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Advances in Ecological and Environmental Research, 35-44 | Science Signpost Publishing

Development of Molecular Markers for Purity Testing in Thai Jasmine Rice

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Received: August 31, 2016 / Accepted: October 01, 2016 / Published: December 15, 2016

Abstract: Purity testing in paddy rice is sometime difficult by using phenotypic characters alone. Molecular markers can be more accurate technique for purity testing. Simple sequence repeats (SSRs) markers are widely spread throughout the rice genome and are used for purity testing in rice. DNA banding patterns from an automated DNA sequencer showed more allelic variability than those from a polyacrylamide gel electrophoresis technique. An average allelic pattern detected by using an automated DNA sequencer is 9.38 alleles per locus. The marker RM20B showed the highest allelic variation of 20 alleles, while the marker RM165 and Glu-23 showed the least allelic variation of 3 alleles. Those markers that can differentiate the rice varieties KDML105, Pathumthani 1 and Chainat 1 are RM21, RM20A, RM20B, RM209, RM3, GT11, RM232 and RM235. When using polyacrylamide gel electrophoresis technique, there was less allelic variation than detected by an automated DNA sequencer. An average allelic pattern detected by using polyacrylamide gel electrophoresis is 4.53 alleles per locus. The marker RM20A showed the highest allelic variation of 9 alleles, while the marker RM165, B03 and Glu-23 showed the least allelic variation of 2 alleles. Those markers that can differentiate the rice varieties KDML105, Pathumthani 1 and Chainat 1 are RM21, RM20A, RM20B, RM209, RM3, RM248, GT11, RM204 and RM3627. Those markers used for purity testing can be classified into 3 groups; 1) those used for identifying soft and hard cooked rice, 2) those used for identifying glutinous and non-glutinous type and 3) those used for identifying aromatic and non-aromatic variety.

Key words: molecular markers, rice purity testing, allele standard, SSR markers

1. Introduction

There are several rice varieties that have been produced in Thailand and sold in both domestic and international markets. Different rice varieties have different physical grain qualities and cooking qualities. For cooking and eating qualities, they depend a lot on the variety's amylose content. Mixing different

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varieties with different grain qualities can lead to poor cooking and eating qualities. The problem will be more serious if a low amylose content variety is mixed with a high amylose content one.

Currently, Thai Jasmine rice is very popular and can be sold at a very high price, leading to a problem of mixing Thai Jasmine rice with other rice varieties that have poorer qualities. Contamination can be occurred at several stages of rice production, including unintentionally seed mixing with other varieties during seed preparation, during harvesting, and during grain processing. However, contamination can also occur intentionally by the traders.

Purity testing can be conducted since the beginning of production process, i.e. field inspection by evaluation of agronomic traits, such as plant type, leaf color, grain shape, grain color, and etc. However, these traits, sometimes, can not be used to differentiate rice varieties. The most accurate method to identify rice varieties is to conduct a DNA test. Simple Sequence Repeats (SSRs) or microsatellites are molecular markers consisting of repeating units of 1-6 bases and that are interspersed throughout the genome of eukaryotes, including rice. SSR markers are suitable for conducting DNA fingerprinting, diversity study, chromosome identification, gene mapping, QTL mapping and marker-assisted selection. More than 3,200 loci of SSR markers have been mapped onto the rice genome (McCouch *et. al*, 2002). The SSR markers are, therefore, suitable to use for developing markers for purity testing in rice.

