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Identifying the Grain Chalkiness Gene Using Molecular Marker Techniques in Rice (*Oryza sativa* L.)

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Abstract. Chalkiness is a major constraint on rice production because it is one of the key factors determining grain quality (appearance, processing, milling, storing, eating, and cooking quality) and price. In this study, we conducted grain chalkiness gene identification using co-dominant insertion/deletion (INDEL) markers and SSR marker combination on 50 different varieties. The application results in 7 InDel markers and SSR marker on chromosome 7 were recorded. Three primers, InDel 5, InDel 14 and RM21938, associated with grain chalkiness. For the InDel 5 primer, the amplification product was 100%. Use of primer InDel 5 in detection and evaluation of genotype to the chalkiness trait of rice grain on 50 rice varieties indicated the suitability level with phenotypic evaluation was 86% and the unsuitability level was 14%. For the InDel 14 primer, the amplification products were 100%. The suitability with phenotypic assessment was 84% and the unsuitability was 16%. For the RM21938 primer, the amplification product was 94%. The suitability with phenotypic assessment was 76% and the unsuitability was 24%. Thirteen of the selected varieties had grain chalkiness gene both InDel 5, InDel 14 and RM21938. Total 13 varieties were detected from InDel 5, InDel 14 and RM12938 primer combinations also showed high efficiency of the InDel technique in identifying chalkiness gene in rice grain. A cluster analysis was performed and a dendrogram was constructed which evinced the nature of phylogenetic classification among the genotypes of the varieties. These markers could be used for developing quality of rice in breeding program.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops as it consists a primary food source for half of worldwide population, considering that almost one-fourth of the calories consumed by humans derives from this cultivation [1, 2]. Rice quality is always linked with exportability and is also an important factor of market competitiveness, especially when rice is exported to developed nations which demand quality standards. These standards are related to acceptable shape and size of rice grains, high nutritional content, flavor, color, and low chalkiness rate, among others. Percentage of grains of chalkiness (PGWC) is one of the main indices of rice-determining appearance quality. For improvement of milling, eating, and cooking quality, the endosperm of rice varieties should be free of chalkiness since chalky grains have a lower density of starch granules compared to vitreous ones and as it is associated with high levels of damage to the kernel during milling [3]. Since consumers prefer rice free of opaqueness and strong translucence, breeding for rice grains free of chalkiness is within market requirements. Thus, the presence of more than 20% chalky kernels is not generally acceptable in most world markets. It is difficult for breeders to improve rice grain chalkiness using conventional methods, due to the lack of a discrete phenotypic segregation in the progeny. Therefore, the identification of quantitative trait loci for rice chalkiness is necessary for the development of the markers strategies in rice breeding program.

Previous studies have shown that chalkiness of rice grain is an important quality component of rice, as it has a profound influence on eating and milling qualities [1] which be controlled by triploid endosperm genetic effects [2] cytoplasmic genetic effect [4]. It has been reported that the loci controlling the percentage of grain with chalkiness (PGWC), designated as *qPGWC-7*, were located on chromosomes 7 [1]. Zhao et al [5] reported the usefulness of the targeted gene/QTLs that *SSIIa* was the major gene for chalkiness and explained up to 17 and 21 % of the variation of the degree of endosperm chalkiness and percentage of grain with chalkiness by using QK model, respectively. Adding Sun et al. [6] identified 10 common QTLs for the percentage of grain chalkiness and degree of chalky endosperm using single nucleotide polymorphism (SNP) genotyping of a chromosomal segment substitution lines cross population.

Without abundant SNP markers, inDel and SSR markers and have been widely used for this purpose. Because of the development of inDeL markers to discriminate all genome types rapidly in the genus *Oryza* [7]. One merit of co-dominant inDeL markers is easy detection directly by PCR and subsequent gel electrophoresis (Pacurar et al. 2012) [8]. Therefore, evaluation and selection of rice varieties without chalkiness or little chalkiness is important to use the variety as the source of the initial material for rice breeding rice. This study aimed, InDel and SSR markers techniques were used to assist selection varieties carrying genes without chalkiness as materials for breeding new rice varieties.

