The Journal of Animal & Plant Sciences, 26(4): 2016, Page: 1117-1123
ISSN: 1018-7081

SCREENING OF PREVIOUSLY REPORTED MICROSATELLITE MARKERS, ASSOCIATED WITH PANICLE CHARACTERISTICS, FOR MAKER ASSISTED SELECTION IN MALAYSIAN RICE (*ORYZA SATIVA* L.)

N. Supari[1], M. A. Javed[1,*], N. Jahan[1], E. Khalili[1] and S. Khan[1]

[1]Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia (UTM), UTM Skudai 81310, Johor Bahru, Malaysia.
*Corresponding Author: majaved@fbb.utm.my

ABSTRACT

Rice (*Oryzasativa* L.) is an important crop that vital to the lives of billions of people providing almost 23percent of the calories intake. Most of the yield related traits are quantitative in nature. To improve these traits, quantitatively traits loci (QTL) are needed to be identified. Major QTLs have the tendency to be detected across populations and it mainly depends on high phenotypic variations for respective traits. Panicle characteristics are the main determinant of rice yield. Several investigations related to the identification of quantitative trait loci (QTLs) for these traits have been reported. Present studies were carried out to screen the QTLs in Malaysian rice cultivars for the selection of desirable plants through marker assisted breeding. Malaysian rice cultivars were evaluated for three panicle traits; panicle length, number of panicle per plant and number of grains per panicle. Eight microsatellite markers, which have shown association with panicle traits in the literature, were selected to identify the microsatellite loci for panicle traits. Among these microsatellites markers, RM310 distinguished the Malaysian rice varieties based on number of grains per panicle. It is therefore suggested to utilize microsatellite marker RM 310 in molecular screening of rice breeding populations to identify the plant possessing high numbers of grains.

Key word: *Oryzasativa* L., Microsatellite loci, Panicle traits, Panicle length, Grains per panicle

INTRODUCTION

Rice (*Oryzasativa L.*) is one of the important staple foods for the world's population. It is a primary food source of carbohydrate that provides 23 percent of calories intake for human (Subudhi*et al.*, 2006). The production of rice can be increased by increasing the area of cultivation or by improving plant productivity. The limited land for rice cultivation gives only one option to improve plant productivity by two major aspects that are by management practice and releasing high yield varieties. In Malaysia, the rice area accounts for about 11 percent of total agricultural lands. Currently, the rice productivity in Malaysia increases only at average of 2 percent per year, despite the required 4.9 percent per year (Akinbile *et al.*, 2011).

In order to develop high yield varieties, selection of desirable traits should be evaluated at molecular level by marker assisted selection. Among yield components, panicle traits are significantly important. According to Hua *et al.* (2002), the numbers of panicle per plant and numbers of grains per panicle was shown to be highly correlated to yield. Zhang *et al.* (2009) mentioned that spikelets number per panicle is associated with grain number per panicle, reflected the importance of this component of yield. These traits are controlled by polygenes, controlled by quantitative trait loci (Xing and Zhang, 2010). With the emergence of DNA marker technology, a lot of QTLs controlling panicle traits in rice have been identified in different populations and environments.

Many QTLs for panicle traits including panicle number, spikelet number per panicle and grain number per panicle have been identified. Ahamadi*et al.* (2008) identified at least 8 QTLs for panicle length on chromosomes 2, 4, 11 and 12 using a population of 59 advanced BC_2F_5 derived from crosses between the recipient parent, IR64 and Taromemolaei as donor parents. Xing *et al.* (2008) used a BC_2F_2 population and its progeny and identified a QTL (*qSSP7*) controlling the number of spikelets per panicle. By using F_2 and F_3 populations derived from a cross between C3074 and Guanglai 4, Zhu *et al.* (2011) detected that qPN1 was a major QTL that is responsible for the phenotypic variation in rice panicle number. It also affected plant height, panicle length and grain yield per plant. Septiningsih *et al.* (2003) have conducted QTL analysis in two different locations in Indonesia, Bogor and Sukamandi for the same study population of BC_2F_2. RM315 and RM265 were identified to be associated with panicle length in both locations by interval mapping and composite interval mapping. The phenotypic variability ranged from 4.3 to 8.7%. On the other hand, interval marker RM431 and RM315 were only detected in Sukamandi with slightly higher phenotypic variation, 10.10% compared to the previous QTL. These interval

markers were associated with panicle number per plant trait. It is suggested that confirmation of QTL study can assist in selection of microsatellite loci. Thus, the objective of this study is to screen the microsatellite markers for panicle characteristics in Malaysian rice, to be used further in marker-assisted selection in rice breeding programs.

