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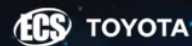
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Genetic diversity of red rice varieties originating from West Java and Banten based on SSR marker related to palatability

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Abstract. West Java and Banten Provinces have diverse local red rice varieties/accessions to support national food security, not only in terms of quantity but also the quality of rice. Good eating quality is closely related to palatability. Palatability is a property that is directly related to the quality of rice feeding, aroma, appearance, taste and texture. This study aimed to analyze the genetic diversity and DNA fingerprint profiles of local red rice accessions from West Java dan Banten using molecular markers related to palatability. A total of 12 red rice accessions and four local red rice accessions from Banten and West Java Provinces were estimated their genetic diversity, respectively. The SSR primers used were Ams (linked with aspartate aminotransferase), GPA (glucosamine-fructose-6-phosphate aminotransferase), GBSS1 (granule-bound starch synthase), CBG (nano cyanogenic β -glucosidase), SS1 (starch synthase), SBE1 (glucosidic linkage of a-polyglucan), RM510 (gel consistency), RM13 (protein content) and RM410 (aromatic). The dendrogram showed two main groups of red rice accessions. The first group consisted of Mayang, Tambleg, Sengkeuhan, Pare Jaketra, Jalawara Hawara, Gadok, Carogol, Beureum Batu, Waren, Segubal, Tampai Beureum and Leger Pondok (similarity reached 80.5%). The second group consists of Kapundung, Cere Beureum and Cireh Hideung with a similarity of 79.5%.

Keywords: Banten, palatability, red rice, SSR, West Java.

1. Introduction

Rice is the main food crop in Indonesia and consumed as staple food. There are various colours of rice depending on the pigment, especially anthocyanin in the pericarp layer, seed coat or aleurons, such as red rice and black rice. The most consumed rice is white one. Red rice contains a high enough vitamin B complex, fibre, essential fatty acids and anthocyanin compounds that are very beneficial to health [1]. Several studies have shown that red rice can be a good source of antioxidants derived from anthocyanin pigments. Islam et al. [2] reported that the red rice population observed in Bangladesh showed differences in coloured pericarp pattern.

Indonesia is known to have a high diversity of rice species and has about 17,000 germplasm accessions [3]. In the 2000s, the number of local rice in the farmers' land had been greatly decreased. Only in certain areas, local rice varieties are still grown by farmers because of the excellent quality and, thus, high selling price. Only a few locations in Banten farmers are still planting local rice



varieties, such as in Cihara, Lebak District. The genetic erosion of rice crops will be more critical if no local rice conservation efforts are made.

Palatability is a property that is directly related to the quality of rice feeding, determined by the fragrance, appearance, taste and texture. The quality of rice flavour is strongly influenced by the physicochemical properties of rice [4]. Some of the genes involved in the synthesis of starch, amino acids, amino sugars and lipids have effects on the physicochemical properties of rice starch which determine the palatability of rice. Therefore, many genetic factors play a role to contribute the rice palatability. Molecular markers have been used to monitor the variation of DNA sequences among varieties. Some molecular markers such as Simple Sequence Repeats (SSR) have been identified to be linked to several genes for essential characters in rice plants [5,6]. Accordingly, we have done research on genetic diversity of West Java and Banten red rice accessions based on palatability SSR markers. The purpose of this study was to analyze the genetic diversity and grouping of accessions of red rice germplasm from the local origin of West Java and Banten based on its DNA fingerprint profile using nine SSR markers linked to the palatability characters.

2. Materials and methods

A total of 16 red rice accessions from Banten dan West Java were used in this study. Red rice accessions from Banten Province are Cere Beureum, Kapundung, Pondok Leger, Tampai Beureum, Segubal, Waren, Beureum Batu, Carogol, Jalahawara, Pare Jaketra, Manikan and Tambleg. Red rice accessions from West Java Province are Cere Hideung, Gadok, Mayang and Sengkuhan. The research was conducted from March to June 2017, in the Greenhouse of Certification Center of Horticulture Plant's Seed at Serang, Banten, and Laboratory of Biotechnology, Faculty of Agriculture, Sultan Ageng Tirtayasa University, Serang, Banten Province.

