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Variation of 20 Accession Tydal Rice Germplasm in Indragiri Hilir District based on Analisis Random Amplified Polymorphic DNA (RAPD)

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Abstract **– Padi (Oriza sativa) is a strategic food commodity for the Indonesian people. Comprehensive informations has been need including molecular information for plant repairs. The purpose of this study was to find out information about the genetic variation of various tidal rice in Indragiry Hilir Regency using molecular analysis. This research was conducted at the Biotechnology laboratory of the Bogor Agricultural Institute from April to May 2017.The genetic material used by 20 tidal rice accessions and other ingredients is chemicals. The results showed that from 68 DNA banding patterns produced from 10 primers produced from 59 polymorphic and 9 monomorphic banding patterns. Based on cluster analysis of 68 DNA band patterns, dendograms were obtained with similarity coefficients of around 56-88% or around 12-44% genetic variation.**

Keywords **– Rice Tydal, Germplasm, RAPD, Genetic Variation.**

I. INTRODUCTION

Indragiri Hilir Regency (Inhil) is one of the districts in Riau Province, and has a large tidal land. Inhil has a tidal area of 11,605.97 km² with a height of 0-4 meters above sea level (Anonimus, 2015). Tidal land use as an area of rice cultivation in Inhil has been long enough cultivated. Various superior varieties and local strains have been cultivated for generations. However, the characteristics of each local tidal varieties are not known for certain. So that it is very difficult to identify the truth or certainty of authenticity as local varieties of tidal rice in Indragiri Hilir regency.

Traditional cultivar identification based on morphologi--cal traits requires extensive observations of mature plants and, in many situations, it lacks definition and objectivity (Wrigley et al. 1987). Furthermore, morphological traits cannot serve as unambiguous markers because of environ--mental influences (Yang and Quiros, 1993). Protein and isozyme electrophoresis has been used in rice (Vithyashini and Wickramasinghe, 2015); (Sing, 2004). The major limitation of these techniques is insufficient polymorphism among closely related cultivars. Because proteins are the products of gene expression, they may vary in different tissues, developmental stages, and environments (Beck--man and Soller 1983). On the other hand, DNA markers, such as RFLPs (restriction fragment length polymor--phism), give a much higher degree of polymorphism and stability. The disadvantages of RFLPs are the laborious procedure involved, the relatively high cost, and the use of radioisotopes. As an alternative, the RAPD (randomly amplified polymorphic DNA) technique developed by Williams et al. (1990) and Welsh and McClelland (1990) provides a faster and easier approach for exploring genetic polymorphism, and also requires very small amounts of DNA. RAPD markers have been successfully used in cultivar analysis in other crops (Hu and Quiros, 1991; Yang and Quiros, 1993).

Based on these ideas the authors are interested in conducting research titled "Rice Tidal Local Attitude in Indragiri Hilir Regency Based on Random Amplified Polymorphic DNA (RAPD) Analysis. Based on the above reasons, the authors are interested to see the level of local tidal rice kinship in Indragiri Hilir District Based on Random Amplified Polymorphic DNA (RAPD) Analysis."

II. RESEARCH METHODS

2.1. *DNA Isolation*

RAPD analysis was conducted at the Bogor Agricultural Biotechnology Laboratory in April 2017. DNA extraction used Doyle and Doyle (1990) modified by Pharmawati (2009) method by increasing EDTA concentration to 50 mM and 2% (v/v) 2-mercaptoethanol addition on extraction buffer and incubation at 55ºC for 14-16 hours.

Table 1. List of Local Tidal Rice Accession used for DNA Analysis

Symbol	Accession Name	Symbol	Accession Name
V1	Lentik Bamban Merah	V11	Acan
V2	Belang	V12	Kodok
V3	Lembu Sawah	V13	Raden
V4	Lentik Bamban Putih	V14	Pagar Sari
V5	Serai Merah	V15	Pulut Putih
V6	Serai Putih	V16	Bujang Berinai
V7	Merah Putih	V17	Karya Merah
V8	Super	V18	Putih
V9	Karanduku	V19	Karya Kuning
V10	Sarang Semut	V20	Arum

Leaf sample was cut, weighed as much as 0,1 g and added 1 ml of extraction buffer (consisting of 2% w / v CTAB, 1.4 M NaCl, 50 mM EDTA, 100 mM Tris-HCL (pH 8), 0.2% (v / v) 2-mercaptoetanol). The leaf sample (Table 1) was crushed and inserted into the tube and then incubated at 65 ºC in the water bath for 30 minutes. Subsequently, 1x chloroform volume was added: isoamethalcohol (24: 1), and centrifugation was performed at 8,000 rpm for 10 min.

The supernatant was transferred to a new tube accompanied by the addition of cold isopropanol and incubated for one night at -20 °C. After incubation, centrifugation is done for 5 minutes at a speed of 8,000 rpm. The isopropanol solution was removed, the DNA pellet was washed with 500 µl ethanol 70% and centrifuged for 5 min. Then ethanol is carefully removed, and DNA is allowed to dry. The DNA pellet was dissolved with 100 µl sterile aquadest. DNA purification was performed using ucleoSpin® Gel and PCR Clean Up Kit based on company instructions.

