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Genetic Relationship Of Local Rice From Kuantan Singingi District Using Sequence Related Amplified Polymorphism (SRAP) Markers

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Abstract

Rice (Oryza sativa L.) is an important food crop that is the staple food of more than half of the world's population because it contains nutrients that the body needs. Information on the genetic diversity of local rice scattered in Kuantan Singingi Regency is still not available. Due to this fact, the genetic diversity of local rice can be identified by DNA analysis. A DNA-based molecular marker that can be applied as a plant genetic marker is SRAP. The SRAP (Sequence Related Amplified Polymorphism) marker system is one of the powerful molecular tools for the clarification of individual sex and estimating the genetic diversity of plant species. This study aims to determine the genetic relationship of rice plants in Kuantan Singingi Regency through SRAP markers. Twenty-four (24) local genotype samples were used in this study. Bulk Segregation Analysis (BSA), wherein one tube 24 samples were mixed, then tested with a combination of primers A to primers P = 16 combinations). Primary combination candidates, calculated based on the number of firm bands generated from each combination. Observational data were processed using Ms. software. Excel and (NTSYS-pc) version 2.02. The results obtained 17 fragments in Primer M, which resulted in a similarity analysis between 71% to 100%. There are two (2) groups at 75% similarity, then there are five (5) groups at 81% similarity and there are four (4) groups that have 100% similarity. The results of this study are expected to be taken into consideration in developing rice breeding strategies in the future.

Keywords: Genetics, Kuantan Singingi, Rice, SRAP

Introduction

Rice is a food-crop commodity that plays an important role in life and the economy in Indonesia. The need for rice is increasing, while rice production is decreasing. Riau is one of the rice-producing areas that have the potential for rice development which is spread in various districts. Kuantan Singingi Regency is one of the areas that have local rice genotypes that can be used to be maximized as prospective elders in assembling New Superior Varieties.

One of the factors that can help increase rice production is the collection of rice germplasm as genetic material. Local rice has a wide genetic diversity and is a source of genes controlling important traits. Genetic diversity is one of the factors that play a role in breeding programs. Lack of information related to the genetic diversity of local rice cultivated in Kuantan Singingi Regency causes the assembly of new high-yielding varieties to be less effective. According to Taha. et al (2013), the availability of information related to genetic diversity is very important and needed to design breeding programs and maintain genetic diversity.

Even though high-yielding varieties now have been widely adopted by farmers, local genotypes are still maintained because they taste according to people's tastes and have specific adaptability. According to Chakravarthi et al., (2006), therefore, characterization and evaluation of germplasm are essential in the breeding program.

Surveys and explorations were carried out by Ezward, Irfan, Nalwida, and Indra in 2019. As a result of these activities, more than 24 genotypes of local rice plants were collected. To find out the detailed identity, morphological characterization was carried out first. However, in many cases, the morphological characterization fails to assess the identity of the details, because many environmental factors affect the morphological performance. Therefore, molecular-based markers need to be applied.

This rice germplasm characterization is important in the conservation of local rice genotypes. There are three main types of genetic markers, namely (1) morphological traits (classical or observable markers) which are phenotypic traits or characters themselves; (2) biochemical markers, the diversity of alleles of enzymes called isoenzymes; and (3) DNA markers (molecular), revealing the diversity of DNA positions. According to Mishra and Slater (2012), a molecular-based marker system is a more effective and efficient technology to distinguish between species and between closely related cultivars compared to the other two markers.

One of the most widely used molecular-based marker systems in recent times is the Sequence-related Amplified Polymorphism [SRAP] developed by Li and Quiros (Li and Quiros, 2001). Some of the advantages of using SRAP compared to other marker systems are that it is multi-locus and multi-allele. These characteristics make the SRAP marker system more efficient for genetic diversity analysis, gene mapping, and fingerprint genotyping.