The research used a descriptive qualitative method including data collection, data analysis and data interpretation. The procedure started with the rice seed seedling for up to 21 days after planting (DAP), and then the DNA was isolated from the young leaves. The DNA isolation used the CTAB method to produce DNA for good PCR amplification. A PCR reaction used nine SSR primers related to rice palatability (Table 1) using standard SSR method and the amplicons were migrated by electrophoresis using 3% agarose gel. Ethidium bromide was used for staining, and then the bands were visualized under UV with Geldoc. The DNA patterns were scanned for the presence of bands and DNA profiles embedded in binary data for "cluster tree analysis". Summary statistics (gene diversity index, PIC and heterozygosity) for all primer was calculated based on the polymorphic alleles.

Table 1. The SSR primers based on palatability gene.

Primer	Chr	Sequence		Linked gene
		Forward (5'-3')	Reverse (5'-3')	
AMs	2	CTTCCAAGGACCCATCCT	CCCAACATCTCCGTCAGAAT	Aspartate aminotransferase ²
GPA	11	AATACGCGGCCTTCTCTAT	TTGATCCGAATGGGTCAAAT	Glucosamine-fructose-6-phosphate aminotransferase ²
GBSS1	6	CAAATAGCCACCCACACCAC	CTTGCAGATGTTCTTCTGATG	Granule-bound starch synthase ¹
CBG	10	AGCTTCCCTAATGGCTTCGT	ATTTGCCAACTTTTGGATGG	Non-cyanogenic β -glucosidase ²
SS1	6	GATCCGTTTTTGCTGTGCC	CCTCCTCTCCGCCGATCCTG	Starch synthase ¹
SBE1	6	ATTTCTTTGGCCACAGGCGA	CCCAGATTCGGAACAAGAAC	Glucosidic linkage of a-polyglucan ³
RM510	6	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	Gel consistency ⁴
RM13	5	TTGGATTGTTTTGCTGGCTCG	GGAACACGGGGTCGGAAGCGAC	Protein content ⁵
RM410	9	GCTCAACGTTTCGTTCTCTG	GAAGATGCGTAAAGTGAACGG	Aromatic ⁶

Chr = chromosome.

¹[5], ²[6], ³[7], ⁴[8], ⁵[9], ⁶[10].

3. Results and discussion

The isolated DNA was diluted to get uniform concentration of about 10–50 ng/μl for PCR. The PCR amplification results were presented in Figure 1.

DNA fingerprint profiles in digital values provided easy identification of rice accessions/varieties. Digital values of DNA patterns based on 9 SSR markers are shown in Table 2. The values showed uniqueness of each accession. Bereum Batu and Waren have the same digital value pattern. The same values were also found between Manikam, Mayang and Tambeleg rice accessions.

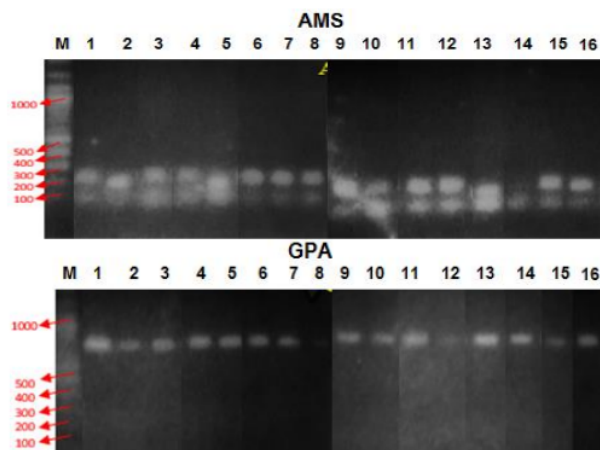


Figure 1. PCR amplification results using molecular markers of AMS and GPA SSR primers based on palatability. M = 100 bp DNA ladder, 1 = Cere Hideung, 2 = Cere Beureum, 3 = Kapundung, 4 = Pondok Leger, 5 = Tampai Beureum, 6 = Segubal, 7 = Waren, 8 = Beureum Batu, 9 = Carogol, 10 = Gadok, 11 = Jalahawara, 12 = Pare Jaketra, 13 = Sengkeuhan, 14 = Tambleg, 15 = Mayang, 16 = Sengkuhan.

