Review article

Microsatellite Markers and their Application on Genetic Diversity Studies of Rice Landraces (*Oryza sativa* L.) in Myanmar - A Review

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Abstract Myanmar is one of the centers of genetic diversity of rice as it has heterogeneous geographical and ecological conditions such as hills and mountains. Since 1988, owing to market oriented economic policy and international rice price, traditional varieties have been replaced by improved varieties. Landraces are known for the excellent adaptation to the local conditions; however, their productivity is very low. Increased productivity of the landraces through high input application has not been possible because they are not responsive to the inputs. However, these landraces and traditional varieties possess very useful and important traits for further breeding and genetic improvement of rice. Thus, there is an urgent need for germplasm collection, their conservation and characterization to study the diversity of traditional rice varieties in Myanmar. In recent years, DNA sequence variations have been used as markers for genome analysis since they are independent of environment and growth stages of plant and more reliable than the other phenotypic or biochemical markers. Of the different types of molecular markers, microsatellites have been utilized most extensively, because they can be readily amplified by PCR, present large amount of allelic variation at each locus, are highly informative, and require small amounts of DNA. They have become the versatile molecular markers not only for germplasm diversity studies but also for exploration of targeted gene. In Myanmar, some research on rice landrace diversity by using microsatellites has been done. Therefore, this review aims to take stock of the current status and summarize genetic diversity studies of traditional rice varieties in Myanmar, through the use of microsatellite markers.

Keywords microsatellites, genetic diversity, rice landraces, Myanmar

INTRODUCTION

Myanmar is known as a rice-based agriculture country and large fraction of national economy rely on the surplus from rice export. From historical time to now, the country has a long history of rice production. Moreover, culture, religion and belief, politics and national economy were influenced by rice and rice production. Therefore, civilization of Myanmar was established based on rice and

rice cultivation. Since 1988, owing to market oriented economic policy and international rice price, traditional varieties have been replaced by improved varieties. However, Myanmar traditional landraces are still under cultivation by resource poor farmers who practice subsistence farming (Yamanaka et al., 2011) and the outcome is low yield. Although the output is lower, these landraces have wide adaptation to local or harsh conditions and they are assumed as a harbor of great genetic potential for rice improvement.

Landraces are also an integral part of cultural heritage due to their close link with the local territory as well as the community with their associated traditional uses, knowledge and habits. Landraces are, however, threatened and suffering from genetic erosion. Major contributing factors include climate change, habitat loss, environmental degradation and changes in farming practices, leading to the introduction of new pests and diseases, a collapse of pollinators, and increased drought and salinization. These precious and irreplaceable resources urgently need to be conserved before they disappear. Therefore, in order to prevent the permanent loss of landraces, research activities on genetic diversity, conservation and utilization become critical issues for the agriculture sector in Myanmar.

Nowadays, several techniques are available for the detection of genetic diversity, i.e. for identifying DNA polymorphism, including the microsatellite ones. This marker is easily amplified by PCR, requires no large initial amounts of DNA samples and has high polymorphism due to a large variation in the number of repetitions. Furthermore, the microsatellite loci have co-dominant multi-allelic expression which permits the discrimination of homozygous and heterozygous genotypes, facilitating the characterization of different populations by allele frequency analysis (Bruford, 1996). Therefore, genetic diversity data, based on microsatellites, can be used for monitoring the genetic variability of species and support management actions to prevent the loss of genetic diversity over time. Tens of thousands of potential SSRs have been identified in rice, and over 25,000 have been developed as molecular markers (Temnykh et al., 2000; McCouch et al., 2002; IRGSP, 2005). These markers are currently being used to develop high density genetic maps, genotype rice accessions, determine the genetic structure, optimize the assembly of core collections, and for marker-assisted breeding (McCouch et al., 2002; Yu et al., 2003; Garris et al., 2005). The limiting feature of the application of these markers is the need for prior sequence information for developing primers for locus-specific PCR amplification. This limitation is alleviated for the economically important species and the ones closely related, since primer sequences of the SSR DNA markers and the amplification conditions are available in the published reports and the rice annotation project database (rap-db). Generation of complex banding patterns for SSR loci could be due to various reasons such as type of repeat, non-optimization of PCR conditions and the nature of genome (Mausa, 2014).

OBJECTIVE

This review aims to make an overall assessment of the current status and summarize genetic diversity studies of traditional rice varieties in Myanmar, through the use of microsatellite markers.