Materials and Methods

An experiment tested 50 different rice varieties from the Gene Bank of the Genetic and Plant Breeding department, Cuu Long Delta Rice Research Institute, Vietnam. After harvesting the seeds of each variety were dried using solar heat to obtain 14% moisture content, stored at room temperature for 3 months, then dehulled and scored for PGWC. PGWC was evaluated according to Dela Cruz and Khush [9]. The following levels were used for classifying endosperm chalkiness of milled rice including level 0 (no chalkiness), level 1 (less than 10% chalkiness), level 5 (10 – 20% chalkiness), and level 9 (more than 20% chalkiness). Three replicates of 100 grains per variety were assessed visually. The mean percentage of chalky grains represented the PGWC level for that line.

DNA preparation and polymerase chain reaction (PCR) protocol

Rice total DNA was extracted from fresh leaves using the cetyltrimethyl ammonium bromide (CTAB) method by Lang [10]. Using a mini scale procedure in a labeled 1.5 ml centrifuge tube in ice, DNA suitable for PCR was prepared. The young leaf was ground using a polished glass rod in well of a Spot Test plate (Thomas Scientific) after adding 400 µl of extraction buffer (50 mM tris-HCl pH 8.0, 25 mM EDTA, 300mM NaCl and 1% SDS). Another 400 µl of the extraction buffer was added and mixed into the well by pipetting. Thereafter, 400 µl of lysate was transferred to the original tube of leaf sample. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA was precipitated using absolute ethanol. DNA was then air-dried and resuspended in 50 µl of TE buffer. An aliquot of 1 µl was used for PCR analysis. DNA quality and quantity were spectrophotometrically determined.

PCR amplification was performed in 10 mM Tris-HCl (pH=8.3), 50mM KCl, 1.5mM $MgCl_2$, 1 unit of TAKARA *Taq*, 4 nmole of dNTP, 10 pmole of primer, with 30ng of genomic DNA per 25 ml using a thermal cycler 9600 (Perkin-Elmer). The PCR reactions were denatured at 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min. The final extension was set at 72 °C for 5 min. After PCR, 13ml of loading buffer (98% formamide, 10mm EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) were added to the total volume of PCR product. The samples were electrophoresed on 3% agarose gel and which was then stained with ethidium bromide and viewed. InDel markers in specific genomic regions were developed from a BLASTN alignment between the genome sequences (Table 1) [1]. Genomic sequence was obtained

from the International Rice Genome Sequencing Project (IRGSP) [11]. SSR marker was identified from the Gramene database [12].

Table 1. Primer sequences and the BAC/PAC location of markers location used for identifying of chalkiness gene.

Marker name	BAC/PAC location	Forward primer (5'-3')	Reversed primer (5'-3')
RM21938	OSJNBb0040H10	CCAAATTGCTTCCTCGGATATAGG	CGGATTTAGGGAGTTCGTGTTCG
InDel 5	OSJNBb0018H10	CAGCTATGTGTAGCTTCG	GTGCTCATTGGGCGGTTT
InDel 14	OSJNBb0040H10	TCATGATCAATGCACAA	AAGACTCCAAGACAACT
InDel 1	OSJNBb0040H10	TTGATTTCCCTGCTATAATACATGT	GGATCATCTGTCGTACCGTTCAAGCG
InDel 3	OSJNBb0040H10	CGTGTTCGTTCGTGCGATTGCTGCT	GATGAGTCCCCAAGAACAAAACTGAGC
InDel 7	OSJNBb0018H10 OSJNBb0039M16	AGAATTCAGATTCGTTGT	GTGTGTTTTCCTGTGCCG
InDel 15	OSJNBb0040H10	AACCCATAAGAAGAGGAT	AATGAGGAAGGAAGCAAT
InDel 17	OSJNBb0040H10	CGATTGTTATTTGGTATA	TATTGGTTGTAGGACTGT

Data analysis

All measurements were conducted in triplicates. An analysis of variance (ANOVA) for all data was performed using the SAS 9.1 software. Means of varieties were compared by Dunan's Multiple range test.

Pair–wise comparisons of the lines based on the presence or absence of unique and shared polymorphic products were used to calculate genetic similarity coefficients. Similarity coefficients were calculated using Nei and Li distance measure [13]. in the NTSYS–PC Numerical Taxonomy and Multivariate Analysis System [14]. The lines were clustered based on similarity coefficients using the unweighted pair group method using arithmetic averages (UPGMA) clustering algorithm.

Results

Phenotype of chalky rice

Regarding levels of the chalkiness, highly significations were observed among the varieties between primary branch and second branch traits. (Table 2)

Table 2. Phenotype analysis of the chalky rice on secondary and primary panicle branches of 50 varieties.

