Molecular Characterization of Selected Rice (*Oryza sativa* L.) Genotypes

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Abstract

Twelve simple sequence repeat (SSR) markers were used to fingerprint and assess genetic diversity among 32 selected rice genotypes. This study was carried out at Biotechnology laboratory, Department of Agricultural Research (DAR). All loci were found to be polymorphic, and the frequency of most common allele at each locus ranged from 3.12% (RM 1347, RM 1359, RM 8136, RM 474 and RM 1353) to 90.48 % (RM 8216). The range of polymorphic information content (PIC) values was from 0.27 to 0.77 (RM 514, RM 413) and 0.75 (RM 474). RM474 was found the best marker for the identification of studied genotypes as revealed higher PIC values and showed highest polymorphism. A total of 43 alleles were detected at 12 loci, the number of alleles per locus ranged from 2 (RM 510, RM 447, and RM 8216) to 6 (RM 474), average of 3.58. The dendogram revealed 5 well distinguish groups based on 0.27 similarity level showing a higher genetic relationship. So, it was denoted that there were considerable genetic variations among the studied genotypes.

Introduction

Rice is main stable food and major energy source for most of Asian people. It plays crucial role in Myanmar economy. It is cultivated in all agro-climatic conditions throughout the country. The area under rice is 7.7 million ha with the national average yield of 4.2 ton/ha which is lower than compared with the yields of some other Asian countries (MOAI, 2015). At present, rice production is self-sufficiency with Myanmar current population. Myanmar rice breeding program has been conducted to develop new rice varieties with increased yield, improved quality, pest and disease resistance and stress tolerance in order to meet increasing population and export in future. All of the rice research activities including pre-breeding evaluation, rice varietal improvement and agro-techniques are main challenges for our food security in accordance with the increasing population (DAR 2016).

There are many methods for germplasm evaluation which is one of the essential pre-breeding activities to produce new improved varieties. Characterization of varieties based on morphological characters is not very reliable because major characters have low heritability and most of the quantitative characters may be affected by environmental factors and growth practices. For this purpose, identification of different genotypes at molecular level is imperative. The DNA-based markers are promising and effective tools for measuring genetic diversity in plant germplasm and elucidating their evolutionary relationships. They are more reliable, and remain unaffected across different growth stages, seasons, locations and agronomic practices. DNA markers are predominantly used in molecular characterization and diversity studies due to their abundance and repeatability (McCouch et al. 2002). Among the polymerase chain reaction (PCR) based markers, the microsatellites also known as simple sequence repeats (SSRs) are useful genetic markers because they detect high levels of allelic diversity. They are co-dominant, ease of application, high reproducibility, rapid analysis, low cost, easy scoring patterns and distributed throughout the genome (Chen et al. 1997). More than 20,000 microsatellite markers have been mapped to specific locations in rice genome (www.irgsp.org) (Pervaiz et al. 2009).

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Molecular markers can be used to examine DUS test for distinctness or other characters that satisfy the criteria for characteristics set out in the general introduction if there is a reliable link between the marker and the character. A combination of phenotypic differences and molecular distances can be used to improve the selection of varieties to be compared in the growing trial if the molecular distances are sufficiently related to phenotypic differences. The method does not have more risk for selecting a variety from the variety collection which should be compared to candidate varieties in the DUS growing trial. The guidance on the use of biochemical and molecular markers in the examination of DUS is explained in documents TGP/15 of UPOV. Information on genetic diversity and relationships among rice genotypes from Myanmar is currently very limited for applying not only in rice breeding but also in the case of DUS examination. Therefore this study was carried out with the following objectives:

to investigate genetic diversity of selected rice genotypes

to assist for the selection of diversified parents for rice breeding programs and

to evaluate the genotypes which will be participate in future DUS test by means of SSR markers.

