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Molecular Characterization of Malaysian rice Germplasm by Using Microsatellite Markers for Variety Identification

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Abstract. The present investigation was committed to distinguish the cultivated Malaysian rice varieties using microsatellite markers. Twenty commercial Malaysian rice varieties were characterized using ten microsatellite DNA markers. Three primers (RM231, RM262 and RM223) identified four varieties based on unique band pattern. The results indicated that varieties MR263 by the size of 170bp and MR253 (180bp) were identified by primers RM262 and RM231, respectively. The microsatellite primer RM223 exhibited two sets of bands, since single band is just made by two varieties Mahsuri and Malinja showed the size of 130 bp. By the primer RM574, eight varieties gave the size of 180 bp and twelve varieties gave the size of 190 bp, but no specific identification could be done by this marker and also rest of the primers. It is suggested that addition of more microsatellite primers would be used to identify the other varieties to provide precise information to rice scientist regarding molecular identification.

Keywords: Malaysian rice, variety identification, microsatellite markers

1. Introduction

About 3 billion people around the world use rice as a basic and staple food which affords 50 to 80% of their daily calories. The cultivated amount for rice is more than 150 million hectares, and the world production is around 600 million tons per year [1]-[3]. In Malaysia, two types of rice are cultivated: upland rice in Sabah and Sarawak about 165,888ha and wetland rice in Peninsular Malaysia about 503,184ha [3]. Rice is one of the best plants for the study of genetic characterization and genome structure because among cereals, it has a small genome size of 430 megabase pairs (Mbp) as equated to the meaningfully large genome sizes of wheat, barley, maize, and sorghum (about 16000, 5000, 3000, and 750 Mbp, in turn), it is diploid [1], [4] and has a considerable level of genetic polymorphism [5], [6]. Microsatellite markers based on simple sequence repeats (SSR) have been developed in many crop species, including barley, rice, grapevine, maize, Brassica tomato and soybean. These markers can detect simple sequence length polymorphism (SSLP) and are quickly relocating restriction fragment length polymorphisms (RFLPs) for many kinds of genetic approaches, largely because their technique is easy, it just need the small amount of starting DNA, rapid turn-around time, the comparatively low cost for the users and high power of genetic resolution [7].

Generally rice varieties are recognized and identified based on morpho-biochemical traits. Majority of the traits are quantitative in nature and therefore it misguides the plant scientist to recognize a particular variety and it is often difficult to use their criteria. Molecular characterization of these varieties would be the

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ultimate solution. So, as fiscal and human resources are limited in many countries, interregional, regional and international cooperation and coaction in those researches which related to the rice should propose some practical solutions which can help to increased food in the whole world.

Moreover, cooperative efforts directed towards strengthening the flow of rice information—through the present modern processed communication facilities—among international establishments, members of the Mediterranean countries, universities and others working on rice in the regions with high vary of temperatures, ought to be significantly encouraged [8].

The aim of the current research was to identify appropriate microsatellite markers for molecular characterization of Malaysian rice cultivars to distinguish the rice varieties at molecular level.

2. Materials and methods

2.1. Plant material and genomic DNA extraction

The seeds of twenty cultivated Malaysian rice varieties were obtained from MARIDI (Malaysian Agricultural Research and Development Institute). Ten seedlings of each variety were grown in glass house and leaves were harvested at seedling stage to extract the genomic DNA. These extractions of genomic DNA were carried out by CTAB method [9].

2.2. Polymerase Chain Reaction (PCR) amplification

All after extracting the DNA samples from rice leaves, Polymerase Chain Reaction (PCR) were amplified. PCR were carried out with Invitrogen by life technologies kit, in a volume of 25 µl containing 10x PCR Buffer, 10µm each of the dNTPs, 0.5µm of each of the primer (The list of SSR markers which were used in this study was described by details in Table 1 and had been retrieved from Rice Gene database - www.gramene.org), 1.25 unit Taq DNA polymerase, 200 ng template DNA and a suitable amount of sterile deionized water.

These amplification was carried out in a MJ Gradient Thermo cycler (Bio-Rad Laboratories) as follows: 5 minutes at 94℃ followed by 35 cycles of 1 minutes at 94℃, 1 minutes at 55℃, 2 minutes at 72℃ and 7 minutes at 72℃ for final extension [10].

Table 1: List of primers with detail.

Primer	Primer sequences	SSR repeat motifs
RM231	**F:**CCAGATTATTTCCTGAGGTC **R:**CACTTGCATAGTTCTGCATTG	**(GA)16**
RM262	**F:**CATTCCGTCTCCGCTCAACT **R:**CAGAGCAAGGTGGCTTGC	**(CT)25**
RM574	**F:**GGCGAATTCTTTGCACTTGG **R:**ACGGTTTGGTAGGGTGTCAC	**(GA)11**
RM223	**F:**GAGTGAGCTTGGGCTGAAAC **R:**GAAGGCAAGTCTTGGCACTG	**(CT)25**

2.3. Electrophoresis and gel staining

For detecting and monitoring the PCR products, electrophoresis is a routine technique. In this technique, monitoring is done by comparing fluorescence bands which is made with DNA ladder and the separation that is based on the mobility of charged macromolecules under the electric field.

