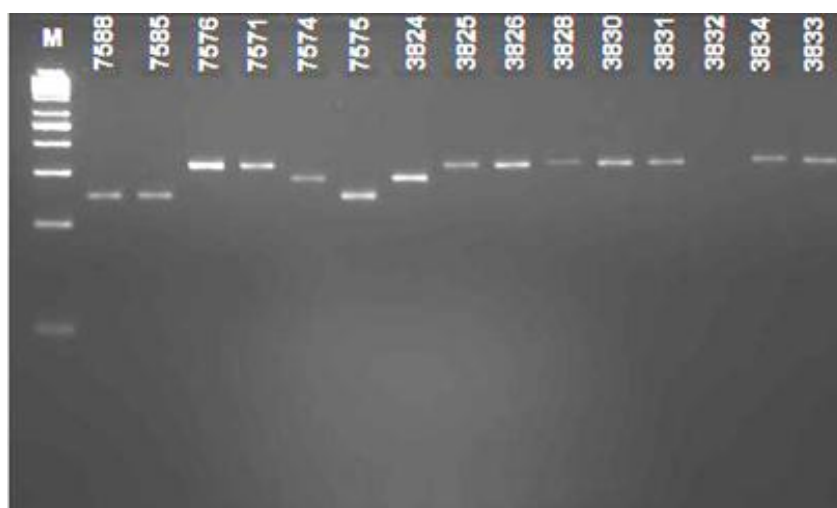
Genetic diversity among markers and states

The expected heterozygosity ranged from 0.3467 (RM249) to 0.8360 (RM257) with an average of 0.7057. But observed heterozygosity was zero for all markers except RM166 (0.0200) and RM229 (0.0400). The PIC values ranged from 0.3747 (RM249) to 0.8848 (RM257) (Table 1).

The highest PIC value was recorded for RM257 followed by RM224, RM164 and RM250 markers. The G_{ST} and gene flow (N_m) were 0.9957 and 0.0011, respectively (Suppl. Table 1). The effective number of alleles (n_e) ranged from 1.6000 (RM249) to 4.6452 (RM250) with the average of 2.8900 for Peninsular Malaysia, while the effective number of alleles (n_e) ranged from 1.4506 (RM249) to 6.0000 (RM257) with an average of 2.9785 for Sabah. The Nei's gene diversity (h) ranged from 0.3750 (RM249) to 0.7847

Table 2. Genetic diversity of 50 Malaysian upland rice accessions among 10 markers for Peninsular Malaysia and Sabah.

Markers	Effective number of alleles	Nei's gene diversity	Shannon's index
Peninsular Malaysia			
RM108	3.2267	0.6901	1.2271
RM 164	2.7170	0.6319	1.1177
RM 166	2.3607	0.5764	0.9800
RM 224	3.1429	0.6818	1.2129
RM 229	1.7916	0.4418	0.8706
RM 249	1.6000	0.3750	0.5623
RM 250	4.6452	0.7847	1.5715
RM 257	3.8919	0.7431	1.7466
RM 317	2.5556	0.6087	1.1323
RM 437	2.9691	0.6632	1.2319
Mean	2.8900	0.6197	1.1653
Sabah			
RM108	2.8403	0.6479	1.0695
RM 164	3.1589	0.6834	1.2409
RM 166	1.6230	0.3839	0.6301
RM 224	5.3419	0.8128	1.7279
RM 229	2.0704	0.5170	0.7731
RM 249	1.4506	0.3107	0.4896
RM 250	3.3137	0.6982	1.2834
RM 257	6.0000	0.8333	1.8503
RM 317	2.5098	0.6016	1.1034
RM 437	1.4760	0.3225	0.5858
Mean	2.9785	0.5811	1.0754

**Fig 2.** SSR banding pattern of 50 accessions of Malaysian upland rice accessions amplified by primer RM224.

(RM250) with an average of 0.6197 for Peninsular Malaysia and it ranged from 0.3107 (RM249) to 0.8333 (RM257) with an average of 0.5811 for Sabah.

The Shannon's information index (*I*) ranged from 0.5623 for RM249 to 1.7466 for RM257 with an average of 1.1653 for Peninsular Malaysia and it ranged from 0.4896 for RM249 to 1.8503 for RM257 with an average of 1.0754 for Sabah (Table 2).