Table 1. List of selected genotypes for molecular characterization

Sr. No.	Varieties Name	Code No.in field exp:	Group (mor:ch)	Sources
1.	JX5 R	3	IV	Hybrid Rice Section, DAR
2.	IR58025B	6	IV	Hybrid Rice Section, DAR
3.	Shwe Pyi Htay	8	I	Rice Section, DAR
4.	Yadanar Toe	10	VII	Rice Section, DAR
5.	Ma Naw Thu kha	11	IV	Rice Section, DAR
6.	Pyi Taw Yin	13	VI	Rice Section, DAR
7.	Sin Thu Kha	14	IV	Rice Section, DAR
8.	Ya Ane lo -1	21	I	Rice Section, DAR
9.	Sin Thwe latt	24	IV	Rice Section, DAR
10.	Myaung Mya May	27	IV	Rice Section, DAR
11.	Shwe Bo Khun Ni	36	IV	Seed Bank, DAR
12.	Kayin Ma Lay	39	III	Seed Bank, DAR
13.	Zee Gwet	79	XI	Seed Bank, DAR
14.	Maw Taik	81	IV	Seed Bank, DAR
15.	Mee Kauk	84	VII	Seed Bank,

Code No.in field exp: code number in field experiment
Group (mor:ch) : group number in morphological characterization

Materials and Methods

Table 2. DNA profile and polymorphism generated in 32 rice genotypes using 12 SSR pri-

Marker	Chro No.	No. of allele	Size range (bp)	Highest frequency allele (%)	Lowest frequency allele (%)	PIC Value
RM 8136	1	4	52.74-145.24	46.8	3.12	0.64
RM 1347	2	4	88.16-309.74	68.64	3.12	0.53
RM 514	3	5	47.40-179.77	31.2	15.6	0.77
RM 1359	4	4	41.01-129.68	46.8	3.12	0.59
RM 413	5	5	84.09-327.04	31.2	6.24	0.77
RM 510	6	2	85.72-235.22	87.36	12.48	0.23
RM 1353	7	3	64.04-370.46	87.36	3.12	0.23
RM 447	8	2	83.33-612.51	74.88	24.96	0.38
RM 6971	9	3	105.48-300.00	74.88	9.36	0.51
RM 474	10	6	96.24-613.51	46.8	3.12	0.75
RM 5349	11	3	54.00-164.97	71.76	6.24	0.49
RM 8216	12	2	68.04-249.51	90.48	9.36	0.17
Total/avg		43/3.58	72.52- 161.70	758.16/63.18	99.84/8.32	6.03/0.5

Materials

A total of 32 traditional and improved rice cultivars, which were components of morphological characterization of core collected rice genotypes of field experiments, were used in this study (Table 1). Twelve SSR markers were selected in view of their high capacity of detecting polymorphism in rice and for being distributed across the 12 linkage groups of this species and applied for testing. The name of the markers and their located chromosomes were shown in Table 2.The original source and motifs for these markers can be found in the rice genes database (http//ars-genome.cornell.edu/rice). The size of polymorphic polymerase chain reaction (PCR) products was measured accurately following the M13 tail PCR method of Schuelke (2000).

Methods of DNA extraction and genotyping of PCR marker loci

Total DNA was extracted from 1 g ml of fresh leaves of 30 days old single seedling by CTAB (cetyltrimethyl ammonium bromide) method (Murry and Thompson, 1980). The DNA was spooled out, washed twice with 70% ethanol, and dissolved in TE (10 mM Tris,0.1 mM EDTA, pH 8.0) containing 25 µg/ml RNase-A, incubated at 37º C for 30 min and extracted with chloroform: isoamyl alcohol (24:1 v/v). DNA was checked for its quality and quantity by 1% agarose gel electrophoresis using a standard containing 100 ng/µl genomic lambda DNA.

The PCR amplification and gel electrophoresis reactions were undertaken in a final volume of 13 mL, containing 0.3 mM of each primer, 1 U of the enzyme Taq polymerase, 0.2 mM of each dNTP, 1 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.3 ml of DMSO (50%), and 7.5 ng of DNA template. The PCR implications were performed on the 2720 thermal cycler using the following procedure one pre-cycle for initial denaturation step at 96ºC (2 min) , each cycle comprised of 1 min denaturation at 94°C, 1 min annealing at 55°C, and 2 min extension at 72°C amplification condition: 30 cycles of 94 ºC (30 sec) , 55 ºC (1 min), 72 ºC (1 min), and a final extension step at 72 ºC (7 min). The DNA fragments were electrophoresis on 3.0% agarose gels in 0.5 TBE buffer at 150 V for 1hr and stained with ethidium bromide. After straining, gels were documented using gel documentation unit (UV -trans-illuminator).

