Molecular Characterization of Rice (*Oryza sativa* L.) Genotypes From Yangon Region in Myanmar using SSR Markers

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Abstract

Rice (*Oryza sativa)* is one of the most important stable crops, consumed daily more than half of the world population. In Asia, Myanmar is center of rice genetic diversity as there are numerous rice varieties under diverse agro-ecological zones. In order to estimate genetic diversity of rice (*Oryza sativa* L.) germplasm in Yangon region of Myanmar, 102 genotypes from different parts of region were analyzed by 12 microsatellite (SSR) markers. In SSR characterization, the mean value of Polymorphic information content (PIC) was found 0.69 for all accessions. The maximum and minimum Polymorphic information content (PIC) values were found to be 0.79 and 0.21 for the primers RM229 and RM201 respectively.The total number of alleles was 91 and the average number of alleles per locus was 7.58. Average gene diversity was 0.72 indicating high genetic diversity among the genotypes. Phylogenetic cluster analysis of SSR data based on UPGMA and Nei's genetic distance divided into eight groups. These results reflect the high genetic differentiation existing in rice germplasm. The results from molecular data will improve in the part of rice varieties improvement programme. This study will support the information for breeders in rice breeding programme.

Keywords: Genetic diversity, *Oryza sativa*, SSR, PIC

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INTRODUCTION

Most of the world's rice is cultivated as daily diet in Asia, which constitutes more than half of the global population (Rahman, Molla, Alam, & Lutfur, 2009). Rice (*Oryza sativa* L.) genetic resources are widely available in the worldwide (Chakravarthi & Naravaneni, 2006). Rice is the most important commodity in the agricultural sector in Myanmar. Therefore, local rice varieties should be genetically characterized.

Myanmar is one of the origins of rice genetic resources in Asia which spreads along the Himalayas from Iran to Myanmar consists of very diverse varieties (Glaszmann, 1986). Many local varieties have been grown in Myanmar for centuries. The areas including Assam in India, Myanmar, Laos and Yunnan in China could provide the richest spectrum of rice varietal diversity (Chang, 1976). For a long time, landraces, traditional and local rice varieties in Myanmar are grown over the whole country, under diverse agro-ecological zones such as flood-prone area, rain fed lowland, upland, and hilly regions. Among these regions, present research focused on rain fed lowland area, especially, Yangon Region.

Genetic divergence among the genotypes plays an important role in the selection of parents having wider variability for different characters. Genetic diversity can be evaluated with morphological traits, seed proteins, isozymes and DNA markers. Development of new biotechnological techniques provides increased support to evaluate genetic variation in both phenotypic and genotypic levels. In contract to morphological traits, molecular markers are powerful tools in the assessment of genetic variation, in the elucidation of genetic relationships within and among species and have demonstrated the potential to detect genetic diversity and to aid in the management of plant genetic resources (da Silva, 2005; Song, Huang, Shi, Zhu, & Lin, 2007; P. Virk, Newbury, Jackson, & Ford-Lloyd, 2000). Molecular markers have been widely used to study the genetic variation and diversity of breeding materials, which were less influenced by temporal, spatial and environmental conditions (Hamza, Hamida, Rebaï, & Harrabi, 2004). In rice, molecular markers have been used to identify accessions (Olufowote et al., 1997; P. S. Virk, Ford-Lloyd, Jackson, & Newbury, 1995) to determine the genetic structure and pattern of diversity for cultivars of interest (Yang, Jana, & Clarke, 1991; Zhang, Maroof, Lu, & Shen, 1992) and to optimize the assembly of core collections.

Several types of molecular markers are available for the extent of genetic variation in rice. Among them, microsatellite markers detect a significantly higher degree of polymorphism in rice and are especially suitable to evaluate the genetic diversity among

closely related rice cultivars (Miah et al., 2013). In rice, SSR markers are distributed relatively uniformly through the genome and have been detected a high level of allelic diversity in genotypes and distantly related species (McCouch et al., 2001). The SSR markers have been used to characterize genetic diversity in genotypes and improved germplasm (Huang et al., 2012) SSR markers are class of repetitive DNA sequences usually 2.6 bp that are distributed throughout whole genome and are flanked by highly conserved region(Chambers and Avoy 2000). The identification of rice genotypes and their inter-relationships is essential and it can be done by molecular markers.