RESULTS AND DISCUSSION

In Myanmar, assessment of genetic diversity of rice germplasm have been conducted by using various characters: viz: morphological, physiological, biochemical, and molecular characters. Among them, molecular markers have been used for genome analysis since they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell. Wunna et al., (2016) studied the genetic diversity of 175 rice accessions from Myanmar, including landraces and improved types from upland and lowland ecosystems in five different areas. They evaluated on the basis of polymorphism data for 65 microsatellite markers and confirmed high genetic diversity with average polymorphism information content (PIC) value of 0.82 per locus with the range of 0.519 to 0.919. The large range of PIC values for the respective accessions provides greater confidence for the assessment of genetic diversity and relationships. Cluster

analysis based on the polymorphism data from SSR markers clearly differentiated the rice accessions into two groups. Group I, with 85 accessions, corresponded to Indica Group and was dominant in all upland regions except for the Northeastern. Group II, with 90 accessions, corresponded to the Japonica Group and was dominant in the Southern (lowland) region (Table 1).

Table 1 Relationships among groups, areas, and rice cultivation ecosystems

Ecosystem	Landrace or improved	Area	No. of accessions (%)		
			Group I	Group II	Total
Upland	Landrace	Northern	16 (9.1)	2 (1.1)	18 (10.3)
		Western	11 (6.3)	1 (0.6)	12 (6.9)
		Southeastern	29 (16.6)	4 (2.3)	33 (18.9)
		Northeastern	12 (6.9)	18 (10.3)	30 (17.1)
		Sum	68 (38.9)	25 (14.3)	93 (53.1)
	Improved	Northeastern	0 (0.0)	7 (4.0)	7 (4.0)
	Subtotal		68 (39.4)	32 (18.3)	100 (57.7)
Lowland	Landrace	Southern	3 (1.7)	50 (28.6)	53 (30.3)
	Improved	Southern	12 (6.6)	2 (1.1)	14 (8.0)
	Subtotal		15 (8.6)	52 (29.7)	67 (38.3)
Others					
Upland	Improved	Unknown	1 (0.6)	0 (0.0)	1 (0.6)
Lowland	Landrace	Unknown	1 (0.6)	6 (3.4)	7 (4.0)
	Subtotal		2 (0.11)	6 (3.4)	8 (4.6)
Total			85 (48.6)	90 (51.4)	175 (100.0)

Pawsan rice cultivars, quality rice, seems to be originated in Myanmar and due to their delightful aroma and good eating quality, they are recognized as premium rice cultivars in Myanmar. Min Soe Thein et al., (2014) collected 38 Pawsan cultivars mainly from Ayeyarwaddy region and documented the variation of genetic structure of these collected Pawsan cultivars in order to fingerprint, conserve and exploit potential cultivars for breeding program. Twenty two SSR loci of Pawsan cultivars with two controls generated 112 alleles with an average of 5.09, and PIC ranged from 0.22 to 0.80 with an average of 0.54. SSR genotyping revealed that pair-wise genetic similarity of Pawsan cultivars ranged from 14% to 84% and averaged of 49%, and Pawsan group was separated from two controls (IR36 and Koshihikari). However, genetic similarity of Pawsan group was closer to Koshihikari (japonica). Among the local varieties in Myanmar, Meedon rice, which is one of the five rice varietal groups in Myanmar, is important for local adaptability, grain quality, premium price and market availability. Minn San Thein et al., (2012) carried out a study to assess genetic diversity and to analyze population structure of Meedon rice germplasm conserved in Myanmar Seed Bank using SSR markers. 154 accessions of Meedon rice germplasm were analyzed with nine SSR markers. A total of 86 alleles were detected with an average of 9.6 alleles per locus. All the loci were found to be polymorphic, and there were considerable genetic variation among accessions with mean values of expected heterozygosity (HE) = 0.5774 and polymorphic information content (PIC) = 0.5496. High frequency of rare alleles was identified, among which 35 unique (accession-specific) alleles were observed. Based on cluster analysis, the three population groups consist of 105 accessions, 43 accessions and 6 accessions indicating the inclusion of non-Meedon rice accessions, which was about one-third of the germplasm, and it was a large proportion. This study revealed that the small number of SSR markers is possible for discriminating Meedon and non-Meedon rice. Yamanaka et al., (2011) conducted a study on genetic variation in Myanmar by using 12 SSR markers. 41 out of 83 strains were obtained from the Myanmar seedbank. The other 42 strains were directly sampled from agricultural fields in the areas where the seed-bank materials were originally derived. The gene

diversity were 0.809 in seed-bank and 0.826 in on-farm. This suggested that on-farm material was comparatively more diverse than the seed-bank samples.