Statistical analysis

$$PIC = 1 - \sum_{i=1}^{n} f2ij$$

The basic statistics were calculated using the genetic analysis package, Power Marker version 1.31 (Liu and Muse, 2005) for diversity measurements among the studied genotypes at each microsatellite locus, allele frequency and rare alleles (accession-specific alleles). The PIC value of a marker was calculated according to a simplified version (Anderson et al. 1993).

where, f_{ij} = the frequency of jth allele for marker i, i = marker, n = number of alleles

The PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency). Pair-wise similarity matrices based on DNA profile data were determined using Jaccard's similarity coefficient (Sneath and Sokal 1973). The characters were scored as 1 and 0 for the presence and absence of the fragment, respectively. Genetic relationships among the genotypes were calculated using UPGMA cluster analysis of the similarity matrix obtained from the proportion of shared amplification fragments. The dendrogram was constructed by using software package PHYLIP (Felsenstein 1993) to infer phylogenetic relationship.

Results and Discussion

DNA polymorphisms

DNA profile and polymorphism generated in 32 rice genotypes using 12 SSR primers were shown in Table 2. A total of 43 alleles were detected at 12 loci, the number of alleles per locus ranged from 2 to 6, with an average of 3.58. The marker RM 474 from chromosome 10 showed the highest number of alleles. It indicated that the studied rice varieties were high in genetic variation. In the analysis of esterase isozome, the highest genetic diversity was found in the area covering Myanmar, Thailand, Laos, Yunnan Province of China (Nakagahra

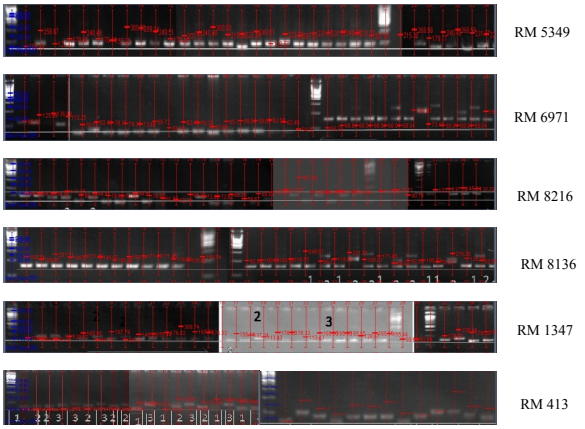

Figure 1. DNA profiles of the 32 rice genotypes generated by 12 SSR primers

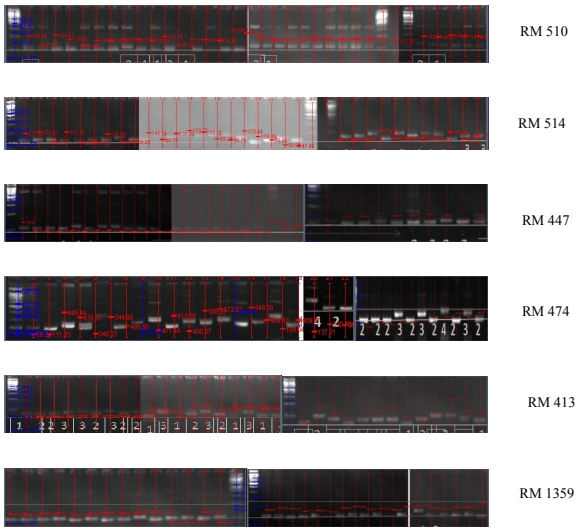

Figure 2. DNA profiles of the 32 rice genotypes

1984). It was found that 34 PCR markers with 2 to 5 alleles per locus and 13 SSR markers in 176 Myanmar local rice germplasm with 2 to 6 alleles per locus (Ohm Mar Saw et al. 2007). This result indicated that SSR markers have been efficiently employed for cultivar identification and genotyping in rice. The results presented in this study indicated that selected Myanmar rice genotypes were diverse at molecular level.