N_0	Variety name	Level 0		Level 1		Level 5		Level 9	
		Primary branch	Second branch	Primary branch	Second branch	Primary branch	Second branch	Primary branch	Second branch
1	OM8108	34.00j-n	28.00q-t	16.00e-q	12.33h-p	29.00b-i	25.00b-h	21.00a	34.67a
2	OM10252	83.33a-d	68.67a-i	7.67j-q	5.67m-p	7.33j-n	10.33h-l	1.67k-m	15.33d-n
3	OMCS2012	90.67a	92.00a	4.67m-q	1.67p	4.00mn	4.33j-l	0.67m	4.67n-q
4	OM6707	72.33a-h	63.33c-l	9.67i-q	5.33m-p	10.00h-n	10.33h-l	8.00b-m	19.67c-i
5	TLR378	19.67m-n	23.33st	24.33d-i	21.00d-j	57.33a	47.67a	2.00k-m	8.00j-q
6	TLR606	70.67a-h	70.33a-h	11.33i-q	6.33l-p	12.67g-n	8.33i-l	5.33d-m	15.00e-n
7	TLR463	88.00ab	75.00a-e	2.33p-q	3.00p	3.67mn	5.33j-l	6.00c-m	13.67e-o
8	OM10050	53.00f-k	49.67f-q	17.67e-p	10.00j-p	16.00e-n	15.67d-l	13.33a-d	24.67a-e
9	TLR601	51.67g-l	54.00e-o	20.33e-l	22.33c-i	18.00d-n	16.00d-l	10.00b-j	7.67j-q