SRAP has several advantages, including a simple system, has a reasonable throughput rate, approaches several co-dominant markers, targets open reading frames (ORFs), and facilitates isolation for sequencing (Zhao et al. 2009). SRAP is very effectively used to analyze genetic diversity (Shaye, et al. 2018).

Previously, only exploration research had just been carried out on local rice genotypes in Kuantan Singingi Regency. Morphological characterization has also not been carried out on these 24 local rice genotypes. However, to see the relationship between genotypes, it is more accurate to do it molecularly. Therefore, this study aims to determine the genetic kinship of local rice in Kuantan Singingi Regency through SRAP markers.

Materials and Methods

Twenty-four (24) local genotype rice seeds were obtained from several villages in the Kuantan Singingi Regency, through exploration activities. The 24 local genotypes are : Padi Sironda putih (PL01), Padi saronda merah (PL02), Padi Pandan wangi (PL03), pulut hitam (PL04), Padi ronda putiah (PL05), Padi singgaro merah (PL06), Padi kuning umur panjang (PL07), Padi ros (PL08), Padi samo putiah (PL09), Padi limbayang (PL10), pulut karate (PL11), Padi sokan umur panjang (PL12), pulut benai Peboun Hulu (PL13), Padi singgam putih (PL14), Padi singgam kuriak (PL15), pulut kari (PL16), pulut benai Kinali (PL17), padi kuning (PL18), Padi putih (PL19), pulut lupo ka laki (PL20), Padi kuning (PL21), Padi Gondok (PL22), Padi saronda kuning (PL23), Padi katiok putih (PL24).

Molecular characterization research has been carried out from February 2021 to March 2021. Molecular characterization activities have been carried out at the Greenhouse and Biotechnology Laboratories, Faculty of Agriculture and Biomedical Laboratory, Faculty of Medicine, Andalas University, Padang. The activity refers to the research that has been carried out by Subositi and Rohmat, (2013).

A total of 24 local rice genotypes were sown in plastic cups using soil media and manure with a ratio of 2: 1. Furthermore, the nursery cups were stored in a greenhouse and watered using a hand sprayer to maintain humidity.

Leaves of rice plants that are 1-2 weeks old after seedlings are cut from the middle to the ends using scissors that have been sterilized using 70% ethanol (Sulistyowati, Angelita, and Enung, 2018). The leaves were put in a 1.5 ml tube and placed in a box filled

with ice. Furthermore, the leaf samples were stored in the freezer and used as DNA isolation material.

DNA isolation

Rice DNA isolation was carried out according to the Thermo Fisher Scientific Gene JET Plant Genomic DNA Purification Mini Kit product manual. A total of 3-4 rice leaves were taken and put in a refrigerator at -80o C before being isolated. The sample was weighed as much as 0.5 g and then ground using a mortar and a gun until smooth. Then the sample was put into a 1.5 ml tube and 500 L of lysis buffer A was added. The solution was homogenized for 20 seconds using a vortex. Then 50 L of lysis buffer B and 10 L of RNAse were added. Then inverted for 20 seconds and incubated at a temperature of 65oC in a water bath for 10 minutes.

After that, 130 L of precipitation solution was added and inverted 2-3 times. Incubated on ice for 5 minutes and centrifuged for 7 minutes at a speed of 12,000 rotations per minute (rpm). The supernatant was taken as much as 500 L and transferred into a 1.5mL tube. Added 400 L of gDNA binding solution and 400 L of 96% ethanol and inverted until homogeneous. The supernatant was transferred into the spin column as much as the volume of the solution and centrifuged for 3 minutes at 10,000 rpm. Discard the liquid in the tube, and the remaining solution is put into the spin column and the centrifugation is repeated. Discard the liquid along with the column, then add 50 l of elution buffer in the center of the spin column membrane.