The frequency of common allele at each locus ranged from 3.12 % to 90.48 % (RM 8216). The markers RM 1347, RM 1359, RM 8136, RM 474 and RM 1353 possessed common allele of 3.2 %.On an average, 23.17 % of the 32 rice genotypes shared a common allele at any given locus. It was found that the minor allele percentage of 1.04 % in RM 413 and 1.82 % in RM 474. The range of polymorphic information content (PIC) values gave 0.27 to 0.77 with an average of 0.5. The highest PIC value

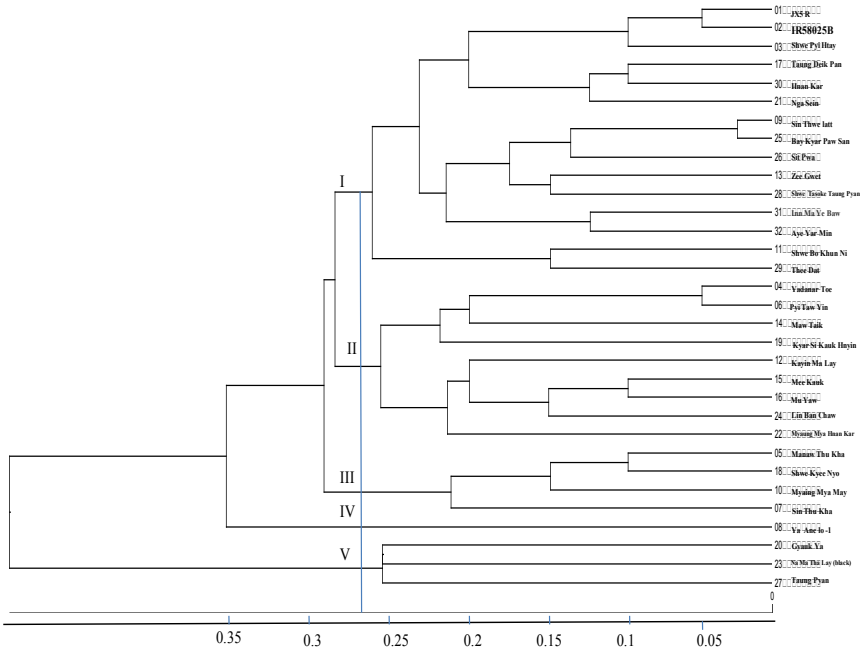

Figure 2. UPGMA cluster dendrogram showing the genetic relationships among selected rice genotypes based

(0.77) was obtained for RM 514 and RM 413 followed by RM 474 (0.75), RM 8136 (0.64), RM 1359 (0.59), RM 1347 (0.53), RM 6971 (0.51), RM 5349 (0.49), RM 447 (0.38), RM 510 and RM 1353 (0.23) and RM 8216 (0.17) respectively. These PIC values revealed that RM 514 and RM 413 were considered as the best markers for 32 genotypes (Table 2). Polymorphisms of studied genotypes were seen in the DNA profile of 12 SSR markers for 32 selected genotypes (Figure 1).