2.4. Identification of unique bands

The PCR products were identified based on the molecular weights. To estimate the PCR product size 100 bp ladders was used.

3. Result and Discussion

The gel electrophoresis of PCR products for each microsatellite marker was shown in the following Figures. Each microsatellite marker indicated different band among varieties. According to McCouch et al.

(2001) [11], microsatellites, also known as simple sequence repeats (SSRs), are simple tandemly repeated di- to tetra-NT sequence motifs flanked by unique sequences. These markers are often polymorphic and have already been demonstrated to be a powerful tool in genotype identification and variety protection, seed-purity evaluation and germplasm characterization, diversity studies, gene analysis and this is important especially for protection of germplasm [12].

Each microsatellite marker indicated different band among varieties. Results from gel electrophoresis have been tabulated in table 2. This table includes size and location of each band, which could be used for determination of different varieties of rice in this research.

Table 2: Number of bands and band size exhibited by microsatellite markers in cultivated Malaysian rice cultivars

No.	Primer Variety	RM262		RM231		RM574		RM223	
		*B	*S	B	S	B	S	B	S
1	**Malinja**	1	140	1	190	1	180	1	**130**
2	**Mahsuri**	1	140	1	190	1	190	1	**130**
3	**Ria**	1	140	1	190	1	190	1 2	300 140
4	**Bahasia**	1	140	1	190	1	190	1 2	300 150
5	**Murni**	1	140	1	190	1	190	1 2	300 140
6	**Masria**	1	140	1	190	1	190	1 2	300 140
7	**Jaya**	1	140	1	190	1	190	1 2	300 150
8	**Sri M.II**	1	130	1	190	1	190	1 2	300 140
9	**Pulut Siding**	1	130	1	190	1	190	1 2	300 140
10	**Sebarang**	1	140	1	190	1	190	1 2	300 150
11	**Muda**	1	140	1	190	1	190	1 2	300 140
12	**MR84**	1	140	1	190	1	190	1 2	300 150
13	**MR81**	1	140	1	190	1	190	1 2	300 140
14	**MR159**	1	130	1	190	1	180	1 2	300 140
15	**MR167**	1	140	1	190	1	180	1 2	300 140
16	**MR211**	1	140	1	190	1	180	1 2	300 150
17	**Maswangi**	1	140	1	200	1	180	1 2	300 140
18	**MRM16**	1	130	1	200	1	180	1 2	300 140
19	**MR253**	1	130	1	**180**	1	180	1 2	300 140
20	**MR263**	1	**170**	1	200	1	180	1 2	300 140

* **B**: Number of bands, **S**: size of bands based on bp: base pairs; Underlined figures are unique bands.

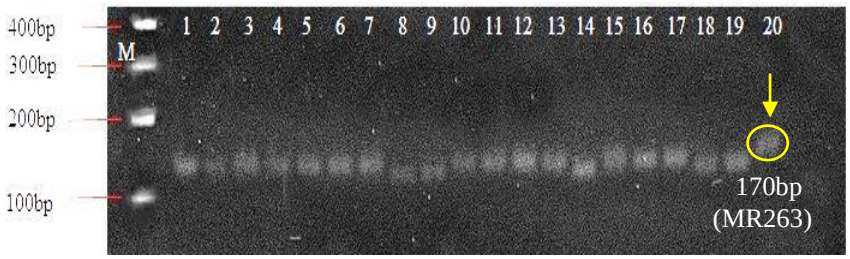
RM262: In this primer, fourteen varieties yielded 140 bp and five varieties gave 130 bp and also a unique band is made by variety MR211 (Lane 20) at 170 bp. Fig 1 (a) shows the band patterns of twenty rice varieties.

RM231: Using this primer, sixteen varieties indicated the size of amplified sample DNA as 190bp, three varieties as 200bp and one variety as 180 bp approximately. The 180 bp band size could be used for identification of MR253 (Lane 19) rice variety. Fig 1 (b) shows the band patterns of twenty rice varieties.