Then incubated for 5 minutes and centrifuged at 10,000 rpm for 1 minute. The DNA isolation results were stored in a -20oC refrigerator. The quality of the isolated DNA was determined using electrophoresis. 1 g of agarose was weighed and added 100 mL of 0.5 x TBE and put into a Schott bottle. Then, the agar was heated in the microwave for 1.5 minutes at medium-high temperature and 10 L of ethidium bromide was added. Agar is put into a gel tray that has been prepared previously and incubated for 30 minutes until it solidifies. Electrophoretic cocktails were prepared with 1 L 10 x BPB, and 2 L DNA samples. Then 2 L DNA (50 ng/μL) as standard.

After the agar solidifies, the comb is removed and the agar barrier is opened, Buffer 0.5 x TBE was put into the electrophoresis bath until the gel sank a few mm. After that, the cocktail was put into the existing well and run at a voltage of 100 volts for 30 minutes. For visualization, put it on the gel documentation under a UV transilluminator. The isolated genomic DNA was measured for absorbance at wavelengths of 260 nm and 280 nm (□ 260/280). Record the absorbance value.

The method used in this experiment is descriptive. The descriptive method was carried out on the analysis of the DNA banding pattern obtained from each genotype. The results of DNA isolation were checked with Electrovoresis and Biodrop.

Molecular Detection with SRAP Method

Molecular detection was carried out by amplifying rice DNA using the SRAP method according to Maulidah, et.al (2019) and Oktavioni, et.al (2019).

Bulk Segregation Analysis (BSA), wherein one tube 24 samples were mixed, then tested with a combination of primers A to primers P = 16 combinations). Primary combination candidates, calculated based on the number of firm bands generated from each combination.

The results of DNA isolation were amplified in vitro using the Biometra-Germany PCR (Polymerase Chain Reaction) machine. Each genotype was tested using 3 primer combinations. The primers used included SRAP primers: H (Me2 + Em4), M (Me4 + Em1), N (Me4+Em2). So the total PCR reactions of 24 plant genotypes were 72 reactions.

Each PCR reaction consists of the following cocktails : KOD (20-25 ng/μL) : 13 μL, Primer F (10 μ/mm) : 1 μL, Primer R (10 μ/mm) : 1 μL, DNA template : 3 μL, Nuclease Free Water : 7 μL, Total : 25 μL.

Amplification was carried out using the RTJ-18 PCR program and was seen as follows: Stage 1, Pre-denaturation 94 ^{o}C for 5 minutes, Denaturation 94 oC for 1 minute, Annealing 35 oC for 1 minute, Extension 72 oC for 1 minute, Continue to 2, Loops 5.

Stage 2, Denaturation 94 oC for 1 minute, Annealing 50 oC for 1 minute, 72 oC for 1 minute, Continue to 5, Loops 20, Extension 72 oC for 1 minute, 4 oC for 0 minutes. After the amplification was completed, the results were visualized by electrophoresis. Amplification results: 3 μL, 1 kb DNA ladder: 2 μL.

The amplification process was declared successful if the visualization results showed the presence of one DNA band. If there is a band, it is given a score of 1, and if not it is given a score of 0.

Data Analysis

The DNA fragments produced from each individual/genotype from each primer were calculated by measuring the base length of the fragment. Note the size of the fragment. The presence or absence of DNA fragments in each accession was given a different score, a score of 1 if there were fragments and a score of 0 if there were no DNA fragments. The similarity index was calculated using the Dice similarity index formula. Cluster analysis and dendrogram construction were carried out using the Unweighted Pair Group Method Using Arithmetic Method (UPGMA). Analysis of the principle of coordinates (Principal Coordinate Analysis) each individual. Then

proceed to the analysis of population genetic diversity. The data was processed using a computer program (software) NTSYS 2.02.

Results and Discussion

DNA Isolation

The success of DNA extraction is influenced by the type of plant, the material used (Retnaningati, 2020), and the chemical content contained in the plant tissue (Pharmawati, 2009). In this study, the rice plant samples used were fresh young leaves. Young leaves produced more DNA than samples from old leaves. This is because young leaves are composed of cells that grow actively, not yet contain many polyphenolic compounds and other secondary metabolites.