MATERIALS AND METHODS

Plant materials: Fifteen commercial rice varieties from Malaysia were used for microsatellite analysis (Table 1). Seeds were collected from Seberang Perai MARDI station, Penang. The plants of each variety (in triplicate, four plants per replicate) were grown in glass house till maturity. The phenotypic data for three panicle characteristics (panicle length, number of panicle per plant and number of grains per panicle) was recorded at maturity. The data was analysed by Duncan's Multiple Range Test (DMRT) to compare the means of these traits.

Genomic DNA extraction: Genomic DNA was extracted from the rice leaves using CTAB method following protocol described by Japelaghi*et al.*, (2011).

Selection of microsatellite markers: Eight rice microsatellite primer pairs (RM431, RM240, RM223, RM315, RM262, RM310, RM544 and RM208) were selected for three panicle characteristics. RM240, RM223 and RM431 were selected for panicle length; RM431, RM315 and RM262 for numbers of panicle per plant; and RM544, RM310 and RM208 for number of grains per panicle. The QTLs associated with these microsatellite primers showed a high phenotypic variation within the mapping populations. The information for these microsatellite markers was retrieved from Rice Gene database (www.gramene.org). Details of these markers are given in Table 3.

PCR amplification and microsatellite marker analysis: Polymerase chain reaction were performed in a total volume of 25µL containing 1x PCR reaction Buffer, 0.1µM of dNTPs mix, 0.2µM of forward and reverse primer, 1 unit of BIORON Taq DNA polymerase, 200 ng DNA template and a suitable amount of sterile deionized water. The PCR reaction was carried out in a MJ Gradient Thermocycler (Bio-Rad Laboratories) with PCR condition as the following: 94°C at 5 minutes, 35 cycles of 94°C at 1 minute for denaturing the DNA, 57°C at 1 minute for annealing step, 72°C at 1 minute for elongation and final extension at 72°C for 5 minutes. PCR product was analysed in 3% agarose gel. The product size was determined by using 50 bp DNA ladder (Fermentas). The polymorphism of these microsatellites is shown in Figure 1. Red line reflects the pattern of polymorphism and grouping of genotypes/varieties.

RESULTS

Phenotypic characterization: Three panicle characteristics were observed in this study; panicle length, numbers of panicles per plant and numbers of grains per panicle. The means of these traits are differentiated by DMRT in Table 3. The maximum panicle length was observed in Mahsuri while the minimum was variety Jaya (30 cm and 20.4 cm, respectively). Significant differences for panicle length were observed for the plant material used in the study. Variety MRQ 74 (Maswangi) showed maximum panicle number (18) while variety Mahsuri and Jaya exhibited the minimum numbers of panicle per plant (8).The number of grains per panicle is the main yield component in rice. Maximum numbers of grains per panicle were recorded in MR106 (231), whereas the minimum numbers of grains per panicle were counted in Malinja (64). A wide range was observed for numbers of grains per panicle compared to other panicle characteristics in the present study.