In Myanmar, the classical way of assessing genetic diversity has been utilized on the study of morpho-agronomic variability and importance for the utilization in rice improvement program. Therefore, molecular marker technique is needed to access genetic variability of local rice genotypes accurately. Therefore, the present study was carried out; to assess the genetic diversity of rice genotypes from Yangon Region in Myanmar and to determine the genetic relationship between genotypes from Yangon Region.

Materials and Methods

Plant materials

A total of 102 rice genotypes were used in this study. All these accessions were obtained from Seed Bank, Department of Agricultural Research (DAR), Ministry of Agriculture, Livestock and Irrigation (MOALI), Republic of the Union of Myanmar.

DNA extraction and PCR Assay using SSR marker

Rice genomic DNA was extracted from 21-day-old seedling leaves collected from each cultivar, by modified CTAB method described by Song et al. (2007). Markers were chosen according to their location on the rice genetic map and their suitability for high-throughput genotyping. Twelve SSR markers distributed on the 12 chromosomes were employed to analyze population structure. SSR markers information is available in GRAMENE (http://www.gramene.org/microsat/ssr.txt). To evaluate the genetic diversity and determine the genetic relationship between genotypes by using twelve SSR markers, most of the laboratory activities were done at Division of New Genetics Laboratory of Advanced Center for Agricultural Research and Education (ACARE), Yezin Agricultural University (YAU).

The polymerase chain reaction (PCR) was performed in a total volume of 25µl per reaction containing 2.5 µl of template DNA(5ng / µl), 1µl of each forward and reverse

primers, 1.25 µl dNTPs (10mM), 0.2µl Taq polymerase (5 U/µl), 0.3µl of MgCl2 (50 mM) and 2.5µl 10×PCR buffer. The PCR amplification was carried out on a thermal cycler at an initial temperature of 94°C for 5 min, each cycle comprised of 1 min denaturation at 94°C, 55°C for 30 s (primer annealing occurred with most of the primers while some were adjusted), 2 min extension at 72°C with a final extension for 7 min at 72°C and then stored at 4°C at the end of 35 cycles. The PCR products were analyzed by electrophoresis on 3% agarose gel. The gels were stained in 0.5mg/ml ethidium bromide and were documented using UVPRO (Uvipro Platinum, EU) gel documentation unit.

Genotype Score and Data Analysis

For each markers, alleles for the data set were scored according to size of base pairs of the 100bp ladder DNA marker. This procedure was conducted for each marker until all alleles were scored with the smallest-largest-sized alleles representing the start of the first scoring and end of the last scoring respectively. Genetic similarities were estimated from the matrix of binary data using jacquard similarity coefficient. To infer genetic relationships and phylogeny, the similarity coefficients were used for cluster analysis of the rice cultivars utilizing the complete linkage method. Basic statistics were calculated using the genetic analysis package Power Marker V 3.23 (Liu & Muse, 2009) or diversity measurements at each microsatellite locus, including the total number of alleles (NA), allele frequency, major allele frequency (MAF), gene diversity (GD), and polymorphism information content (PIC). Genetic distances between each pair of accessions were measured by calculating the shared allele frequencies using Power Marker V3.23. Correlation and path analysis were computed by using The UPGMA tree was drawn by MEGA 6 program software (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

Results and Discussion

Allelic polymorphism and Genetic Diversity

Information available on these alleles present in different genotypes will be very useful for developing the mapping populations for genome analysis as well as in applied breeding programs. Molecular-based biological and geographical diversity differed with respect to allelic richness, frequency of rare alleles, the common and most frequent alleles, and group-specific unique alleles.