2.2. *Aplification and Electrophoresis*

Amplification and electrophoresis reactions used Williams *et al* (1990) methode. Amplification were performed in volumes of 25 /d containing lOmM Tris-Cl, pH 8.3, 50 mM KC1, 2 mM MgCl2, 0.001 % gelatin, 100 /tM each of dATP, dCTP, dGTP and TTP (Pharmacia), 0.2 /tM primer, 25 ng of genomic DNA, and 0.5 unit of Taq DNA polymerase (Perkin Elmer Cetus). Amplification was performed in a Perkin Elmer Cetus DNA Thermal Cycler programmed for 45 cycles of 1 min at 94°, 1 min at 36°, 2 min at 72°, using the fastest available transitions between each temperature. Amplification products were analyzed by electrophoresis in 1.4% agarose gels and detected by staining with ethidium bromide. Annealing temperatures above 40° in the thermal cycling profile.

With some combinations of primer and genomic DNA template a nondiscrete size range of amplification products, appearing as a 'smear' as visualized on a gel, could be converted to discretely sized bands by reducing the concentration of either the polymerase or the genomic DNA. Annealing temperatures above 40° in the thermal cycling profile prevented amplification by many of the 10 base oligonucleotides tested (Tabel 2).Visualization is done with UV transluminator and photographed using Polaroid 667 film. A total of ten RAPD primers were attempted in PCR-RAPD reaction for DNA fragment amplification.

The genetic diversity of PCR - RAPD products is determined by scoring based on the appearance of the band. The bands appearing on the gel are assumed to be RAPD alleles. The diversity of RAPD alleles is determined by the difference of allele migration on the individual gel of each sample. Based on the presence or absence of the ribbon, the ribbon profile is translated into binary data. The emerging ribbons are coded 1 (present) and 0 (none) (Ferreira and Grattapaglia, 1994). This data is used to create a matrix of genetic similarities using the SIMQUAL (Similarity for Qualitative Data) procedure. This similarity matrix is used for Sequential Agglomerative grouping analysis, Hierarchical Method With Arithmatic Average (SHAN) clustering using UPGMA (Unweighted Pair Group Method with Arithmetic Average) method using NTSYS-PC (Numerical Taxonomy and Multivariate System)) version 2.2. The cophenitic correlation coefficients specified were : r > 0,9 (very Good), 0,8 < r < 0,9 (good fit), r < 0,7 very poor fit (Rohlf, 2000) computer The level of polymorphism (PIC) was calculated for each microsatellite marker. The value of PIC is measured by using online software (www.genomics.liv.ac.uk /animal/Pic1.html) (Mulsanti 2011).

Table 2. List of RAPD primers used for kinship analysis of Tidal Rice in Indragiri Hilir District

No	Primer	Basa Sekuen	T Anealing (ºC)
1	OPE8	TCACCACGGT	36,8
2	OPE20	AACGGTGACC	32,7
3	OPH1	GGTCGGAGAA	33.2
4	OPH7	CTGCATCGTG	31.7
5	OPH8	GAAACACCCC	32.3
6	OPH11	CTTCCGCAGT	35.1
7	OPM1	GTTGGTGGCT	33.2
8	OPM2	ACAACGCCTC	33,7
9	OPM20	AGGTCTTGGG	31,8
10	OPA4	AATCGGGCTG	34,1

III. Results and Discussion

The PCR results of 10 RAPD primers tested on 20 tidal rice samples in Indragiri Regency can be read and calculated, so the results can be analyzed. Number, allele length, number and percentage of polymorphic alleles, heterozigisity and PIC of the ten RAPD primers produced are listed in Table 3 and 4. The results showed that the ribbon pattern of PCR results from the ten primers showed different ribbon patterns. The size of the resulting bands varies between 250-2700 bp. The apparent ribbon pattern of the ten primers is 68 banding patterns with an average of 6.8 bands per primer. The highest number of ribbon pattern is found in OPE 8 and OPH 11 which is 10 band pattern while the lowest band number is found in OPM 1 which is 2 band pattern. The longest ribbon size is found on the OPE 8 primer of 2700 bp while the shortest ribbon size is in OPM 20 of 250 bp

Table 3. Number, Allele length, Number and percentage of polymorphic alleles.