Cluster analysis

According to cluster analysis, 50 accessions of upland rice from Peninsular Malaysia and Sabah were divided into seven groups at 0.30 similarity coefficient level based on microsatellite polymorphism (Fig 3). The accessions of Peninsular Malaysia placed in group I, III, and V, while the

accessions of Sabah was grouped at IV, VII and VI. Group II was common between two states. The genetic similarity coefficient ranged from 0.11 to 1.00 among the 50 accessions of upland rice (Suppl. Table 2). Group I consisted of 12 accessions which were from Peninsular Malaysia.

Group II contained 2 accessions, 03824 from Peninsular Malaysia and 07509 from Sabah. Group III and V were collected from Peninsular Malaysia that consisted of 6 and 2 accessions, respectively. All members of group IV and VII were collected from Sabah.

Group VI consisted of 16 accessions and all members, except two accessions, 03825 and 03830, were collected from Sabah (Table 3). Inter cluster similarity coefficient among 50 accessions of upland rice is presented in the Table 4. The lowest similarity coefficient i.e. highest genetic distance was

Table 3. Grouping of Malaysian upland rice accessions according to cluster analysis based on 10 SSR markers.

Groups	Rice accessions
Group I	06040,06048, 06067, 06068,06050,06044,06071, 06041,06045, 06070,06043, 06059,
Group II	03824, 07509
Group III	03828, 03832, 03834, 03833, 03831, 03837
Group IV	07531, 07534, 07535
Group V	03838, 03835
Group VI	07537, 07538, 03826, 07574, 07588, 07585, 07540, 07575, 07541, 07543, 07544, 07576, 07571, 07539, 03825, 03830
Group VII	07545, 07546, 07597, 07596, 07595, 07590, 07594, 07589, 07508