The primer was chosen because it produces a band pattern that can amplify clearly. According to Yuniastuti et al (2010), stated that primer selection was carried out to look for primers that could amplify a clear and large number of DNA bands. In addition, primer selection was also carried out to find primers that produced polymorphic bands (Figure 1).

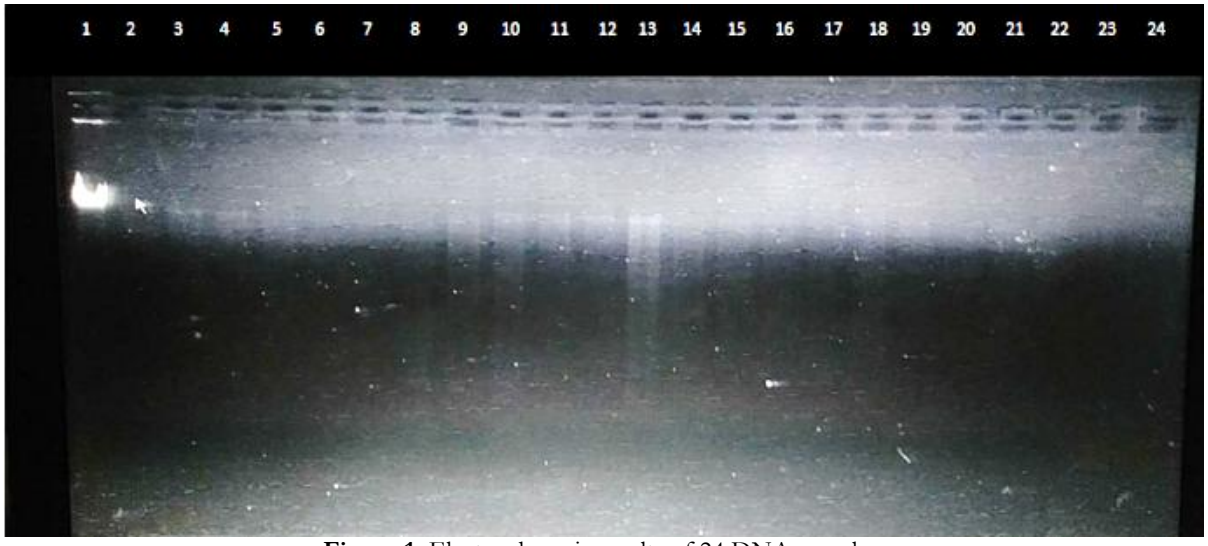

Figure 1. Electrophoresis results of 24 DNA samples

When electrophoretic some samples are not visible on the DNA bands or are very thin in quality. To verify the number of DNA samples, Biodrop analysis was carried out by increasing the DNA concentration. Visual quality if the band looks thick because the concentration is high and if the band looks bright then the quality is good.

To determine the quantity of sample DNA, the DNA concentration was measured using Biodrop. The results of DNA concentration with Biodrop can be seen in Table 1.

DNA purity level : genotype 06 = 1.8, genotype 07 = 2.25, genotype 08 = 2, genotype 09 = 1,4, genotype 10 = 2, genotype 11 = 2, genotype 12 = 1.8, genotype 13 = 1,6, genotype 14 = 1,6, genotype 15 = 1,6, genotype 16 = 1,6, genotype 17 = 2,3, genotype 19 = 2, genotype 20 = 2, genotype 21 = 2,3, genotype 22 = 1.5. According to Sambrook and Russell (1989), DNA is said to be pure if it has an absorbance value between 1.8-2.0. The DNA purity value lower than 1.8 indicates that the DNA sample is contaminated with protein, whereas if the value is higher than 2.0, it means that the DNA sample is contaminated by RNA. DNA samples can be continued for the amplification process.