2. **Methodology**

Materials

1. Six rice cultivars for making allele standard; CT9993-5-10-1-M, IR36, IR64, Azucena, Lemont and Nipponbare. Eleven rice lines/varieties used as standard control; KDML 105, IR77924-UBN-27-39, RD33, Pathumthani 1, RD6, Chainat 1, RD15, Niew Ubon 2, Hawm Pitsanulok 1, Phitsanulok 2 and Phitsanulok 3. Ten rice varieties from the National Rice Genebank; Niew Sanpatong, Jao Haw, Jekchuy garbkeaw, Kao Niew Dam, Sangyod Phattalung, Kao Gaw Diew, Pinkaew 56, KDML 105, RD6 and Kao Tah Haeng 17.
2. Automatic DNA sequencer (Bechman)
3. Sequencing gel electrophoresis (BIO-RAD, Power PAC 3000) and agarose gel electrophoresis (Owl Separation Systems Inc., Model A2)
4. Chemicals necessany for DNA extraction, PCR reactions and DNA staining.
5. Thirty-two loci of Simple Sequence Repeats (SSRs) markers

Methods

1. DNA extraction from leaf tissue samples

- Rice plants were grown in pots and leaf tissue samples were collected at 30 days. Fifty grams of leaf tissue were put into liquid nitrogen and the samples were ground manually.

- DNA extraction was conducted following a procedure described by Saghai-Maroof *et al*. (1984)

2. DNA amplification using SSR markers

- DNA amplification by a polymerase chain reaction (PCR) using a procedure described by Liang and Pardee (1997).

3. Fragment analysis using polyacrylamide gel electrophoresis.

- After DNA amplification by SSR markers, the PCR products were then separated by using polyacrylamide gel electrophoresis. The PCR products were loaded onto 4% polyacrylamide gel. The gel was then run through an electric field under 1X TBE buffer (pH 8.0).

- The DNA banding patterns were visualized using a silver staining protocol.

4. Fragment analysis using an Automated DNA Sequencer.

- An automatic DNA sequencer was also used to conduct a DNA fingerprinting. The multiplexing PCR reactions could be prepared, 3 loci could be determined simultaneously by using 3 different fluorescent dyes tagging to each marker.

3. Results and Discussion

1. DNA banding patterns of allele standard derived from an automatic DNA sequencer.

An automatic DNA sequencer could generate more variation of DNA fragments since the machine could differentiate fragments with only 1-base different. Samples of fragment analysis derived from an automatic DNA sequencer were shown in figure 1, which analyzed fragments amplified with the marker RM17 in rice varieities KDML 105, Chainat 1, Pathumthani 1, RD33, RD6, RD15, Niew Ubon 2 and Hawm Pitsanulok 1. The sizes of DNA fragments of each variety were 177, 158, 185, 177, 177, 177, 158 and 177 bases, respectively.

An average allelic variation per locus was 9.38 alleles, which the locus RM20B gave the highest number of 20 alleles while RM165 and Glu-23 gave the lowest allele number of 3 (Table 1). The markers that could be used to differentiate the varieties KDML 105, Pathumthani 1 and Chainat 1 were RM21, RM20A, RM20B, RM209, RM3, GT11, RM232 and RM235 (Table 1).

2. DNA banding patterns of allele standard derived from polyacrylamide gel electrophoresis

The variation of DNA fragments obtained from polyacrylamide gel electrophoresis was lower than that obtained from an automatic DNA sequencer. An average number of alleles per locus was 4.53. The RM20A locus gave the highest allele number of 9, while RM165, BO3 and Glu-23 gave the lowest allele number of 2 (Table 2). Some markers, such as RM17 and RM3, could be used to differentiate the DNA bands by polyacrylamide gel electrophoresis (Figures 2 and 3) Those markers that could be used to differentiate the rice varieties KDML 105, Pathumthani 1 and Chainat 1 were RM21, RM20A, RM20B, RM209, RM3, RM248, GT11, RM204 and RM3627 (Table 2).

4. Conclusion

The use of SSR markers, which are interspersed throughout the rice genome, for studying genetic diversity and conducting DNA fingerprints can be considered as a convenient and rapid technique. These markers can also be used for purity testing. The loci suitable for purity testing should be those that generate a high number of allelic variations. From this study, 3 groups of markers used for detecting rice varieties with different grain qualities were obtained. First, those markers used to determine cooking and eating qualities, separating soft cooked rice from hard cooked rice, for example, GT11, RM3 and RM17. Second, those markers used to determine starch type, separating non-glutinous from glutinous varieties, i.e. Glu-23 and RM225. Third, those markers used to separate aromatic from non-aromatic varieties, i.e. B03, RM204 and RM248 (Table 3).