Microsatellite analysis: Figure 1 shows the banding pattern of each microsatellite for fifteen rice varieties used in the present study. Polymorphism patterns are shown by red line. Monomorphism was observed in all varieties except Maswangi (MRQ74) for RM 240 which showed a band size of 120 bp, compared to 140 bp in other varieties (Fig 1. A). RM 223 showed a monomorphism except Jaya, Sekencang, Pulut Siding. Varieties Jaya and Sekencang showed a band size 160 bp and the band size of Pulut Siding was 130 bps compared to a band size 150 bp as shown by other varieties (Fig 1. B). RM 431 exhibited a monomorphism (Fig 1. C). The marker polymorphism and phenotypic differences for panicle length did not match. The banding pattern of rice varieties for RM 315 was monmorphic (Fig 1. D). RM 262 showed a polymorphism (Fig 1. E). Jaya, MR211 and MR106 showed a band size 140 bp compared to other band size (150 bp) in other varieties. Although, RM 262 differentiated the varieties in two groups but phenotypic data for Jaya, MR211 and MR106 did not show match with band size. Therefore, microsatellite markers that are associated with panicle length and number of panicle per plant showed no correlation between the trait data and band patterns. The banding patterns of RM 544 and RM 208 markers (Fig 1. F and H, respectively) did not show matching with phenotypic data of numbers of grains per panicle. However, RM310 discriminated the rice varieties into three groups (Fig 1. G). MR263, Mahsuri, Jaya, Sri Malaysia II, Pulut Siding, MR211 and Malinja exhibited a band size 80 bps (group 1). MR106 and MR127 showed a band size of 100 bps (group 2) while the band size of Pulut Malaysia I, Maswangi (MRQ74), Kadaria, MR159 and Sekencang was 110 bps (group 3). The numbers of grains per panicle

ranges from 64 to 100, 185 to 231 and 125 to 168 in group 1, group 2 and group 3, respectively. It shows a matching between the phenotypic data and bad patterns.

Table 1. Details of Malaysian rice varieties employed in the present study

No.	Accession No.	Variety Name
1.	00581	Jaya (Malaysia)
2.	00826	Mahsuri
3.	00839	Malinja
4.	02123	Pulut Malaysia I
5.	02672	Sri Malaysia II
6.	04552	Sekencang
7.	04554	Kadaria
8.	04555	Pulut Siding
9.	04633	MR84
10.	07487	MR106
11.	07489	MR127
12.	08638	MR159
13.	11629	MR211
14.	11787	Maswangi(MRQ74)
15.	-	MR263

Table 2.Phenotypic evaluation of three panicle characteristics in Malaysian rice varieties.