10	OM6562	39.67i-n	40.00k-s	21.00e-k	25.00c-f	34.33b-f	22.67b-i	5.00f-m	12.33f-q
11	OM3673	28.67k-n	31.67o-t	22.33d-j	17.00d-m	33.00b-g	18.33c-k	16.00ab	33.00ab
12	OM10373	27.67k-n	26.00q-t	20.33e-l	22.67c-h	38.33a-d	24.67b-h	13.67a-c	26.67ad
13	OM10418	48.00g-l	48.67g-q	17.00e-p	10.33j-p	27.00b-k	23.00b-i	8.00b-m	18.00c-k
14	OM10450	62.33b-i	48.67g-q	18.67e-n	16.00e-n	14.67e-n	15.67d-l	4.33g-m	19.67c-i
15	OM6564	85.67a-d	69.33a-h	4.00n-q	6.33l-p	8.00i-n	14.33e-l	2.33j-m	10.00i-q
16	OM284	25.67l-n	29.33p-t	31.33b-e	21.00d-j	35.67b-e	26.67b-f	7.33c-m	23.00b-g
17	OM138	93.67a	85.67a-c	1.33q	3.33p	2.67mn	6.67j-l	2.33j-m	4.33n-q
18	OM279-1	49.00g-l	24.00r-t	18.00e-o	24.67c-g	28.00b-j	32.00bc	5.00e-m	19.33c-i
19	TLR594	46.67h-m	48.00g-r	21.67e-k	20.67d-j	26.00b-l	19.67c-j	5.67c-m	11.67h-q
20	OM10375	71.33a-h	60.00d-m	9.33i-q	8.00k-p	6.33k-n	9.00i-l	13.00a-e	23.67a-f
21	OM10383	70.00a-h	49.33f-q	16.00e-q	10.67i-p	5.00l-n	11.00g-l	9.33b-k	29.00a-c
22	TLR368	31.33k-n	24.00r-t	42.33a-c	43.33a	26.00b-l	29.67b-e	0.33m	3.00o-q
23	OM70L	60.00c-j	58.33d-n	12.00h-q	6.33l-p	19.33d-n	17.67c-l	8.67b-l	17.67c-l
24	TLR444	84.67a-d	81.00a-d	7.00j-q	4.33n-p	6.00k-n	9.00i-l	2.33j-m	5.67n-q
25	TLR390	14.00n	14.67t	28.33b-g	26.00c-e	46.33ab	26.33b-g	11.33b-h	33.00ab
26	TLR391	90.33a	73.33a-f	5.00l-q	5.67m-p	3.33mn	8.67i-l	1.33lm	12.33g-p
27	TLR393	54.33e-k	47.00h-s	29.67b-f	24.33c-g	12.33g-n	14.67e-l	3.67h-m	14.00e-o
28	TLR394	82.00a-d	60.33d-l	7.33j-q	7.33k-p	8.67h-n	14.67e-l	2.00k-m	17.67c-l
29	TLR395	52.00g-l	44.33i-s	16.00e-q	10.67i-p	26.00b-l	23.33b-i	6.00c-m	21.67b-h
30	TLR397	19.67m-n	35.67m-t	27.00c-h	21.33d-j	42.00a-c	30.33b-d	11.33b-h	12.67f-p
31	CanTho 3	74.00a-g	68.33a-i	14.33f-q	13.33f-p	9.33h-n	11.33f-l	2.33j-m	7.00k-q
32	TLR456	38.33i-n	47.33g-s	37.67a-d	34.00a-c	16.67e-n	11.67f-l	7.00c-m	7.00k-q
33	TLR442	74.00a-g	71.33a-g	11.33i-q	8.33k-p	10.67h-n	13.67f-l	4.00h-m	6.67l-q
34	TLR402	54.33e-k	57.67d-n	15.67f-q	18.33d-k	18.00d-n	18.67c-j	12.00b-g	5.33n-q
35	OM10000	31.00k-n	34.33n-t	50.00a	40.00ab	16.33e-n	19.67c-j	2.67j-m	6.00m-q
36	OM10037-3	59.00d-j	52.67e-p	15.33f-q	13.33f-p	18.00d-n	15.33d-l	7.67c-m	18.67c-j
37	TLR604	40.33i-n	28.33p-t	20.00e-m	21.33d-j	28.67b-j	38.00ab	11.00b-i	12.33g-p
38	OM10029	29.33k-n	43.33j-s	43.67ab	28.67b-d	23.67c-m	22.33c-i	3.33i-m	5.67n-q
39	OM10258	80.33a-e	63.33c-k	13.00g-q	13.00g-p	5.67k-n	8.67i-l	1.00lm	15.00e-n
40	OM28L	40.00i-n	39.00l-t	14.67f-q	20.33d-j	42.67a-c	26.67b-f	2.67j-m	14.00g-p
41	OM4900	86.33a-c	90.33ab	6.33k-q	5.67m-p	6.33k-n	3.00kl	1.00lm	1.00pq
42	OM6161	82.33a-d	80.67ad	9.33i-q	5.00n-p	7.33j-n	10.00h-l	1.00lm	4.33n-q
43	TLR396	91.67a	89.00ab	3.00o-q	3.00p	4.67l-n	4.33j-l	0.67m	3.67o-q
44	OM7374	60.33c-j	66.00b-j	20.00e-m	10.67i-p	19.00d-n	13.00f-l	0.67m	12.33g-p
45	OM10041	52.00g-l	44.67i-s	24.00d-i	15.33e-o	19.00d-n	26.00b-j	5.00f-m	14.00e-o
46	OM10396	39.00i-n	35.67m-t	19.00e-n	18.00d-l	29.67b-h	29.33b-e	12.33b-f	17.00d-m
47	CanTho2	71.67a-h	64.33c-j	12.00h-q	11.67h-p	13.33f-n	16.00d-l	3.00j-m	8.00j-q
48	OMCS2000	80.00a-f	76.00a-e	7.33j-q	9.67j-p	7.67i-n	10.00h-l	5.00e-m	4.33n-q
49	IR64	86.33a-c	91.33a	6.33k-q	4.00op	4.33mn	3.00kl	3.00j-m	1.67pq
50	KDM105	88.67ab	90.67a	7.67j-q	5.00n-p	2.00n	2.67l	1.67k-m	1.67pq
F		8.97**	9.02**	6.04**	8.19**	4.64**	4.81**	4.33**	7.26**
CV (%)		22.65	22.06	45.68	40.81	58.36	45.16	66.89	41.17

Note: Coefficient of variation (CV), ** significant at P< 0.01

The level 0 of four varieties (TLR391, OMCS2012, TLR396 and OM138) were more than 90% primary branch number. While there were four varieties (OM4900, KDML105, IR64 and OMCS2012). OMCS2012 had high percentage of level 0 both primary branch and second branch traits.

There were two varieties (OM10000 and TLR368) had high percentage of level 1 in second branch trait (40 % and 43.33 %, respectively). The level 1 only one variety as OM10000 had higher percentage than all verities in both primary branch and second branch traits (50%).