UPGMA cluster dendrogram showing the genetic relationships among selected rice genotypes based on 12 SSR markers revealed 5 well distinguish groups at 0.27 similarity level (Figure 2).The group I was a largest group including 15 genotypes, JX5 R (1), IR 58025B (2), Shwe Pyi Htay (3), Taung Deik Pan (17), Hnan Kar (30), Nga Sein (21), Sin Thwe Latt (9), Bay Kyar Paw San (25), Sit Pwa (26), Zee Gwet (13), Shwe Tasoke Taung Pyan (28), Inn Ma Ye Baw (31), Aye Yar Min (32), Shwe Bo Khun Ni (11) and Thee Dat (29). Group II was the second largest group consisting 9 genotypes and followed by group III, V and IV with 4, 3 and 1 genotypes respectively. The name of the genotypes including in the group II were Yadanar Toe (4), Pyi Taw Yin (6), Maw Taik (14), Kyar Si Kauk Hnyin (19), Kayin Ma Lay (12), Mee Kauk (15), Mu Yaw (16), Lin Ban Chaw (24) and Myaung Mya Hnan Kar (22). Group III contained Ma Naw Thu Kha (5), Shwe Kyee Nyo (18), Myaung Mya May (10) and Sin Thu Kha (7) varieties. The group V was a second smallest one including Gyauk Ya (20), Na Ma Tha Lay (black) (23) and Taung Pyan (27). There was only one genotype in group VI, Ya Ane lo -1 (8) (Table 3). It was also noted that Ya Ane lo-1(8) genotype have the distinctness character of short growth duration. The studied rice genotypes were redundant genotypes from different collection sites. The results presented in this study indicated that experimental genotypes were diverse at molecular level. It can be said that there was a reliable link between the molecular and morphological characterization because most the genotypes in the same group in morphological characterization were also occurred in the same group in molecular. The knowledge in genetic variation is important for breeders to fully draw the potential of germplasm. Therefore, the information generated from this experiment would allow to select appropriate parents within Myanmar rice cultivars for further breeding program and the selection of reference varieties to be compared in DUS growing trial.

Table 3. Cluster membership of 32 rice genotypes based on 12 SSR molecular markers characterization

Cluster	No.of genotypes	Name of Genotypes
I	15	JX5 R (1), IR58025B (2), Shwe Pyi Htay (3), Taung Deik Pan (17), Hnan Kar (30), Nga Sein (21), Sin Thwe Latt (9), Bay Kyar Paw San (25), Sit Pwa (26), Zee Gwet (13), Shwe Tasoke Taung Pyan (28), Inn Ma Ye Baw 31, Aye Yar Min (32), Shwe Bo Khun Ni (11), Thee Dat (29)
II	9	Yadanar Toe (4), Pyi Taw Yin (6), Maw Taik (14), Kyar Si Kauk Hnyin(19), Kayin Ma Lay (12), Mee Kauk (15), Mu Yaw (16), Lin Ban Chaw (24), Myaung Mya Hnan Kar (22)
III	4	Ma Naw Thu Kha (5), Shwe Kyee Nyo (18), Myaing Mya May (10), Sin Thu Kha (7)
IV	1	Ya Ane lo - 1(8)

Conclusion

In the present study, a total of 12 SSR markers were used across 32 selected rice genotypes for their characterization and discrimination. All microsatellite markers were showed polymorphism. In a total of 43 alleles, the number of alleles per locus ranged from 2 to 6 alleles (RM 474), with an average of 3.58 alleles across 12 loci. The polymorphic information content values ranged from 0.17 to 0.77. The RM 474 was found the best marker for the identification of studied genotypes as revealed by higher PIC values (0.75) and showed highest polymorphism. There was wide range of frequency allele percentage. RM 8216 occurred as most common allele possessing highest frequency allele percentage of 90.48. The microsatellite marker based molecular fingerprinting could serve as a sound basis

in the identification of genetically distant accessions as well as in the duplicate sorting of the morphologically closed accessions.

Molecular characterization of the genotypes gives precise information about the extent of genetic diversity which helps in the development of an appropriate breeding program. Therefore the present study revealed a considerable genetic diversity present in the 32 rice genotypes at molecular level and a limited number of SSR markers efficiently grouped these genotypes into 5 clusters. Clustering pattern obtained in the present study revealed that individuals in clusters were evenly distributed. The results of evaluating molecular and morphological similarity of studied rice genotypes confirmed that the two kinds of similarity may coincide but there was no correlation between them confirming the relevant literature (Lopez et al. 2008). Nevertheless both similarities are worthwhile to calculate despite the different genetical background if parental relation is important. Morphological trait based DUS test does not take pedigree into account while molecular similarity can reflect it more precisely. Most of the genotypes represented in this clustering pattern were similar with respect to most of the morphological characterization except one or two genotypes. For this selected genotypes, combination of phenotypic differences and molecular distances can be used to improve the selection of varieties from this selected genotypes to be compared in the DUS trial.

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Abbreviation

UPOV = International Union for the Protection of New Varieties of Plants

DUS = Distinctness, Uniformity and Stability

TGP/15 = Technical Guidelines Protocol /15