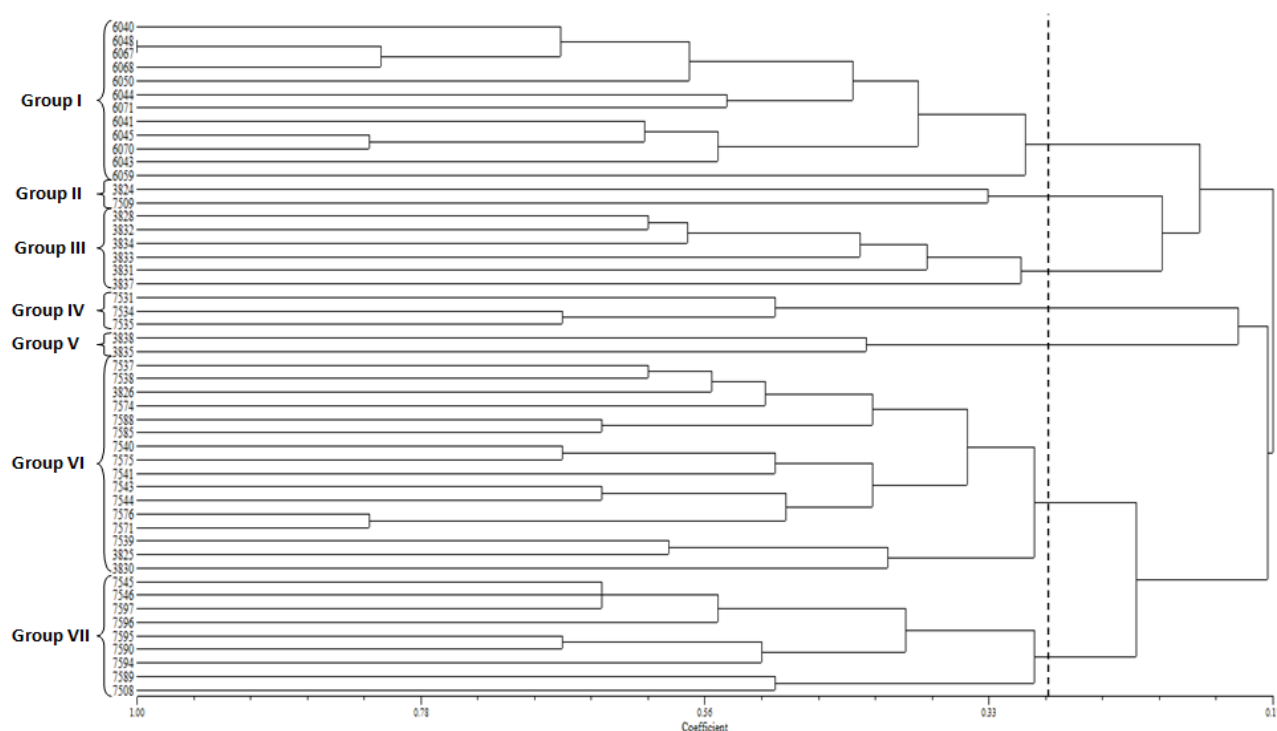


Fig 3. Cluster analysis of 50 upland rice accessions based on microsatellite polymorphism

recorded between groups V and VI followed by groups I and V, groups III and VII and groups III and IV. The highest similarity coefficient was found between groups VII and VI followed by groups II and VI, groups II and III and groups I and III.

Principal component analysis (PCA)

PCA was computed based on 10 microsatellites polymorphism. According to PCA 50 accessions of upland rice were divided into 7 groups (Fig 4). Group I, II, III, IV, V, VI, and V consist of 9, 5, 5, 6, 3, 14, and 8 members, respectively. The groups of PCA were more than 75% similar to groups of cluster analysis.

The accessions of group I in PCA and cluster analysis were the same, except 3 accessions (6071, 6041, and 6059), which were belonged to group I of cluster analysis. Group III in PCA had 5 members including 3828, 3833, 3831, 3838, and 3837, of which four of them were repeated at group III of cluster analysis, except the accession, 3838.

The most of accessions of groups VI and VII of PCA was repeated at groups VI and VII in cluster analysis. Principal components (PCs) indicated 51.1% of total variation among the accessions. The first three components showed 24, 15.3 and 11.8% variations, respectively (Table 5). PC1 indicated

24% of total variation. All accession in PC1 had positive contribution and ten of them such as accessions 07576 (0.602), 07540 (0.589), 07575 (0.587), 07538 (0.584), 07539 (0.574), 07544 (0.571), 07571 (0.560), 07585 (0.555), 07543 (0.554), and 07537 (0.549) had the high eigenvectors. Thirty one and 25 accessions had negative eigenvectors in PC2 and PC3, respectively.

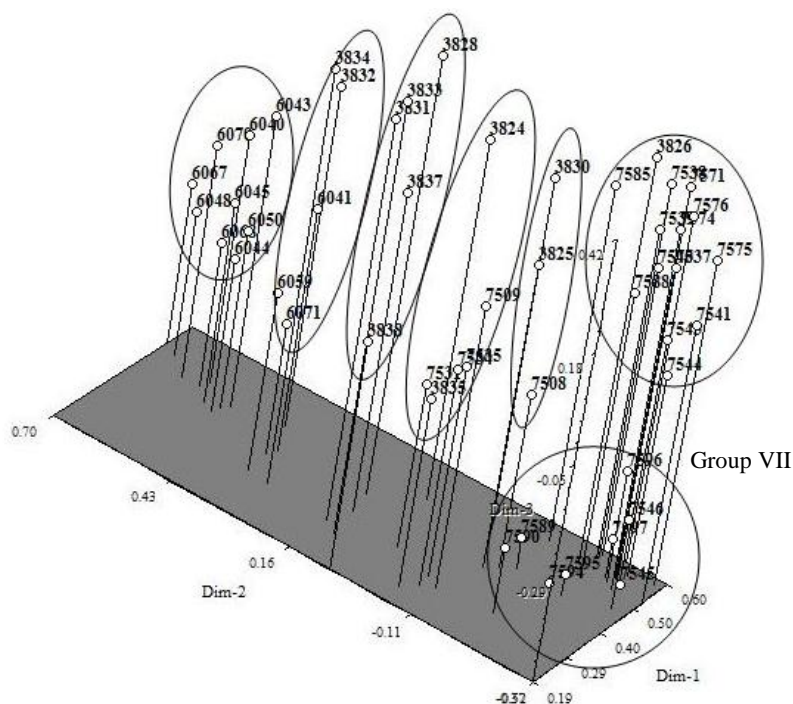
Discussion

The genetic diversity parameters like, expected heterozygosity, Shannon's and Nei's index, and PIC values had positive correlation to the number of allele. The RM257 marker had the highest Shannon's information index (2.0050), Nei's gene diversity (0.8273), and expected heterozygosity (0.8360) and on the other hand, RM 249 showed the lowest Shannon's information index (0.5269), Nei's gene diversity (0.3432), and expected heterozygosity (0.3467). Rice plant is a self-pollinated crop; therefore, SSR markers should indicate only one allele per locus and observed heterozygosity should be zero.