Development of molecular markers for purity testing is a useful technique for controlling the quality of rice products. It is an accurate and reliable method since it did not depend on environment. The customers are more confident when they buy products with better quality control system.

References

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Saghai Maroof, M. A., K. M. Soliman, R. A. Jorgensen and R. W. Allard. 1984. Ribosomal DNA spacer length polymorphism in Barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acd. Sci. USA 81: 8014-8018.

Table 1. Genotypic data from an automatic DNA sequencer of some SSR markers that showed high and low allelic variation.

Cultivar	Marker					
	RM20A	**RM20B**	RM206	RM17	Glu23	RM165
CT9993-5-10-1-M	10	11	3	1	2	1
IR36	13	15	4	4	2	3
IR64	5	6	11	4	2	3
Azucena	10	3	15	4	1	2
Lemont	2	3	3	1	2	2
Nipponbare	1	1	13	1	2	1
KDML 105	**8**	**8**	9	3	2	3
RD33	8	8	9	3	2	3
IR77954-UBN-27-39	8	18	14	1	2	3
PTT1	**4**	**4**	4	4	2	3
RD6	11	11	9	3	3	3
CNT1	**14**	**19**	4	1	2	3
RD15	8	8	9	3	2	3
NUB2	3	5	1	1	3	3
HPSL1	6	6	7	3	2	3
PSL2	6	6	4	1	2	3
PSL3	9	16	11	4	2	3
NSPTGB	15	20	10	1	3	3
JHGB	16	2	2	2	2	1
JCGB	7	10	12	4	2	2
NDGB	12	14	8	1	3	3
SYGB	7	17	6	4	2	1
KKDGB	8	8	12	4	2	2

PK56GB	8	12	5	1	2	3
KDMLGB	8	8	9	3	2	3
RD6GB	11	12	9	3	3	3
KTH17GB	9	9	10	1	2	3
Allelic variation	**16**	**20**	15	4	**3**	**3**

Table 2. Genotypic data from Sequencing Gel of some SSR markers that showed high and low allelic variation.

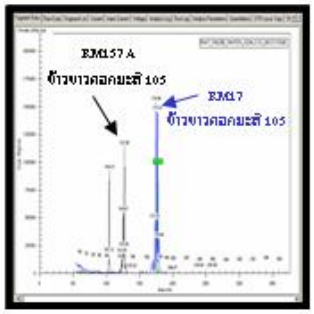
Cultivar	**Marker**					
	RM20A	**RM20B**	RM1	RM165	B03	Glu23
CT9993-5-10-1-M	4	4	3	1	1	1
IR36	1	7	1	1	2	1
IR64	1	7	1	1	2	1
Azucena	6	6	5	1	1	1
Lemont	5	5	8	1	1	1
Nipponbare	5	5	6	2	1	1
KDML 105	**1**	**1**	1	1	1	1
RD33	1	1	1	1	1	1
IR77954-UBN-27-39	1	1	1	1	1	1
PTT1	**8**	**4**	1	2	1	1
RD6	4	4	3	1	1	2
CNT1	**2**	**2**	2	1	2	1
RD15	1	1	1	1	1	1
NUB2	3	3	4	1	2	2
HPSL1	1	7	1	1	1	1
PSL2	1	7	7	1	1	1
PSL3	4	4	7	1	1	1
NSPTGB	4	4	3	1	1	2
JHGB	9	5	4	2	1	1