Performing PCR reaction with Indel markers

Among the seven InDel primers (InDel 1, InDel 3, InDel 5, InDel 7, InDel 14, InDel 15, and InDel 17), InDel primers as InDel 5, InDel 14 linked to chalkiness gene were found to be highly polymorphism among 50 rice varieties in this study. (Fig.1). The results show that the primer amplified 95% of the samples and that all of the samples recorded polymorphism. Based on the difference between the alleles exhibiting via bands on the gel, it can identify the difference between the varieties of genetics.

Primer InDel 5, the results obtained showed that the number of samples generating the different band with two alleles was often clear in the position of molecular marker size. The difference of quantity and position of bands can show the difference of DNA sequence between the varieties. To observe the bands with the size of 190bp in comparison with standard ladder corresponding allele A carrying chalkiness gene. For the band with the size of 200bp in comparison with standard ladder corresponding allele B without carrying chalkiness gene. The primer InDel 5 identified chalkiness gene for 36% of varieties and 64% varieties to the non-chalkiness gene. (Fig. 1)

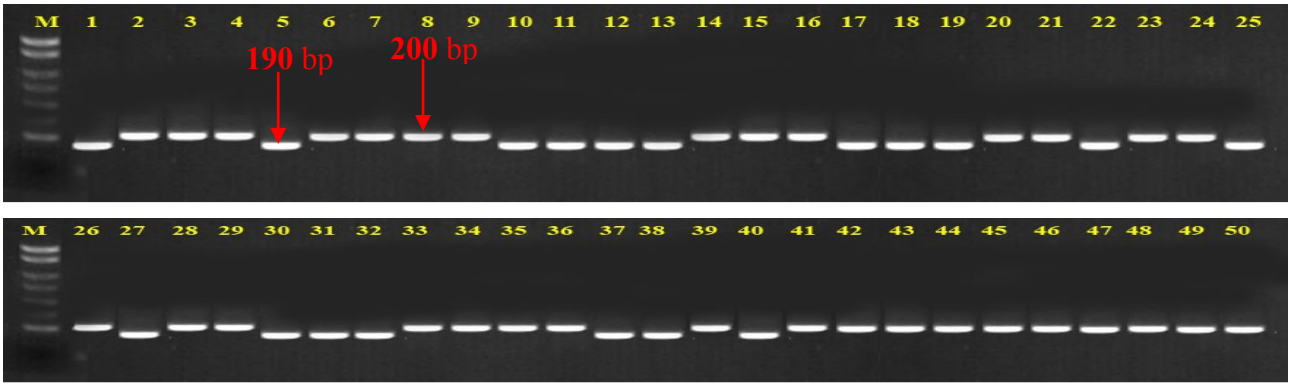

Figure 1. Detection of chalkiness gene in different rice verities by primer InDel 5 observed on the agarose gel 3%.

Primer InDel 14, Results obtained a few samples produced the different bands with two alleles often clear at the position of the band, which can show the difference of DNA sequence between the varieties. Based on bands recorded both alleles, allele A (210bp) and allele B (220bp). The varieties with a combination of allele A (210bp) and allele B (220bp) bands will have chalkiness gene and non-chalkiness gene, respectively compared with the standard ladder. The primer InDel 14 showed 42% varieties carrying chalkiness gene in the 50 varieties, 58% varieties non-chalkiness gene. (Fig. 2)

In Case of using SSR marker located gene affecting the chalkiness trait on the rice grain helped assess the genetic linkage of the rice varieties. The SSR marker locus primer RM21938 on chromosome 7 showed two alleles often clear at the position of the band, which can show the difference of DNA sequence between the varieties. Based on bands recorded both alleles, allele A (200bp) and allele B (210bp) will have chalkiness gene and non-chalkiness gene, respectively in comparison with standard ladder. The seventeen varieties (34%) were identified carrying with chalkiness gene and 64% varieties non chalkiness gene. (Fig. 3)