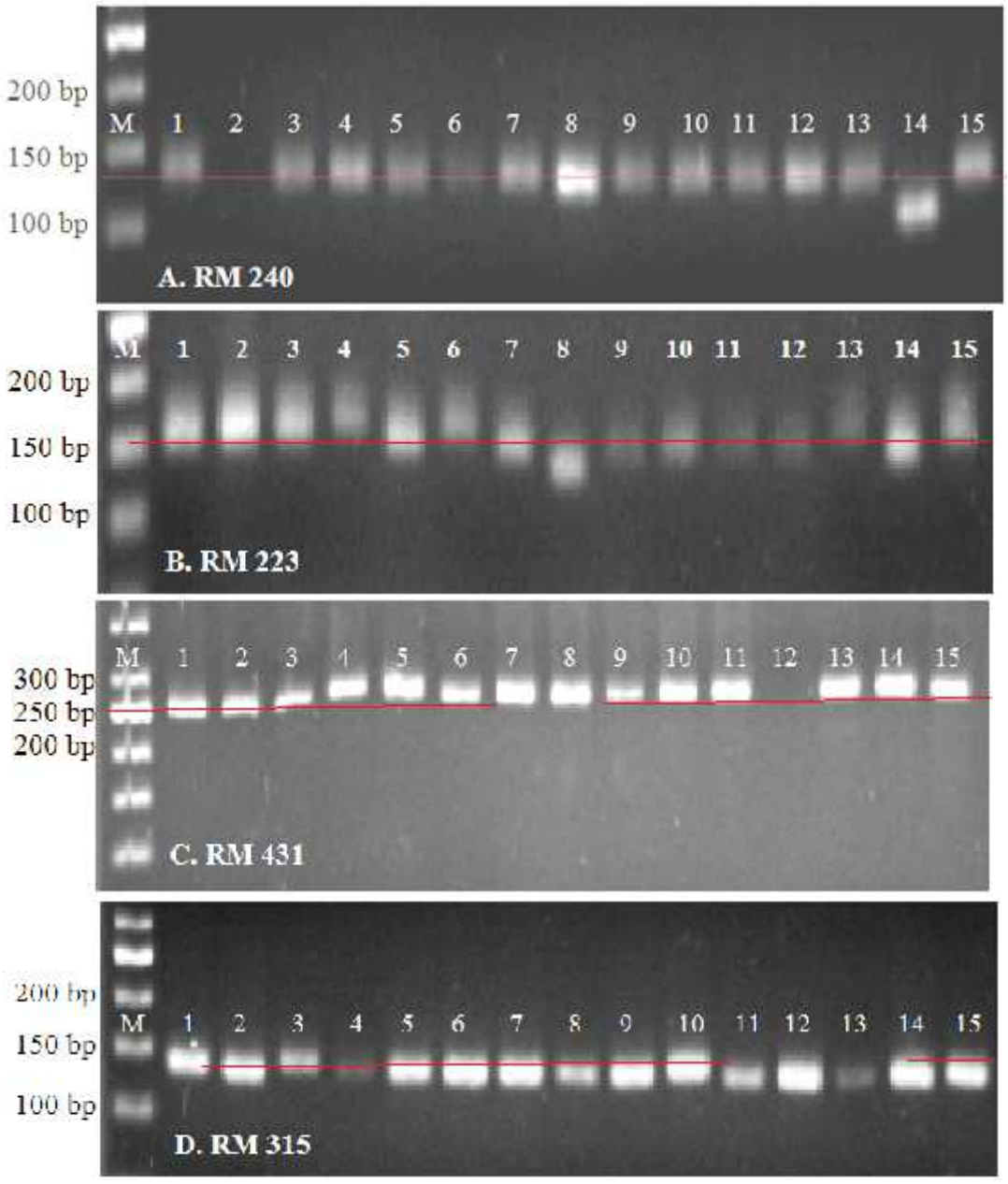
Sr. no.	Variety	Means of panicle traits		
		Panicle length (cm)	No. of panicle per plant	No. of grains per panicle
1	Malinja	29.7^{gh}	12^{abc}	64^{a}
2	Mahsuri	30^{h}	8^{a}	76^{abc}
3	Pulut Malaysia I	27.6^{efgh}	13^{abcd}	137^{f}
4	Jaya	20.4^{a}	8^{a}	100^{bcde}
5	Sri Malaysia II	$26.5^{abcdefg}$	13^{abcd}	73^{ab}
6	Sekencang	24.1^{abcde}	9^{a}	128^{def}
7	Kadaria	23.2^{ab}	16^{bcd}	140^{f}
8	Pulut Siding	28.2^{fgh}	12^{abc}	70^{ab}
9	MR84	23.7^{abc}	15^{bcd}	125^{def}
10	MR106	27.2^{cdefgh}	17^{cd}	231^{h}
11	MR127	27.9^{fgh}	11^{ab}	185^{g}
12	MR159	27.4^{defgh}	15^{bcd}	168^{g}
13	MR211	27.5^{defgh}	12^{abc}	95^{bcd}
14	Maswangi (MRQ74)	23.9^{abcd}	18^{d}	138^{f}
15	MR263	25.2^{bcdef}	17^{cd}	90^{abcd}
	Range	24.4 – 30.0	8.0 – 18.0	64.0 – 321.0

The means are compared by Duncan's Multiple Range Test. The means following the same letter are significantly different at the 0.05 level.

Table 3. Details of selected microsatellite markers associated with loci controlling panicle characteristics as reported in literature.

Locus	Motif	Forward primer	Reverse primer	Panicle characteristics	Literature/ reference
RM223	(CT)25	GAG TGA GCT TGG GCT GAA AC	GAA GGC AAG TCT TGG CAC TG	Panicle length	Brondani*et al.*, 2002
RM240	(CT)21	CCT TAA TGG GTA GTG TGC AC	TGT AAC CAT TCC TTC CAT CC	Panicle length	Deshmukh*et al.*, 2010
RM431	(AG)16	TCC TGC GAA CTG AAG AGT TG	AGA GCA AAA CCC TGG TTC AC	Panicle length, No. of panicle	Swamy*et al.*, 2014 Wickneswari*et al.*, 2012