The 12 SSR primers were used across 102 rice accessions. These SSR markers revealed 91 alleles across the rice accessions. The allelic richness per locus varied widely

among the markers, ranging from 5 to 11 alleles, with an average of 7.58 alleles per locus (Table 1). The highest number of alleles (11) was detected in the marker RM44 and the lowest number of alleles (5) was detected on the marker RM201. The average number of alleles per locus obtained in the present study was smaller than that reported in previous study by Kuroda, Sato, Bounphanousay, Kono, and Tanaka (2007). They reported an average of 9.28 alleles per locus over 7 SSR loci. But greater than finding of who recorded 6.33 alleles per locus using a small set of three SSR markers on 34 varieties. This difference in average allele per locus might be due to diverse nature of genotypes used by different researchers and selection of SSR markers. (Fig. 1, Table 1). Rice accessions shared a common major allele at 12 locus from 0.24 (RM225) to 0.88 (RM201). A moderate level of diversity exists in these loci in rice accessions with the average 0.4 (Table 1).

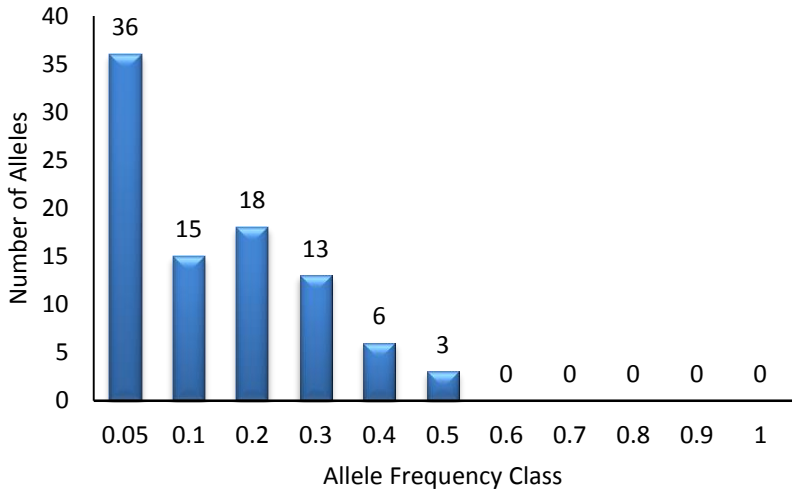

Figure 1. Histogram of allele frequencies for all 91 alleles in the 102 rice accessions.

The result showed that rare alleles (frequency < 0.05) comprised 39.1% of all alleles, whereas intermediate (frequency 0.1-0.5) comprised 60.1% and there was no abundant alleles (frequency > 0.5). Luikart, Allendorf, Cornuet, and Sherwin (1998) mentioned microsatellite datasets from non-bottlenecked population also had a large proportion of rare allele. In this study, the alleles at low and intermediate frequencies were more abundant in rice accessions. This result indicated that studied population had less mutation rate and they were non-bottlenecked natural population.

The gene diversity and PIC values ranged from 0.22 (RM201) to 0.81 (RM229) and from 0.21 (RM201) to 0.79 (RM229), with an average of 0.72 and 0.69, respectively. The major alleles frequency per locus varied from 0.24 (RM225) to 0.88 (RM201) with the average of 0.4 (Table 1).

Table 1. Number of alleles, polymorphic information content and genetic diversity index for 12 simple sequence repeat (SSR) loci in the 102 accessions.

Marker	M A F	No. of observation	NA	RA	GD	PIC
RM 60	0.26	102	6	0	0.8	0.77
RM 208	0.46	102	6	1	0.7	0.66
RM 225	0.24	102	7	2	0.8	0.77
RM 258	0.32	102	10	6	0.79	0.76
RM 44	0.32	101	11	7	0.8	0.78
RM 247	0.4	94	10	7	0.75	0.71
RM 201	0.88	101	5	3	0.22	0.21
RM 31	0.4	99	7	1	0.76	0.74
RM 2	0.39	101	7	2	0.76	0.73
RM 229	0.26	102	8	2	0.81	0.79
RM 237	0.46	91	5	1	0.65	0.59
RM 241	0.34	102	9	4	0.76	0.72
Mean	0.4	99.92	7.58		0.72	0.69
Total			91	36		

MAF=Major Allele Frequency, NA=Number of alleles, Rare allele (RA) =Number of alleles that frequency < 0.05, GD= Gene Diversity, PIC= Polymorphic Information Content.