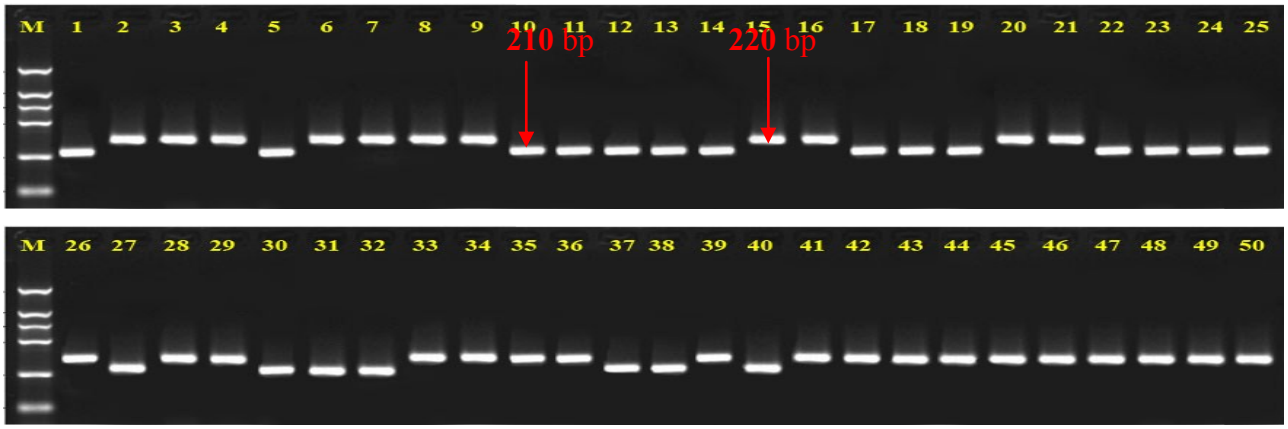

Figure 2. Detection of chalkiness gene in different rice verities by InDel 14 primer observed on agarose gel 3%.

The positions of 16, 17, 18 on Fig. 3 were equivalent to OM284, OM138, OM 279-1 at the three positions without exhibiting bands. Therefore, all of these three varieties did not carry chalkiness gene on rice grain. The position of 21 was equivalent to OM10383, which exhibited 2 (poly allele) carrying gene affecting simultaneously to the high and low chalkiness trait of rice grain.

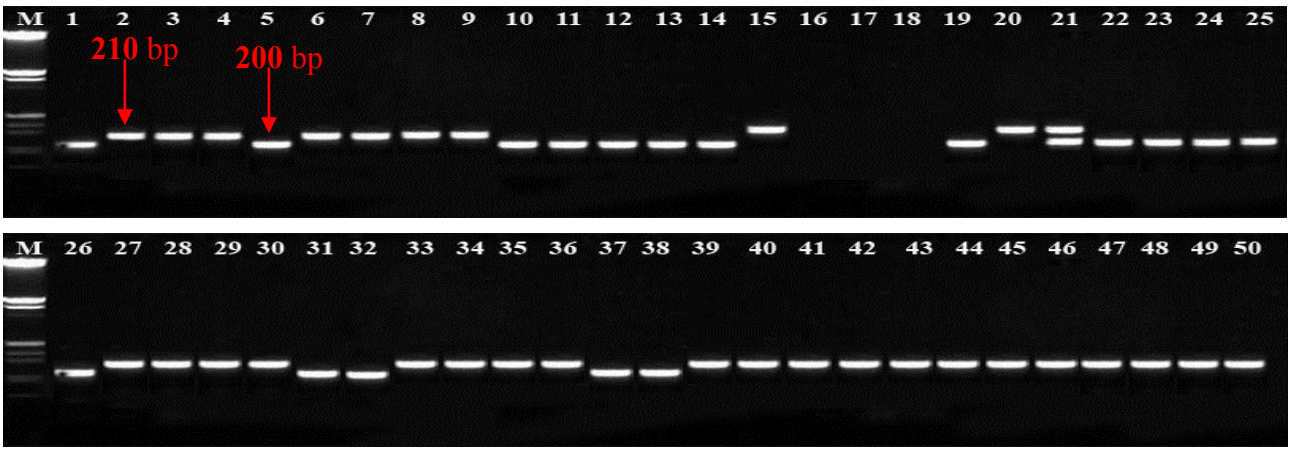

Figure 3. Detection of chalkiness gene in different rice verities by RM21938 observed on agarose gel 3%.

In short, using three primers to predict genotype and phenotype of 50 varieties had the high result. Primer InDel 5 indicated the suitability between phenotype and genotype was 86%, accounting for the highest rate in three primers and the unsuitability was only 14% of the total.

Grouping of varieties and clustering analysis

The dendrogram using UPGMA cluster analysis, the varieties had the similar genetic size in the same group (Fig. 4). Total 50 varieties grouped into 2 main groups with the homologous genetic coefficient of 1.91, the chart can be classified into two major groups A and B. However, in groups A and B focusing numerous varieties were divided into 4 sub-clusters as A1, A2, B1, and B2.