				per plant	Septiningsih*et al.*, 2003
RM315	(AT)4, (GT)10	GAG GTA CTT CCT CCG TTT CAC	AGT CAG CTC ACT GTG CAG TG	No. of panicle per plant	Septiningsih*et al.*, 2003
RM262	(CT)16	CAT TCC GTC TCG GCT CAA CT	CAG AGC AAG GTG GCT TGC	No. of panicle per plant	Marri *et al.*, 2005 Ahamadi*et al.*, 2008
RM310	(GT)19	CCA AAA CAT TTA AAA TAT CAT G	GCT TGT TGG TCA TTA CCA TTC	No. of grains per panicle	Zhang *et al.*, 2006
RM544	(TC)9	TGT GAG CCT GAG CAA TAA CG	GAA GCG TGT GAT ATC GCA TG	No. of grains per panicle	Zhang *et al.*, 2006
RM208	(CT)17	TCT GCA AGC CTT GTC TGA TG	TAA GTC GAT CAT TGT GTG GAC C	No. of grains per panicle	Wickneswari*et al.*, 2012



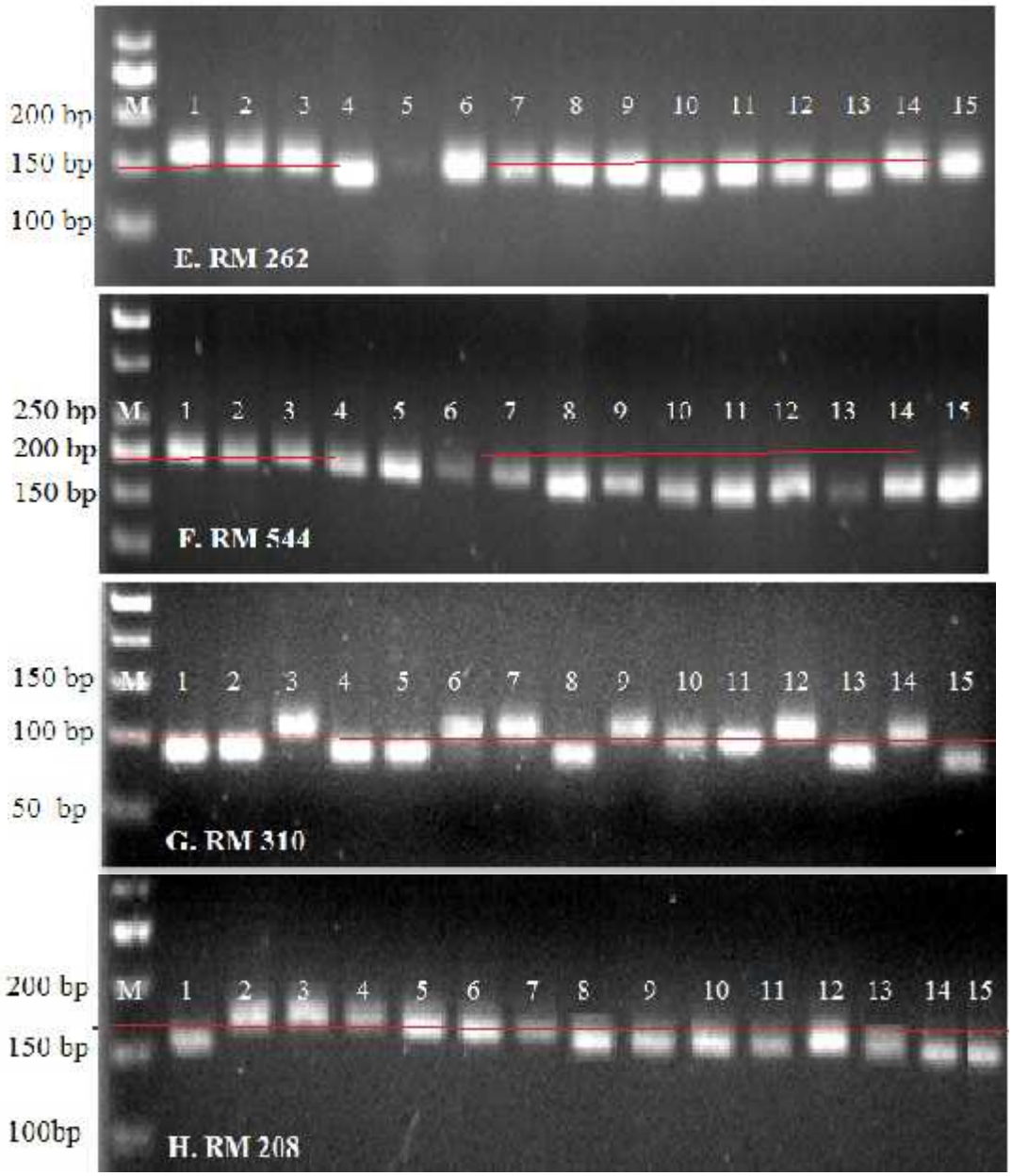


Figure 1. Microsatellites profile of 15 Malaysian rice varieties. Lane M: 50bp DNA ladder; Lane 1: Malinja; Lane 2: Mahsuri; Lane 3: Jaya; Lane 4: Pulut Malaysia 1; Lane 5: Sri Malaysia 2; Lane 6: Sekencang; Lane 7: Kadaria; Lane 8: Pulut Siding; Lane 9: MR84; Lane 10: MR106; Lane 11: MR127; Lane 12: MR159; Lane 13: MR211; Lane 14: MRQ 74; Lane 15: MR263. Figures A to H shows the polymorphism pattern of respective microsatellite markers.

DISCUSSION

Utilization of eight microsatellite markers, associated with loci controlling three panicle characteristics, in the analysis revealed that only one microsatellite marker has been identified which clearly distinguished the rice cultivars into three groups matched with phenotypic evaluation for numbers of grains per panicle.

Zhang *et al.* (2006) employed RM310 and RM126 to map a QTL associated with yield component traits that are spikelets per panicle, grains per panicle, heading date and plant height. The identified QTL explained a high phenotypic variation at 80.2% for grains per panicle. Thus, it is considered as a major QTL. Zhang *et al.* (2009) used nearly isogenic lines (NIL) mainly to conduct fine mapping the major QTLs based on QTL information and linkage analyses. It allows the detection

of major QTLs being expressed as the characteristic of single Mendelian gene. Through NIL, five QTLs for spikelet number per panicle, qSPP8 on chromosome 8, qSPP1 on chromosome 1, qSSP2 on chromosome 2, qSPP3 on chromosome 3 and qSPP7 on chromosome 7 have been mapped as single Mendelian factors (Zhang *et al.* 2006, 2009).