The PIC values, a reflection of allele diversity and frequency among the cultivars, also varied from one locus to another. The PIC values derived from allelic diversity and frequency among the genotypes were not uniform for all the SSR loci tested. A marker with PIC > 0.5 can be considered as highly informative and highly polymorphic, whereas, 0.5 > PIC > 0.25 recognized as reasonably informative and below 0.25 is measured as slightly informative (Marshall, Slate, Kruuk, & Pemberton, 1998). The lower PIC value (0.21) was observed in RM201. The mean PIC value (0.69) for all genotypes observed in this study was higher than the PIC value of 0.5. This indicated that all genotype used in the present study were more diverse. Out of the twelve SSR markers used in this study, 11 markers (RM 60, RM208, RM225, RM258, RM44, RM247, RM31, RM2, RM 229, RM237 and RM241) had PIC value greater than 0.5. Therefore, these markers had to be highly informative and therefore could be utilized in rice genotypes because they were capable of distinguishing between genotypes. Lower PIC value may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes of cluster analysis indicating that the shared allele distance and cluster analysis method were suitable to use the information derived from SSR markers.

Genetic diversity assessment of the improved and indigenous rice genotypes was essential component in germplasm characterization and conservation to identify potential

parents. Genetic diversity was detected not only among the sampled rice varieties but also within the same varieties, particularly within the traditional varieties. Diversity analysis in studies of Yangon rice genotypes evaluated on the basis of PIC. In this study, twelve Microsatellite (SSR) markers which were used to assess the genetic diversity of (102) genotypes ranged from 0.22 to 0.81 of rice cultivars.

Genetic Relationships

The UPGMA cluster analysis of the 102 accessions in this study resulted in eight groups which appeared to be related to their pedigrees. The group I include only 2 accessions; Thet Nu Saba Net Pyan and Nga Kywe Taung Pyan which possess modified glume (wings like structure). Group II comprised 5 accessions. This group contains the old cultivars, Kauk Sann and Mee Kauk. In group III, there were 19 accessions which included different local cultivars such as Nga Cheik, Nga Shink Thway, Ya Gyaw-2. Including Myanmar popular variety Paw San Hmwe and Na Ma Tha Lay, there are 17 accessions are included in the group IV. In group V, different accession Paw San Hmwe and other 6 Myanmar old cultivars were grouped. In group VI, there were biggest group mix with modern and old cultivars in this study which contained 27 accessions. In group VII and VIII also contained 14 and 11 accessions with old and modern cultivars. The UPGMA cluster analysis of the 102 accessions in the present study produced meaningful groupings based on pedigree and/or geographical origins of the accessions. Position of some accessions in the dendrogram gave concerning the origin of some old cultivars. (Figure 2, Table 2).

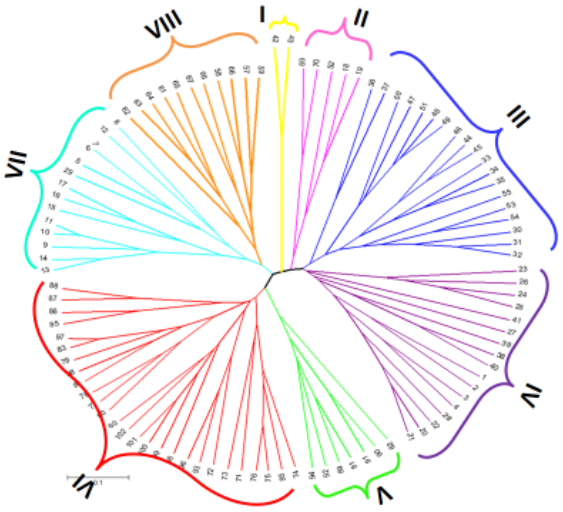

Figure 2. UPGMA dendrogram based on a genetic distance matrix among 102 rice accessions from Yangon Region in Myanmar.