Group A, at the fusion of 0.42 consisted of 29 varieties and were divided into 2 minor groups A1 and A2. Group A1 consisted of 27 varieties (at the fusion ranged from 0.95 – 1.43). Group A1 was divided into 2 sub-cluster (group A1.1and group A1.2) with the fusion of 0.48. Group A1.2 (the same fusion with group A1.1. Group A2 (the same fusion level with group A1) this group included 2 varieties OM284 and TLR391. Comparison with the phenotype of varieties in group A1.2 had low chalkiness rate relatively with level 9 ranged from 0.67 – 24.67%) (Table 2).

Cluster B was further divided into two sub -clusters (B1 vat B2) with fusion level of 1.43 including 21 varieties. Group B1 (the same fusion with group B2): included the remaining varieties and were also divided into 2 minor groups: B1.1 and B1.2. Group B1.2 included 3 varieties OM70L, TLR444, OM10450. Group B1.1 included 15 varieties which had the chalkiness rate of

level 9 ranged from 0.33 – 34.67%. As the comparison with the phenotype, the chalkiness rate was relatively high. Group B2 (with fusion ranged from 0.48-0.95) including 3 varieties TLR393, TLR397, OM28L (Table 2).

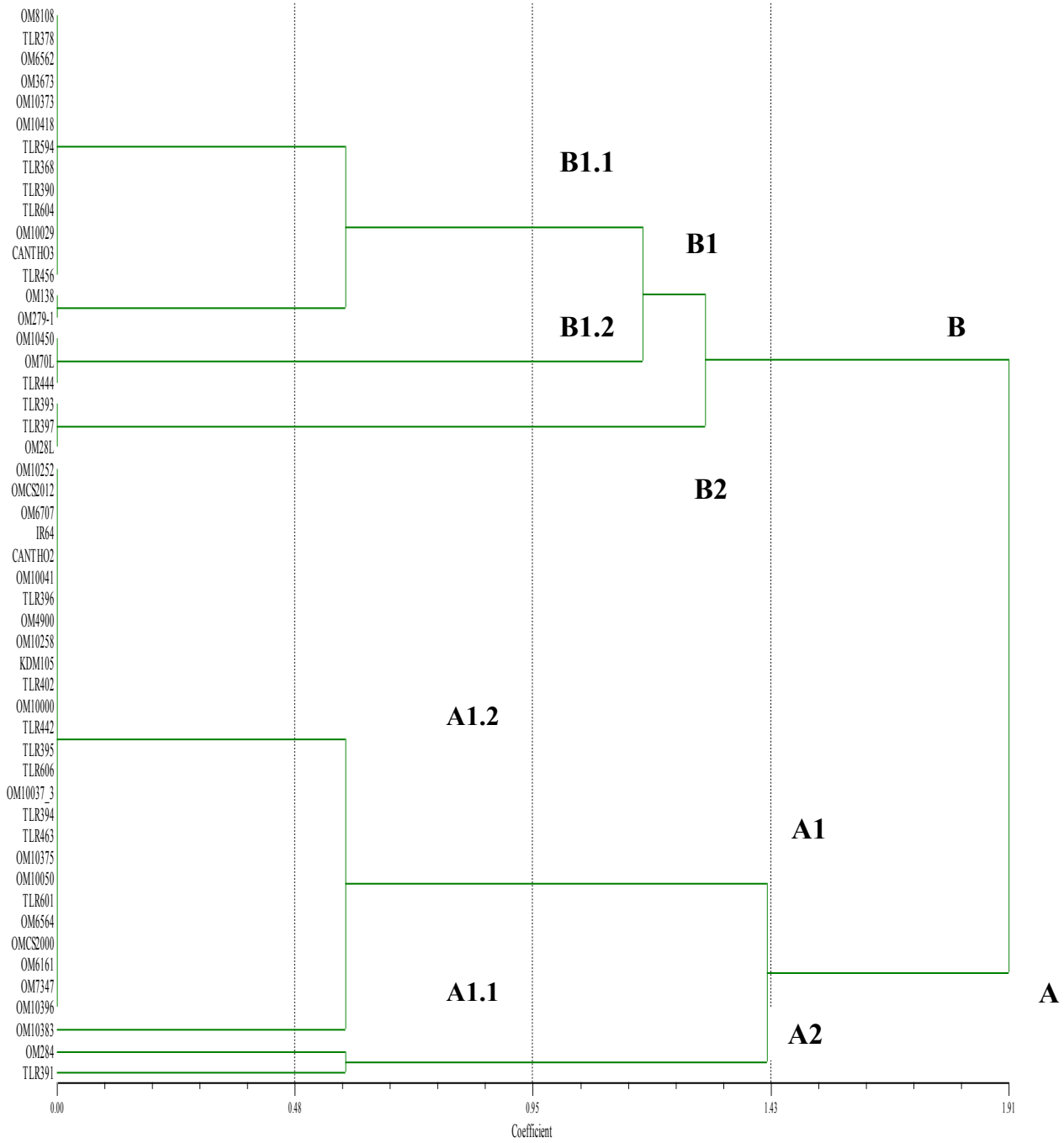

Figure 4. The Cluster analysis dendrogram of 50 varieties according to genotype.

Discussion

Grain quality in rice is a very important character as it dictates a variety acceptance by farmers, marketing and a parent variety other uses in the breeding program. Famer's perception and preference can be used by breeders to develop varieties that might have higher adoption rate and thus higher productivity. Wang et al. [15] reported that compact panicle cultivars had the large variation of physicochemical traits as chalky grain rate and amylose content on the rice panicle. A high percentage of grain chalkiness is a major problem because it diminishes grain quality in rice. Chalkiness of ordinary rice varies from 5% to 50%, and depending on the level of chalkiness is

ranked from 1 to 9. Chalkiness makes trace opacity in the endosperm of the grain, without affecting the quality of cooked rice, because the quality of cooked rice is related to amylose content. For ordinary rice, high chalkiness will affect the rate of broken grain (or broken rice) in milling quality. Adding, the ratio of secondary and primary panicle branches are important factors which in chalk – primary grains fill faster. The current study clearly shows marked differences between the occurrence between secondary and primary panicle branches. The chalky grain rate of secondary branches was substantially lower than that of the primary branches, suggesting the possibility of improving both grain yield and appearance quality (chalkiness) through selecting compact panicle type and increasing secondary branches number. In addition, Xu et al [16] suggested that increasing grain number on the secondary branches of the middle to upper parts be an effective way for improving grain yield and quality. Therefore, evaluation of chalkiness trait of rice needs for closely combination between genotypic and phenotypic assessment.