Isozyme analyses classified majority of the rice cultivars into six groups; group-I corresponds to the indica and group-VI refers to both temperate and tropical japonica. Groups II, III, IV and V were classified as indicas conventionally (Glaszmann, 1987). Recently, a diversity analysis was conducted using DNA markers on Myanmar rice varieties and found that some varieties from isozyme group-V appeared to be japonica types. Thawda et al., (2005) analyzed 31 rice varieties from Myanmar and they reported that the rice varieties, in isozyme group-V (Singh, 2000; Khush et al., 2003) could also be considered as japonica. Therefore, Moe Moe Oo et al., (2009) conducted a research to obtain more information on Myanmar rice varieties from isozyme group-V for proper genetic resource conservation and management. A total of 43 microsatellite primers were utilized and 41 primers showed polymorphism among the 52 accessions. The number of alleles per locus detected by microsatellite primers varied from 2 to 15 with an average of 5.63 alleles per primer. The highest polymorphism was observed at RM334 on chromosome no.5 which showed 15 alleles. According to the cluster analyses, most of the Myanmar rice varieties from the isozyme group-V are genetically closer to japonica and a few of them are found closer to indica. The dendrogram reveals two main groups with the genetic similarity of 0.476. The group-I consisted 5 rice varieties including indica control and 4 Myanmar rice varieties with maximum genetic similarity of 0.520. The group-II was formed cluster by 48 Myanmar rice varieties together with temperate japonica control and tropical japonica control. All the rice varieties in group-II possess japonica type DNA in both nuclear and chloroplast genomes. Therefore, it is assumed that the rice varieties from the group-I are genetically closer to indica and the group-II closer japonica.

Table 2 Number of alleles and gene diversity of rice landraces from Myanmar and standard strains observed for 10 SSR loci

Locus	Alleles/locus		Gene diversity	
	Myanmar	Standard	Myanmar	Standard
RM1	9	4	0.876	0.718
RM7	18	9	0.939	0.839
RM19	10	11	0.930	0.919
RM20A	15	10	0.877	0.911
RM20B	18	8	0.918	0.888
RM120	11	5	0.885	0.851
RM164	17	12	0.919	0.904
RM167	17	10	0.908	0.888
RM241	15	10	0.879	0.888
OSR21	20	14	0.922	0.900
Average	15	9.3	0.905	0.871

According to Htay Htay Aung (2007), SSR analysis revealed high variation among the Myanmar rice varieties. There are 202 alleles in 132 accessions using 34 SSR loci, the Myanmar rice varieties showed 197 alleles. The average alleles per locus for all accessions and Myanmar rice varieties are 5.94, and 5.79, respectively. The average gene diversity for all accessions is 0.71 while for the Myanmar rice varieties, it is 0.70. This indicates that high gene diversity exists among Myanmar rice varieties. The dendrogram based on cluster analysis of SSR markers generated two major clusters. Cluster I consisted of mostly japonica, javanica and high quality aromatic rice types. Cluster II corresponded to the indica group. Glutinous rice varieties are mostly scattered in cluster II with a few included in cluster I. It has been indicated that most glutinous cultivars arose independently from indica types. Myanmar rice varieties represented a genetically diverse and heterogeneous group with multiple alleles at many of the SSR loci. Genetic polymorphism of ten microsatellites loci were examined in 100accessions of rice including 77 landraces from Myanmar, 3 cultivated varieties from Japan, 4 from China, 1 from Vietnam, 4 from Philippines, 5 from Taiwan area, 6 from India and 2 from Indonesia (San San Yi et al., (2005). Those other varieties were representative strains for each country (Oka, 1958). In this study, RM 1 produced the smallest

number of alleles per locus and OSR 21 generated the largest number in both samples. Range of alleles per locus is 9-20 and totally 150 alleles in Myanmar strains and 4-14 and totally 93 alleles for standard strains. Therefore, in Myanmar samples, total and average numbers of alleles are higher than those in standard strains. Gene diversity of Myanmar strain is also greater than standard strains from different Asia countries (Table 2). Oka (1988) also stated that the diversity of Oryza sativa is the highest in Myanmar compared with other Southeast Asia countries because these standard strains are core collection of landraces of Asian rice that seeds were donated from National Institute of Genetics (Japan). In cluster analysis, it produced 5 main clusters. 4 main groups are indica and the other one is japonica rice group.

Table 3 Informative primers

SSR locus	Chromosome	No. of alleles	Gene diversity
RM1	1	12	0.90
RM263	2	17	0.62
RM7	3	18	0.94
RM241	4	15	0.88
RM164	5	17	0.92
OSR21	6	20	0.92
RM11	7	9	0.80
RM72	8	11	0.81
RM3164	9	8	0.80
RM271	10	8	0.84
RM167	11	17	0.91
RM247	12	9	0.77

CONCLUSION

Overall we can conclude that microsatellites are useful markers and they detect a high level of allelic variation and give an understanding of the genetic relationships and diversity among the Myanmar rice varieties. SSRs can also be used not only in rice germplasm evaluation but also effectively in indica-japonica differentiation. According to the survey articles, the genetic diversity of over 600 Myanmar landraces have been evaluated by using 148 SSR loci, which are distributed all over the 12 chromosomes. Despite this, the publications involving genetic diversity measurement using microsatellites are still rare in Myanmar rice varieties because around 7,000 accessions are kept in the Seed Bank at the Department of Agricultural Research, Myanmar. Based on the level of polymorphism detected by individual primers, the most informative primers were identified with different polymorphic bands (Table 3). Therefore, future research studies of genetic variation should be performed on Myanmar rice landraces through the use of those high polymorphic SSR loci.

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