The number of alleles, gene diversity, heterozygosity and PIC values of 16 accessions with the 9 SSR is summarized in Table 3. The number of alleles detected was 15–28 alleles, with an average of gene diversity about 0.93. Heterozygosity is obtained from the calculation of gene frequencies at each locus [11]. Based on heterozygosity values, AMs, GBSS1, SS1, SBE1 and RM13 primers are able to distinguish heterozygosity. Conversely, the primers with a heterozygosity value of 0 are assumed to be unable to distinguish heterozygosity (GPA, CBG, RM510 and RM410). The value of heterozygosity close to 0 is low, while the value of heterozygosity close to 1 is high [11].

The polymorphic alleles were analyzed by calculating how many percents of the polymorphic alleles obtained in each SSR primer used. The level of primers informativeness is determined by the calculation of PIC. PIC value provides an estimate of the distinguishing power of a marker by computing not only the number of alleles in one locus, but also the relative frequency of the alleles of an identified population. PIC value becomes the standard to evaluate the genetic markers based on PCR amplified DNA pattern [12,13]. Therefore, PIC value is divided into three classes: highly informative ($PIC > 0.5$), moderately informative ($0.25 > PIC > 0.5$) and slightly informative ($PIC < 0.25$). According to Table 3, the average PIC value was 0.93, which means that all of the primers were highly informative. This study used nine primers (AMs, GPA, GBSS1, CBG, SS1, SBE1, RM510, RM13 and RM410), where each primer has been linked to genetically different characters.

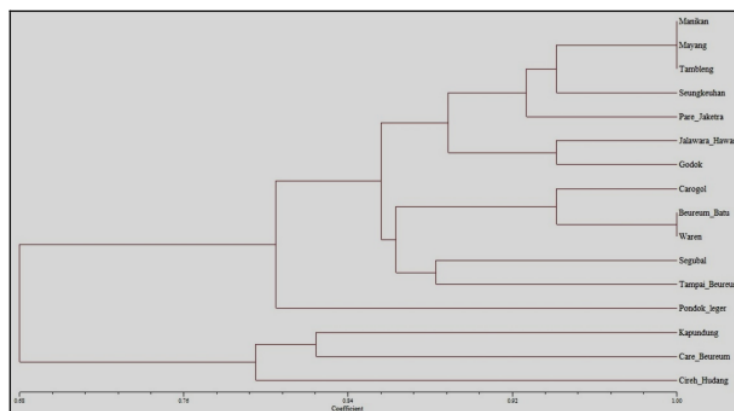


Figure 2. Dendrogram of 16 red rice accessions from West Java and Banten.

Table 2. The digital value of conversion of DNA patterns based on 9 SSR markers.

Accession	Digital values	Accession	Digital values
Manikan	1.011.100.100.101.111	Tambleg	1.011.100.100.101.111
Kapundung	0.111.111.100.101.110	Pondok leger	0.111.100.000.101.111
Carogol	1.011.101.000.101.011	Segubal	1.011.110.000.101.111
Beureum Batu	1.011.101.000.101.111	Tampai Beureum	1.011.100.000.100.111
Care Beureum	0.111.101.000.001.110	Cereh Hideung	0.111.100.101.001.110
Waren	1.011.101.000.101.111	Gadok	1.011.100.000.001.111
Jalawara Hawara	1.011.100.100.001.111	Mayang	1.011.100.100.101.111
Pare Jaketra	1.011.101.100.101.111	Seungkeuhan	1.011.100.110.101.111

Table 3. The number of alleles, gene diversity, heterozygosity and PIC values of 16 accessions with 9 SSR markers in terms of palatability gene.