JCGB	1	5	2	1	1	1
NDGB	7	8	1	1	2	2
SYGB	1	5	2	2	2	1
KKDGB	1	1	2	2	1	1
PK56GB	1	1	1	1	1	1
KDMLGB	1	1	1	1	1	1
RD6GB	4	4	3	1	1	2
KTH17GB	1	1	1	1	3	1
Allelic variation	**9**	**8**	**8**	**2**	**2**	**2**

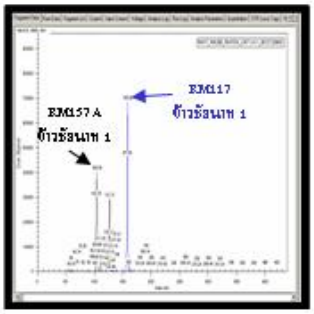
Table 3. Groups of markers identifying different characteristics of Thai rice varieties.

Characteristics	**Markers**
1) Cooking quality (soft vs hard cooked rice)	RM3, RM209,RM20A, RM20B, RM21, RM17, RM149, GT11, RM190, RM239, RM157A, RM261, RM257, RM3627, RM1, RM202, RM206, RM224, RM232, RM235
2) Starch type (Glutinous vs Non-Glutinous)	Glu-23, RM225,
3) Aroma	RM248, RM204, B03

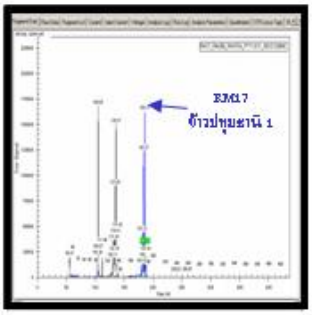
Figure 1. Allele Standard detected by an automated DNA sequencer of KDML 105, CN1, PTT1, RD33, RD6, RD15, NUBN2 and HPSL1 at RM157A and RM17 loci.



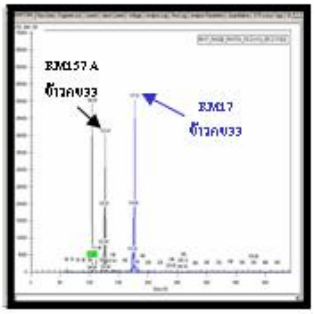
KDML105 : Allele D (177 bp)



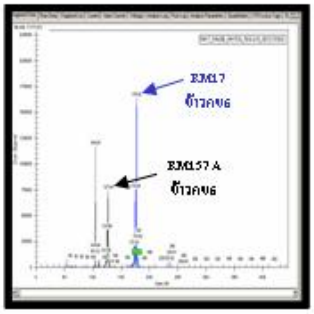
CN1 : Allele B (158 bp)



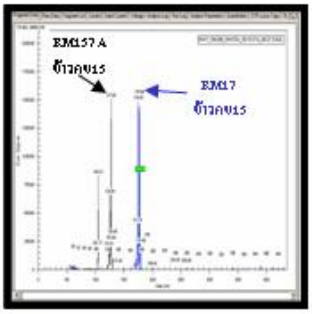
PTT1 : Allele E (185 bp)



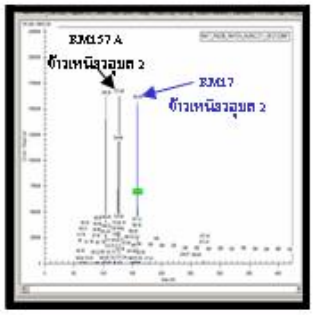
RD33 : Allele D (177 bp)



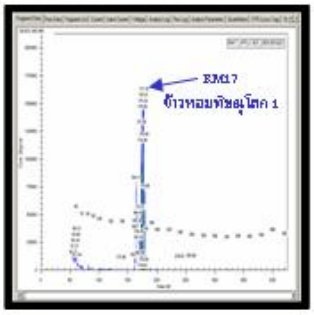
RD6 : Allele D (177 bp)



RD15 : Allele D (177 bp)



NUBN2 : Allele B (158 bp)



HPSL1 : Allele D (177 bp)