SRAP Amplification

The SRAP (Sequence Related Amplified Polymorphism) marker system has become a popular marker for genetic diversity (Li. et al, 2013) and is associated with natural and direct evolutionary processes of plant evolution. SRAP is commonly used to limit and test variation within and between individual samples (Robarrts and Wolfe, 2014).

In addition, it is also believed to be more cost-effective, easy, and reliable, using forward and reverse primers so that there are more alternative primer combinations (Robarts and Wolfe, 2014). SRAP has been widely used to identify the genetic diversity of several types of plants such as arabica coffee from Yemen (Taha. et al., 2013), hybrid robusta coffee (Gimase, et al. 2014] elephant grass (Xie X, et al. 2009), plants cereals (Zaefizadeh and Goleiv, 2009), rice (Dai, et al. 2012), bananas (Youseff, et al. 2011), some medicinal plants (Oktavioni, et al. 2019), Alpinia galanga (Maulidah, et al. 2019).

Table 1. The results of DNA concentration with Biodrop

No	Genotype	DNA concentration was measured using Biodrop
1	Padi Sironda putih	9 ng/µL
2	Padi saronda merah	14 ng/µL
3	Padi Pandan wangi	9 ng/µL
4	pulut hitam	4 ng/µL
5	Padi ronda putiah	4 ng/µL
6	Padi singgaro merah	9 ng/µL
7	Padi kuning umur panjang	9 ng/µL
8	Padi ros	12 ng/µL
9	Padi samo putiah	22 ng/µL
10	Padi limbayang	10 ng/µL
11	pulut karate	8 ng/µL
12	Padi sokan umur panjang	26 ng/µL
13	pulut benai	13 ng/µL
14	Padi singgam putih	10 ng/µL
15	Padi singgam kuriak	10 ng/µL
16	pulut kari	15 ng/µL
17	pulut benai	7 ng/µL
18	padi kuning	1 ng/µL
19	Padi putih	16 ng/µL
20	pulut lupo ka laki	8 ng/µL
21	Padi kuning	7 ng/µL
22	Padi Gondok	21 ng/µL
23	Padi saronda kuning	6 ng/µL
24	Padi katiok putih	8 ng/µL

The KOD used is as follows : A1 = Me1 + Em1, B1 = Me1 + Em2, C1 = Me1 + Em3, D1 = Me1 + Em4, E1 = Me2 + Em1, F1 = Me2 + Em2, G1 = Me2 + Em3, H1 = Me2 + Em4, I1 = Me3 + Em1, J1 = Me3 + Em2, K1 = Me3 + Em3, L1 = Me3 + Em4, M1 = Me4 + Em1, N1 = Me4 + Em2, O1 = Me4 + Em3, P1 = Me4 + Em4 (Figure 2).

Bulk segregant analysis (BSA) is a technique used to identify genetic markers associated with plant phenotypes. The number of firm bands from each combination is A = 9, B = 12, C = 12, D = 9, E = 9, F = 5, G = 9, H = 12, I = 10, J = 12, K = 10, L = 12, M = 12, N = 7, O = 10, 0 = 10. Next, the one that produces the most firm bands is selected. Then each genotype was tested using 3 primary combinations. The primers used included SRAP primers: H (Me2 + Em4), M (Me4 + Em1), N (Me4+Em2). So the total PCR reactions of 24 plant genotypes were 72 reactions.

DNA amplification by PCR on each plant sample (24 genotypes) was carried out. The results of screening of 16 primers were carried out using the BSA method, obtained 3 (three) primer combinations that produced the clearest and best number of DNA band fragments, namely primers H, M, and N. The results of DNA amplification can be seen in Figure 3. Based on the number of fragments produced by this BSA method, then the combination of M primers will be used in the clustering analysis. The combination of M primers yielded 17 fragments for each individual. The resulting fragment size range is 740-5,000 bp for the whole individual. The average number of H, M, and N primary firm bands can be seen in Table 2.