It is suggested that construction of near-isogenic lines and fine mapping can improve the ability to detect genes with small phenotypic effects. In present study, only RM 310 associated with a locus controlling numbers of grains per panicle discriminated the Malaysian rice varieties in three distinct groups with a specific range of numbers of grains per panicle in each group. Eight microsatellites were selected based on phenotypic variability reported by previous studies. It reflects that validity of major QTLs, to detect these across environments and mapping populations, is important (Bai *et al.*, 2012). All traits related to plant yield are quantitative and thus expression of genes depends on environmental conditions (Haung*et al.*, 2013). It may be environmental differences or genetic backgrounds that only one microsatellite marker differentiated the rice varieties for one panicle trait. Septiningsih*et al.* (2003) identified several QTLs for yield components in different environmental conditions and analyzed the data by different tools. Several of these QTLs were identified in the specific location. It would be possible reason that rest of the microsatellite markers could not discriminate the varieties as per phenotypic data. Present studies revealed that RM 310 would be a potential microsatellite marker for marker assisted selection in Malaysian rice breeding programs.

Conclusions: In the current studies, 8 microsatellite markers, associated with panicle traits, were selected based on literature review. Fifteen Malaysian rice varieties were evaluated for three panicle characteristics; panicle length, numbers of panicles per plant and numbers of grains per panicle. Banding patterns of these rice varieties for selected microsatellite markers were analysed. Microsatellite marker RM 310 (associated with a locus controlling numbers of grains per panicle) discriminated the varieties in three distinct groups based on band size. Each group showed a specific range for grains per panicle. It is therefore suggested that this DNA marker has potential to be employed in marker assisted selection for numbers of grains in rice breeding programs.

Acknowledgement: Research Management Centre (RMC) of UniversitiTeknologi Malaysia (UTM) is being acknowledged for the financial assistance Cost Center No.Q.J 130000.2645.09J08.

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