But two markers, RM166 (0.0200) and RM229 (0.0400), indicated two alleles for accessions number 07539 and that accessions wer heterozygote. The genetic differentiation (G_{ST}) was computed as 0.9957. The value of G_{ST} indicated

Table 4. Inter clusters similarity coefficient among 50 accessions of upland rice.

	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Group I	1.0000						
Group II	0.1300	1.0000					
Group III	0.1799	0.1973	1.0000				
Group IV	0.1115	0.0861	0.0784	1.0000			
Group V	0.0624	0.1683	0.1628	0.1372	1.0000		
Group VI	0.1079	0.1978	0.1353	0.1270	0.0600	1.0000	
Group VII	0.0926	0.1169	0.0666	0.1452	0.1401	0.2176	1.0000

**Fig 4.** The PCA of 50 upland rice accessions based on 10 microsatellites polymorphism.

that about 1% genetic diversity existed within accessions and around 99% genetic diversity is among accessions. To study the distributions of genetic variation in the Malaysian upland rice accessions, Nei's (1973) gene diversity statistics were applied. Nei's gene diversity among the accessions of Peninsular Malaysia was 0.6197, while Sabah was 0.5811. Similar results were reported previously by Tue et al. (2007) and Priyanka et al. (2004). Lapitan et al. (2007) analyzed the genetic diversity of Philippines rice and observed 92% polymorphism using 164 SSRs, in which the highest PIC was (0.91) and lowest (0.18) for SSR markers (RM473B and RM420), respectively. In addition, the highest (17) and the lowest (2) number of alleles were observed for RM473B and RM420 markers, respectively. Tu et al. (2007) measured 87% genetic variation among and 13% within the population, so the genetic differentiation was around 0.871. The average of genetic diversity based on Shannon's information index was 1.405 for all of the rice varieties. They recorded heterozygosity among the varieties and it was 0.006, significantly lower than the expected heterozygosity (0.696). The highest PIC value was recorded for RM257 followed by RM224, RM164 and RM250. These markers are more suitable for diversity analysis in upland rice varieties. Similar findings were reported by several authors (Latif et al., 2011; Islam et al., 2007; Priyanka et al., 2004). Genetic similarity coefficient based on Jaccard's method was ranged from 0.0 to 1.0. Fifty accessions of upland rice from Peninsular Malaysia and Sabah clustered into seven groups. The Peninsula

Malaysia accessions placed in groups I, III, and V. The Sabah accessions placed at groups IV, VII, and majority of group VI; and group II is common between two states. Therefore, it is evident from the cluster analysis that most of the accessions are grouped according to their geographical origin. El-malky et al. (2007) clustered 8 genotypes of rice based on 14 SSR markers into two groups. The first group belonged to *indica* and second group to *japonica* varieties. So, these varieties were clustered according to type of rice. Tu et al. (2007) divided 60 varieties of rice from Yunnan province of China into four distinct clusters. The result of this study agreed to the result of current study, because the cluster analysis was correlated to the geographic variation. Group I included varieties from two provinces (Jiangcheng and Mangshi), group II varieties of Jiangcheng state, group III varieties from Yunyang province, and group IV belonged to Mangshi state genotypes. Although in our study, numbers of SSR makers were comparatively less, yet we can determine the genetic diversity among 50 accessions of rice germplasm. Several authors did use 10-15 SSR markers to assess genetic diversity in rice genotypes (Thanh et al., 1999; Latif et al., 2013), indicating that, even the low number of markers can cover the rice genome, if they distribute normally on whole genome. The highest genetic distance was recorded between groups 5 and 6 followed by groups 1 and 5, groups 3 and 7 and groups 3 and 4. Genotypes having greater genetic dissimilarities could be hybridized to obtain maximum heterosis. Similar results were reported by several authors

Table 5. Component loading of the first three principal components for 50 accessions of upland rice by SSR markers.