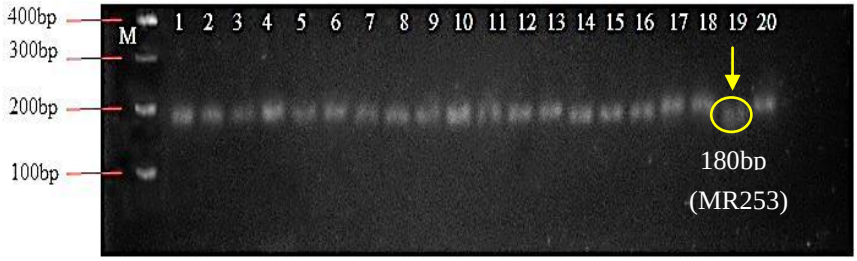
RM574: In this primer, eight varieties yielded 180 bp and twelve varieties gave 190 bp, so no specific identification could be done. Due to no considerable difference in the size of bands, it could be concluded that the primer yields a monomorphic band. The band patterns of twenty rice varieties are shown in Fig 1 (c).

RM223: This primer indicated two sets of bands. In the first band eighteen varieties have shown the size of amplified sample DNA as 300 bp. In the second band, five varieties showed 150 bp, thirteen indicated 140 bp and Mahsuri (Lane 2) variety have resulted in 130 bp and Malinja (Lane 1) variety indicated 130 bp approximately. Since the single bands is just made by these two varieties. It could be concluded that this primer might be capable of specifying these two cultivars. The band patterns of twenty rice varieties are shown in Fig 1 (d).

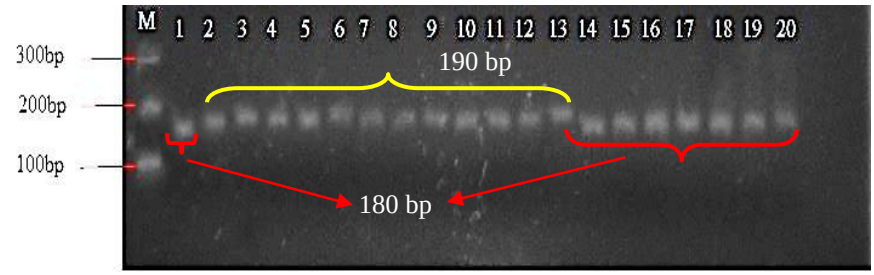
a. Band pattern exhibited by cultivated Malaysian rice varieties, amplified by primer RM262.



b. Band pattern exhibited by cultivated Malaysian rice varieties, amplified by primer RM231



c. Band pattern exhibited by cultivated Malaysian rice varieties, amplified by RM574



d. Band pattern exhibited by cultivated Malaysian rice varieties, amplified by RM223

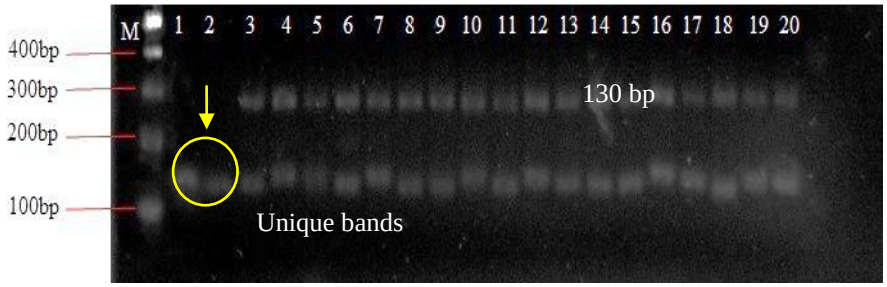


Fig. 1: Band patterns exhibited by four microsatellite markers in twenty cultivated Malaysian rice varieties (a, b, c and d). M is used for 100bp ladder. The numbers show the varieties as described in Table 2.

Lee et al. (2011) investigated genotyping of 53 Sarawak rice cultivars by using microsatellite markers. Polymorphic markers were chosen from the initial screening results in order to obtain microsatellite marker panels that can differentiate the rice cultivars. They suggested using microsatellite markers as a useful tool for the estimation of genetic diversity and cultivar differentiation and present invaluable genetic information for future breeding and association mapping efforts [13]. Also, the main aim of this study is determination useful microsatellite markers for studying genetic characterization among twenty Malaysian rice varieties to expose genetic relationships between these varieties and to evaluate the potential of this technique for identification of rice germplasm. To gain this goal, PCR products of each primer are classified based on their size and quantity of the bands.

Based on the above information, it can be concluded that, cultivar MR253 (Lane 19) could be identified by primer RM231, variety MR263 (Lane 20) could be identified by primer RM262 and varieties Malina (Lane 1) and Mahsuri (Lane 2) could be identified by primer RM223. These varieties determined by one specific primer. Therefore by these primers the identification of some specific Malaysian rice cultivars will be done.

4. Conclusions

Identification of rice varieties at molecular level complements the morphological/agronomic markers. In present study, three microsatellite markers were identified to distinguish the four rice varieties based on unique band pattern. RM231, RM262 and RM223 could be used to identify the four cultivated Malaysian rice varieties (MR263, MR253, Mahsuri, Malinja) at molecular level. The results of present study would be helpful for plant scientist to identify the rice cultivars based on unique microsatellite.

5. Acknowledgments

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