Primer	Number of alleles	Gene diversity index	Heterozygosity	PIC
AMs	17	0.91	0.07	0.91
GPA	15	0.92	0.00	0.91
GBSSI	19	0.92	0.12	0.92
CBG	19	0.94	0.00	0.94
SSI	28	0.96	0.25	0.96
SBE1	28	0.96	0.03	0.96
RM510	19	0.93	0.00	0.93
RM13	25	0.95	0.31	0.94
RM410	14	0.89	0.00	0.89
Total	184	8.38	0.78	8.36
Average	20.4	0.93	0.09	0.93

AMs markers can predict characters that have a sense of taste and aroma, especially aspartate aminotransferase. Lestari et al. [14] used AMs as DNA markers for eating quality of indica rice in Indonesia. The GPA primer amplified 900 bp fragment which may indicate the presence of glucosamine fructose-6-phosphate aminotransferase. In addition, Utami et al. [15] used GPA primers as DNA markers for physical grain in red rice, indicating its support to red rice research in Indonesia.

The GBSSI primer detected the presence of granule-bound starch synthase genes which function to synthesize amylose *in vivo*. CBG markers are markers for the presence of non-cyanogenic glucosidase located on chromosome number 10. CBG markers can predict the character of the taste and aroma [6].

The SSI primer marked the presence of starch synthase 1 which has a function in the catalytic activity of starch biosynthesis [16]. There are variations in alleles found at the locus associated with starch synthesis, one of which is starch synthase 1 on chromosome number 6 (can be marked by primer SS1) and *Waxy* gene or *Wx* also on chromosome 6 using a GBSSI primer (granule-bound starch synthase). The existence of the *Wx* gene contributes to amylose levels.

The SBE1 primers are related to the character of the *Waxy* (*Wx*) gene, the soluble starch synthase I (SS1) gene and the starch branching enzyme 1 (SBE1) gene [17]. Primer RM510 can be used to estimate the presence of characters related to the consistency of gel in rice plants [8]. The consistency of the gel will determine the texture of rice after cooking. Rice with soft gel cooking consistency is soft and remains soft even after cooling. Rice with soft gel consistency is preferred by most rice consumers.

The RM13 primer can predict the characters associated with grain protein content in rice which can be seen with a band measuring at 141 bp [9]. The protein content is an indirect indicator of the quality of rice taste. According to Lestari and Koh [6], low protein content and increased stickiness and texture caused high palatability. The physicochemical properties of varied rice starches are genetically affected.

Figure 2 shows a dendrogram consisting of two large groups of red rice. The first group consisted of Mayang, Tambleg, Sengkeuhan, Pare Jaketra, Jalawara Hawara, Gadok, Carogol, Beureum Batu, Waren, Segubal, Tampai Beureum and Pondok Leger. The second group consisted of Kapundung, Cere Beureum and Cireh Hideung. The difference between germplasms in both groups was 32%. Group 1 has a similarity of 80.5%, while the second group has a similarity of 79.5%. Manikam, Mayang and Tambleg accessions are 100% similar. One-hundred percent similarity, which indicates the same palatability characters, was also found between Beureum Batu and Waren. Similarity coefficients are the standard genetic level between accessions. The greater the coefficient of similarity, the more closely the accession is, thus genetically similar. This information is needed in plant breeding activities. However, to date, the physicochemical/palatability properties of various rice accessions are generally still conventionally determined, which requires a large number of rice samples. DNA markers that are developed quickly can be an alternative method for rapid and efficient evaluation, and can predict their potential physicochemical properties.

4. Conclusions

All nine SSR markers related to rice palatability were able to estimate genetic diversity of local red accessions from Banten and West Java. The red rice accessions from these two provinces were highly diverse as reflected by their high gene diversity index and PIC value. All SSR markers proved their high informativeness as indicated by the PIC values. Most of these rice accessions could be distinguished according to the specific DNA fingerprint profiles generated in this study. The red rice accessions belong to two major groups without considering their local origin.

5. Acknowledgement

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