Accessions	Eigenvectors		
	PC1	PC2	PC3
Eigenvalues	24	15.3	11.8
6040	0.486	0.566	0.018
6041	0.407	0.352	0.007
6043	0.453	0.495	0.110
6044	0.415	0.511	-0.188
6045	0.429	0.537	-0.088
6048	0.505	0.663	-0.206
6050	0.435	0.503	-0.133
6059	0.320	0.353	-0.135
6067	0.527	0.697	-0.172
6068	0.463	0.573	-0.207
6070	0.459	0.611	-0.014
6071	0.386	0.363	-0.241
7531	0.335	0.029	-0.170
7534	0.272	-0.066	-0.058
7535	0.307	-0.061	-0.070
7537	0.549	-0.301	0.151
7538	0.584	-0.239	0.281
7539	0.574	-0.240	0.190
7540	0.589	-0.243	0.104
7541	0.527	-0.375	0.079
7543	0.554	-0.307	-0.003
7544	0.571	-0.310	-0.083
7545	0.540	-0.316	-0.525
7546	0.473	-0.345	-0.333
7597	0.537	-0.284	-0.437
Accessions	Eigenvectors		
	PC1	PC2	PC3
7596	0.529	-0.293	-0.280
7595	0.437	-0.260	-0.479
7594	0.445	-0.226	-0.523
7590	0.300	-0.207	-0.382
7589	0.441	-0.158	-0.458
7588	0.505	-0.253	0.094
7585	0.555	-0.149	0.244
7576	0.602	-0.285	0.228
7571	0.560	-0.293	0.312
7574	0.515	-0.312	0.253
7575	0.587	-0.356	0.177
3824	0.490	0.076	0.255
3825	0.400	-0.114	0.130
3826	0.545	-0.222	0.345
3828	0.423	0.163	0.417
3830	0.492	-0.062	0.245
3831	0.305	0.164	0.337
3832	0.481	0.390	0.213
3834	0.316	0.305	0.365
3833	0.282	0.132	0.401
3837	0.361	0.148	0.164
3838	0.188	0.072	-0.030
3835	0.240	-0.043	-0.118
7508	0.420	-0.135	-0.146
7509	0.287	-0.091	0.083

Table 6. The list of SSR markers used in the present study.

SSR markers	Sequences	Chromosome	Repeat motif
RM257	F: 5'-CAGTTCCGAGCAAGAGTACTC-3' R: 5'-GGATCGGACGTGGCATATG-3'	9	(CT) ₂₄
RM224	F: 5'- ATCGATCGATCTTACAGAGG-3' R: 5'-TGCTATAAAAGGCATTCGGG-3'	11	(AAG) ₈ (AG) ₁₃
RM250	F: 5'-GGTTCAAACCAAGCTGATCA-3' R: 5'-GATGAAGGCCTTCCACGCAG-3'	2	(CT) ₁₇
RM166	F: 5'-GGTCTGGGTCAATAATTGGGTTACC-3' R: 5'-TTGCTGCATGATCCTAAACCGG-3'	2	(T) ₁₂
RM108	F: 5'- TCTCTTGC GCGCACACTGGCAC-3' R: 5'- CGTGCACCACCACCACCACCAC-3'	9	(GGT) ₁₀
RM249	F: 5'-GGCGTAAAGGTTTTGCATGT-3' R: 5'-ATGATGCCATGAAGGTCAGC-3'	5	(AG) ₅ A ₂ (AG) ₁₄
RM164	F: 5'-TCTTGCCCGTCACTGCAGATATCC-3' R: 5'-GCAGCCCTAATGCTACAATTCTTC-3'	5	(GT) ₁₆ TT(GT) ₄
RM317	F: 5'-CATACTTACCAGTTCACCGCC-3' R: 5'-CTGGAGAGTGTGCTAGTTGA-3'	4	(GC) ₄ (GT) ₁₈
RM437	F: 5'-ACACCAACCAGATCAGGGAG-3' R: 5'-TGCTCGTCAATGGTGAGTTC-3'	5	(AG) ₁₃
RM229	F: 5'-CACTCACACGAACGACTGAC-3' R: 5'-CGCAGGTTCTTGTGAAATGT-3'	11	(TC) ₁₁ (CT) ₅ C ₃ (CT) ₅

(Abdullah et al., 2011; Latif et al., 2011; Shabanimofrad et al., 2011; Rafii et al., 2012). To develop suitable upland rice varieties, accessions 03838, 03835, 07537, 07538, 03826, 07574, 07588, 07585, 07540, 07575, 07541, 07543, 07544, 07576, 07571, 07539, 03825, 03830 could be used as parents for future hybridization program.