Zhou et al [1] described *qPGWC-7* as the first fine mapped gene by InDel 14 and InDel 3 for white-belly endosperm in rice, which can explain 60.6% of phenotypic variation in their population. At present in breeding practice, the decrease of rice chalkiness has become one of the main aims in rice quality breeding, especially for *indica* rice. The overall assessment of PCR products was majority were suitable to assess phenotype. However, there were still some varieties exhibiting incorrect comparison with phenotypic analysis. Our results, the primer with the highest suitable amplification product to phenotypic assessment was InDel 5 (86%). Next, to primer InDel 14 amplification products were consistent with the phenotype at 84%, and finally, RM21938 at 76%. Chalkiness is very susceptible to environmental conditions such as temperature, fertilizer, and humidity, which might be an important reason why only a few associations were identified using published markers [17]. Here, we identified for grain chalkiness using 7 InDel markers and SSR marker Techniques polymorphism genotyping in 50 varieties. However, one SSR and two InDel markers were informative among 50 varieties because of low polymorphism among varieties.

The cluster analysis assisted in grouping the genotypes into clusters having specific characteristic traits which may be helpful in selecting parents for future breeding programs. Thus, the cluster analysis provides some varieties which can be utilized for improvement rice quality in future breeding programs.

Conclusion

The evaluating results from genotypic analysis suggested that 3 primers used in PCR – SSR reaction the amplification products were most produced accounting for over 94% of the total amplifiers.

- Results of genotypic analysis: For primer InDel 5 had the amplification product of 100%. Using primer InDel 5 in detection and evaluation of genotype to the chalkiness trait of rice grain on 50 rice varieties showed that in accordance with phenotypic evaluation of 86% and unsuitability of 14%.
- For primer InDel 14 had the amplification product of 100%. The suitability to the genotypic assessment of 84% and the unsuitability of 16%.
- For primer RM21839, amplification product was 94%. The suitability with the phenotypic assessment of 76% and the unsuitability of 24%.
- In this study, 13 varieties were detected from InDel 5, InDel 14 and RM12938 primer combinations also showed high efficiency of the InDel technique in identifying chalkiness gene in rice grain.

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