Data Analysis

The coefficient of similarity of molecular characters observed from 24 local rice genotypes in this study can indicate the closeness of their kinship relationship. Therefore, a closeness test was carried out in the kinship relationship possessed by the 24 local rice genotypes using a dendrogram as shown in Figure 4.

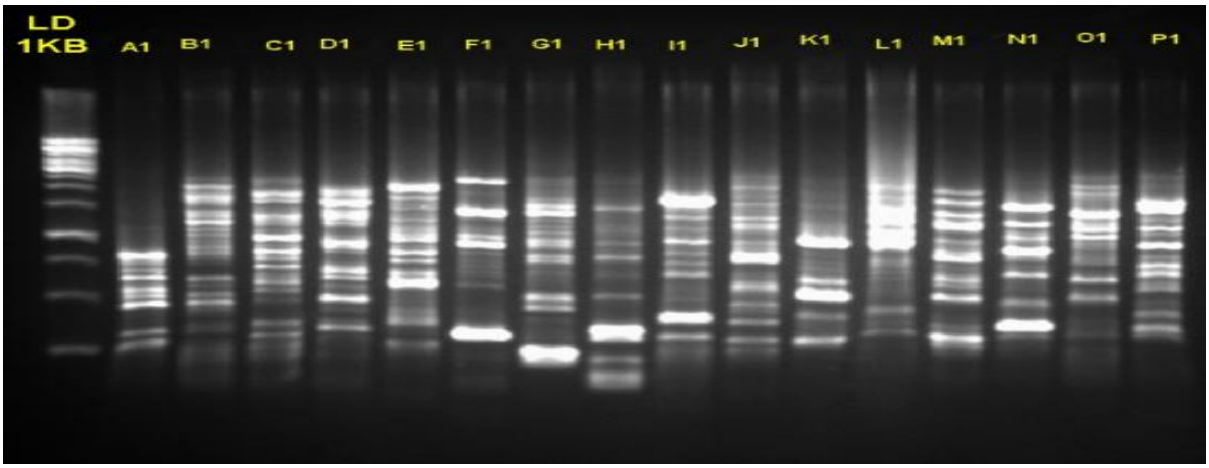

Figure 2. Results of BSA Primary Screening Test of 16 SRAPS primer combinations using one local rice genotype.