Materials and methods

Plant materials

A total of fifty accessions of Malaysian upland rice were obtained from Malaysian Agricultural Research and Development Institute (MARDI) (24 originated from Peninsular Malaysia and 26 from Sabah) (Suppl. Table 3). The experiment was conducted in the field of Universiti Putra Malaysia. Sprouted seeds were sown in the pots. Young leaves were collected at 21 days after sowing. Leaves were put into the vials at -20°C prior use.

Selection of SSR markers

A total of 23 SSR makers were selected for diversity analysis. Out of 23, 10 primers showed strong polymorphism among 50 upland rice accessions (Table 6).

DNA extraction and PCR protocols

Total DNA was extracted from fresh leaves of 21-day-old plants by the hexadecyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1978). The concentration of extracted DNA was measured using Nano-drop machine (Nanodrop 2000c). Touchdown PCR conditions was applied as described by Korbie and Mattick (2008). Each PCR reaction was carried out in a 20 µL reaction volume containing 1µl of 50ng DNA template, 2µl PCR buffer (Dream buffer, contained 20Mm MgCl₂), 0.4µl dNTPs, 1µl forward primer, 1µl reverse primer, 0.1µl of 200µM Taq DNA polymerase, and 14.5µl double distilled water.

Data analysis

Each SSR band was scored as present (1) and absent (0) band for each genotype. The polymorphism information content (PIC) for each SSR locus was calculated according to the

formula, $PIC=1-\sum P_j^2$ where, P_j is the frequency of the j th allele for the i th marker, and summed over n alleles. The all genetic diversity parameters such as percentage of polymorphic loci (PPL), effective alleles number (n_e), gene diversity (h), Shannon's information index, gene frequency, and gene flow (N_m) were calculated by POPGENE software (version 1.3.1). Genetic differentiation of population (G_{ST}) was computed. The G_{ST} measures the proportional amount of variation within subpopulations as compared with the total population and does not specify to identify of alleles involved. When G_{ST} is equal to 0 indicates that subpopulations are identical in allele frequency and 1 when they are fixed for different alleles and always it is positive. Gene flow was computed from G_{ST} , $N_m = (1/4)(1-G_{ST})/G_{ST}$, Where N is the effective population size and m is the fraction of individuals in a population. When/if $N_m < 1$ it indicates that local populations tend to differentiate and when $N_m \geq 1$ shows little differentiation among populations. Genetic similarity was computed based on Jaccard's coefficient and it was used for cluster analysis by UPGMA. The analysis and dendrogram construction were performed using the NTSYS-pc software (version 2.1) (Rohlf, 2002). Principal component analysis (PCA) was computed based on 10 polymorphic SSR markers.

Conclusion

Genetic diversity among 50 accessions was evaluated based on 10 microsatellite markers. A total of 49 alleles were detected and ranged from 74 to 322 bp. Among 10 SSR markers, RM257, RM224, and RM164 indicated high values of genetic diversity compared to other SSR markers due to large number of allele, high PIC, high Shannon's and Nei's index. The all accessions of upland rice that were originated from Peninsular Malaysia and Sabah clustered into seven groups based on Jaccard's similarity coefficient level 0.3. The genetic similarity ranged from 11 to 100%. In cluster analysis, 3 (clusters, I, III, and V) and 2 groups (clusters IV and VII) belonged to Peninsular Malaysia and Sabah, respectively and also 2 groups (clusters II and VI; majority belonged to Sabah) were common between two states. To

exploit maximum heterosis as well as to develop desired variety of upland rice, accessions 03838 and 03835 could be crossed with the accessions, 07537, 07538, 03826, 07574, 07588, 07585, 07540, 07575, 07541, 07543, 07544, 07576, 07571, 07539, 03825, 03830.

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