Table 2. Cluster groups of rice genotypes

Cluster	No. of Accessions	Names of Accessions
I	2	Thet Nu Saba Net Pyan, Nga Kywe Taung Pyan
II	5	Kauk Sann, Shwe Wa Hnan, Tha Zin Tan, Shwe Wa Hmwe, Mee Kauk
III	19	Nga Yun Wa Bo, Phut U, Ya Gyaw-2, Nga Cheik, Sein Shwe San, Nga Shink Thway, Leik Kalay Mee Don, Shwe War Mee Don, C-68-18, Padan Nga Sein, Gwa Kama Kyi, Hnan Su, Hnan Su, Shwe Dinga, Ye Baw Sein, Shwe Chay Gyin, Nga Sein Kalar, Ya Ma Gyi, Let Ywe Zin
IV	17	Lone Thwe Shwe War, C-4-63, Sein Kama Kyi, Thet Nu Saba Net, Nga Kye Du Me, Yodaya, Mercury, A Pyo Gyi Paung Dan Shay, Emata Pin To, Emata Pin To, Nga Yaw Man, Paw San Hmwe, Hnan Su, Shwe Palin, Kamar Kyi Shwe War, Hmaw Bi Nga Kywe, Na Ma Tha Lay
V	7	Pathein Nyunt, Paw San Hmwe, Pawa Nyo, Ye Manaing Kauk Kyi, Shwe War Gyi, Buzayet, Taing Thi Lauk
VI	27	Nga Yar Po, Kywet Thwa, Pin Do Sein, Kauk Yin Shwe War, Kamar Kyi, Sit Pwa, Shwe Wa Mee Don, Kywe Pu, Pan Thit Sa, Ye Kyaw, Hmaw Bi Nga Sein, Shwe Palin, Ma Sein Aye, Shwe Wa Gyi, Taung Paw Khin Sein, B-541-B-KN-58-5-3, Khao Note Tit Own, Khao Kwe Lan, Shwe Sayar, Kauk Hnyin Gyi, Moe Ma Kha Gyi, Byat Nga Kywe, Kan Yoe Tan-1, Shwe Bo (1), Hmaw Bi (3), Kan Yoe Tane (1), Shwe Bo -1
VII	14	Shwe Pu, Nat Pyi Hmwe, Nyaung Aine, Nga Kywe, Nat Pyi Hmwe, Nat Pyi Hmwe, Pyi Daw Aye, Pyi Daw Aye, Kywe Chae Manaing, Bay Kyaung, C-64-1, Leik Kalay Nga Phyu, Shwe Kyi Dauk, Lone Gyi Kar
VIII	11	Go Kaung, Moe Thay, Kauk Hnyin, Nga Sein Thee Dat, Ye Laik Mee Don, Thu Ka De Thee Dat, Nga Sein Gaung Me, Kalai Nga Sein, Kauk Hnyin Khun Ni, Shwe Palin, Hnget Pyaw Nyunt

Conclusion

The diversity and the unique features of the Myanmar rice genotypes examined in this study could be quite relevant to domestic rice development. Assessment of genetic diversity in plants is an integral part of plant breeding programs because it helps plant breeders to develop new crop varieties with desirable qualities. Results from this study revealed genetic diversity of rice germplasm in Yangon, which clearly indicate the importance of continued conservation and utilization in breeding of rice germplasm in this region. It should be included representative rice varieties from similar geographic and climate conditions around Yangon region. Furthermore, it should consider collecting sufficient numbers of individuals within the same rice varieties (in the same regions) and preferably the same varieties from different regions, because rice varieties cultivated by farmers usually contained considerable genetic heterogeneity. These results could be useful for rice breeders in rice improvement strategies on the traits to introduce the new rice varieties.

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