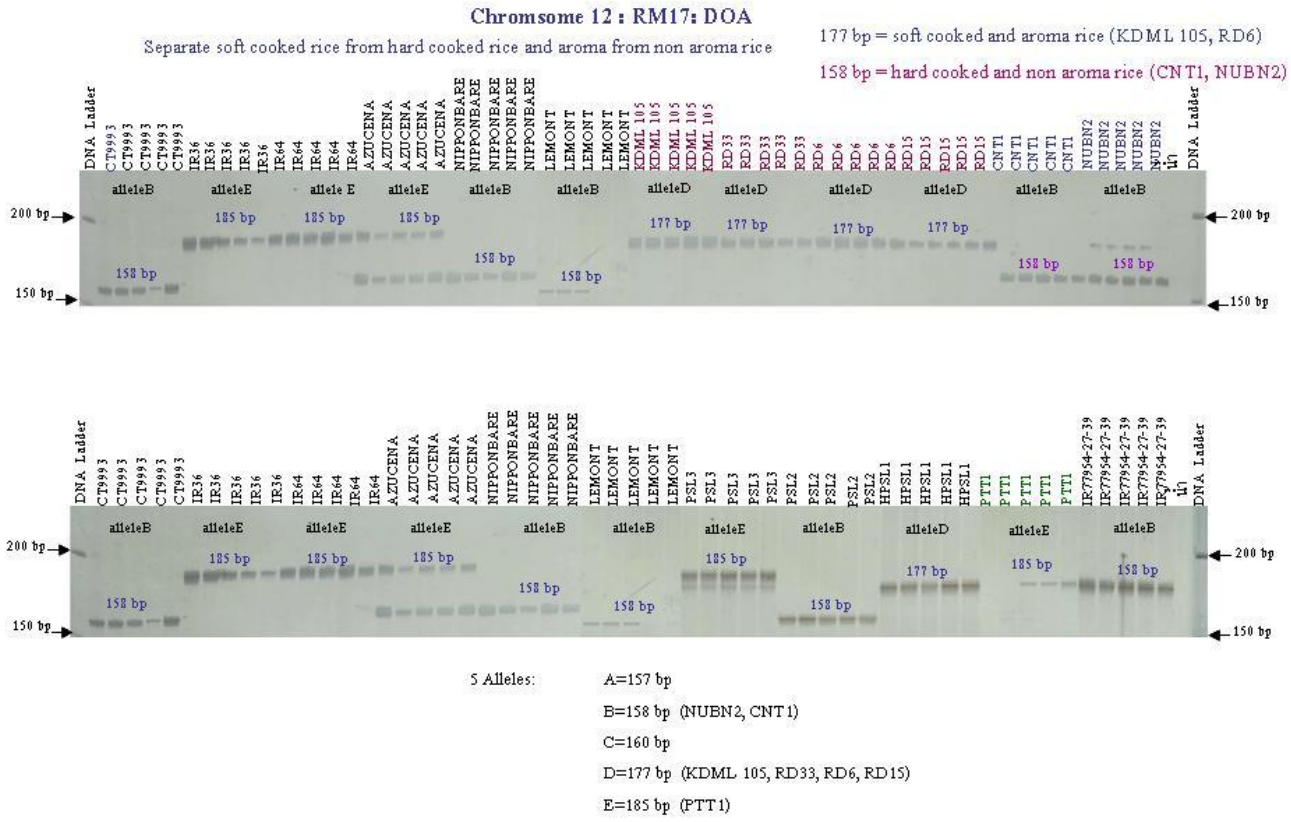
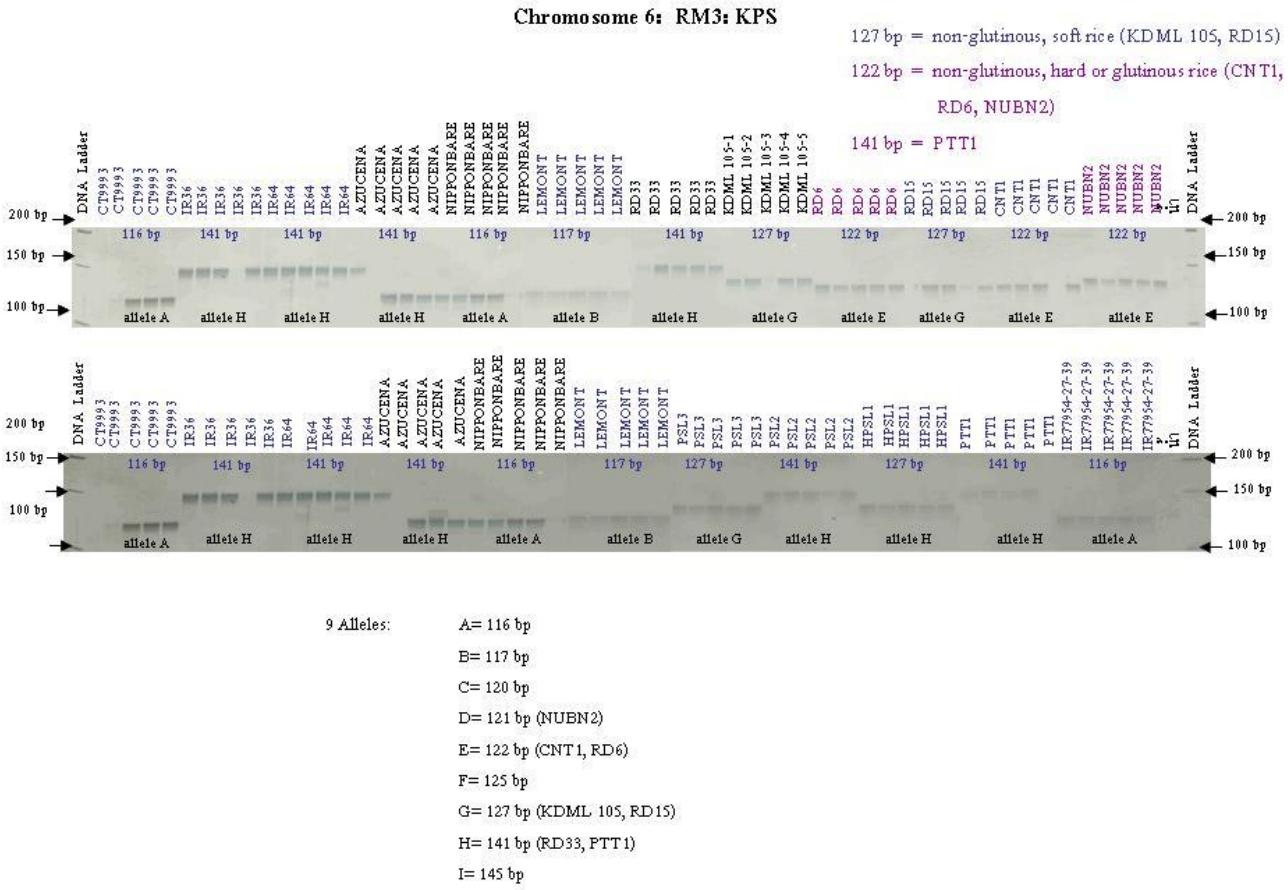


Figure 2. Allele Standard detected by polyacrylamide gel electrophoresis of 17 rice varieties at an RM17 locus.



9 Alleles:
A= 116 bp
B= 117 bp
C= 120 bp
D= 121 bp (NUBN2)
E= 122 bp (CNT1, RD6)
F= 125 bp
G= 127 bp (KDML 105, RD15)
H= 141 bp (RD33, PTT1)
I= 145 bp

Figure 3. Allele Standard detected by polyacrylamide gel electrophoresis of 17 rice varieties at an RM3 locus.