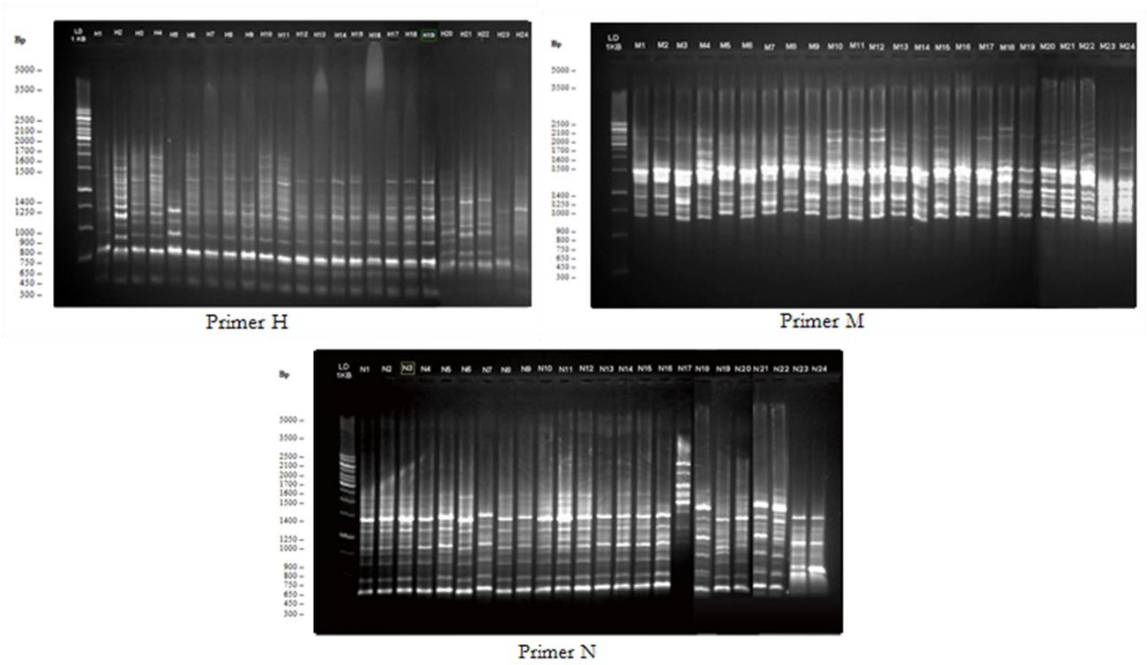

Figure 3. Results of DNA amplification (SRAP primer selection) Primer H, M, and N for 24 samples.

Plant properties can be used in cluster analysis which is useful for determining the close relationship of a plant so that it can be used for identification and description of species-level relationships. The highest phenotype coefficient (KF) is 100% (1,0) there are four (4) kinship groups. While the lowest is 75% (0.75), there are two (2) kinship groups. This indicates that these genotypes are formed from the same population so that the level of kinship is closer. On the other hand, there are genotypes with very different names but very high levels of kinship. This is in accordance with Suskendriyati et al (2000), which states that the differences and similarities of a plant. The smaller the Euclidean distance between several objects being analyzed, the closer the kinship of the object and the more similarities in character it has (Santoso, 2002). Based on 24 local rice genotypes in Kuantan Singingi Regency at a similarity coefficient of 81% (0.81), it consisted of five (5) groups, where group 1 consisted of Padi Sironda putih (01), Padi saronda merah (02) dan pulut hitam (04). Group 2 consists of : Group 2 consists of : Padi Pandan wangi (03) dan pulut kari (16). Group 3 consists of : pulut benai Kinali (17) dan padi kuning (18). Group 4 consisted of : Padi ronda putiah (05), Padi singgaro merah (06), Padi kuning umur panjang (07), Padi ros (08), Padi samo putiah (09), Padi limbayang (10), pulut karate (11), Padi sokan umur panjang (12), pulut benai Peboun Hulu (13), Padi singgam putih (14), Padi singgam kuriak (15), pulut lupo ka laki (20),

Table 2. The average number of H, M and N primary firm bands

No	Genotype	Primary H	Primary M	Primary N
1	Padi Sironda putih	5	6	8
2	Padi saronda merah	10	9	8
3	Padi Pandan wangi	6	7	9
4	pulut hitam	8	9	8
5	Padi ronda putiah	6	6	7
6	Padi singgaro merah	7	7	9
7	Padi kuning umur panjang	4	7	6
8	Padi ros	6	10	9
9	Padi samo putiah	7	8	7
10	Padi limbayang	8	10	7
11	pulut karate	6	9	10
12	Padi sokan umur panjang	5	10	8
13	pulut benai	4	11	7
14	Padi singgam putih	5	7	9
15	Padi singgam kuriak	4	8	9
16	pulut kari	4	7	7
17	pulut benai	7	7	7
18	padi kuning	7	9	7
19	Padi putih	7	8	7
20	pulut lupo ka laki	7	8	7
21	Padi kuning	7	10	6
22	Padi Gondok	6	9	6
23	Padi saronda kuning	5	8	4
24	Padi katiok putih	3	8	4
	Average	**6**	**8.25**	**7.33**

Padi kuning (21), Padi Gondok (22), Padi saronda kuning (23) and Padi katiok putih (24). Group 5 consists of : Padi putih (19).

Conclusion

Based on the research results, there is a diversity of molecular characters. Obtained 17 fragments, which resulted in a similarity analysis of 24 local rice genotypes in Kuantan Singingi district between 71% to 100%. There are four groups of genotypes that have 100% similarity.

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