Primer	Number of alleles	Number of polymorphic alleles	Percentage of Polymorphic Allele	Allele Length
OPE8	10	10	100	300-2700
OPE20	8	8	100	450-2500
OPH1	7	6	85,71	600-2700
OPH7	8	7	87,5	450-2000
OPH8	3	0	100	1100-1500
OPH11	10	8	80	350-1500
OPM1	2	2	100	1000-1100
OPM2	5	5	100	300-1700
OPM20	7	6	85,71	250-2500
OPA4	8	7	87,5	250-1700
Total	**68**			
Average Range			**-**	**250-2700**

Table 4. Heterozygosity and PIC

Primer	Heterozigositas	PIC	Caption
OPE8	0.835	0.814	Very Informative
OPE20	**0.836**	0.814	Very Informative
OPH1	0.832	0.810	Very Informative
OPH7	0.828	0.805	Very Informative
OPH8	0.667	0.586	Very Informative
OPH11	0.810	0.783	Very Informative
OPM1	0.480	0.364	Low Informative
OPM2	0.7505	0.711	Very Informative
OPM20	0.749	0.709	Very Informative
OPA4	0.851	0.833	Very Informative
Rerata	**0.719**	**0.678**	Very Informative

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Based on the ability to provide information, PIC is divided into three categories: PIC> 0.5 = very informative, 0.25> PIC 0.5 = medium and PIC, 0.25 = low (Botstoen *et al*, 1980). All of the primers tested in this study were informative, 9 of them (OPE8, OPE20, OPH1, OPH7, OPH8, OPH11, OPM2, OPM20 and OPA4) having PIC> 0.5 were classified in very informative groups. While OPM1 with PICs 0.364 was classified as moderate. PIC is needed to choose markers that can distinguish between lines used. PIC quantification is the number of alleles that can be produced by a mark and the frequency of each allele in each allele tested. Markers that have a smaller level have little ability to differentiate the samples tested the average value of PIC (0.678) was highly informative, indicating that the RAPD primer can be used to identify the rice varieties being analyzed.

The analysis of ragged germplasm of rice plasma genetic DNA was conducted on 20 tidal rice accessions in Indragiri Hilir Regency. DNA analysis was done by amplifying the sample DNA using 10 RAPD markers. Quantitative size determination of amplified DNA fragments was performed on UV transminator. The output on the UV transiminator monitor screen is taken with Polaroid 667 (Figures 1). Electroforegram Figure shows that the obtained DNA fragment is clear enough to be used in RAPD analysis.

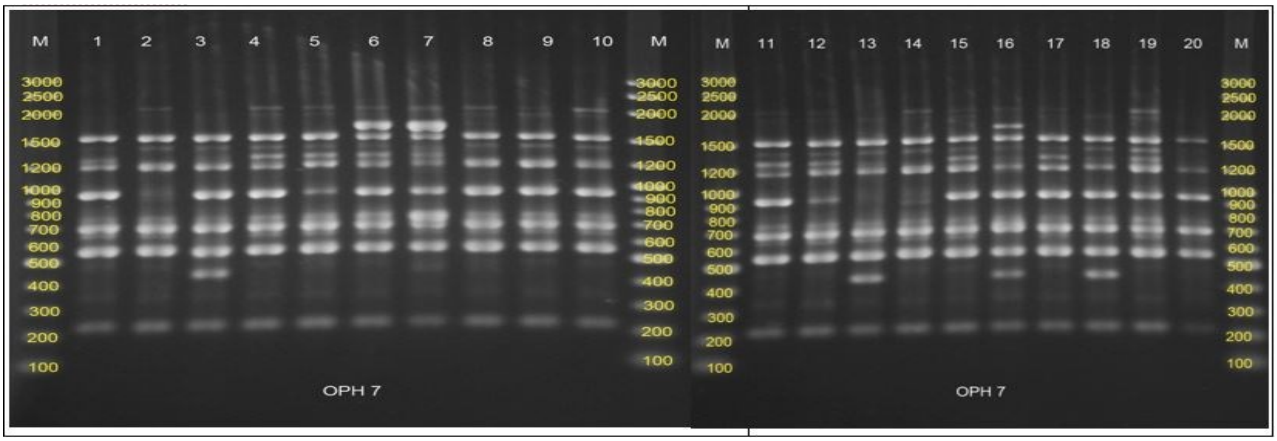

Fig. 1. Electroforegram Result 20 Accession of tidal germplasm on OPH7 primer (Inf: 1-20 samples of germplasm accession, M = Marker).

The ribbons on the algarose gel are the result of genomic DNA amplification. The band pattern can be grouped into two categories namely polymorphic band and monomorphic band. Polymorphic bands are DNA bands that appear on certain sizes but in other samples no DNA bands are found in these sizes. The monomorphic bands are the bands contained in some samples having no variation.

The total number of loci obtained with 10 RAPD primers (OPE8, OPE20, OPH1, OPH7, OPH8, OPH11, OPM1, OPM2, OPM20, OPA4) were 60 loci. The loci were scattered on OPE8 primers by 10 loci, OPE20 primers by 8 loci, OPH1 primers by 7 loci, OPH7 primers of 8 loci, OPH8 primers by 3 loci, OPH11 primers as many as 10 loci, OPM1 primer 2 loci, OPM2 primer 2 loci, primer OPM20 7 locus and primary OPA4 8 locus (Table 2). Unweighted Pair-Group Method Arithmetic (UPGMA) using Numerical Taxonomy and Multivariate System (NTSYS) version 16 can be seen in Figure 3.

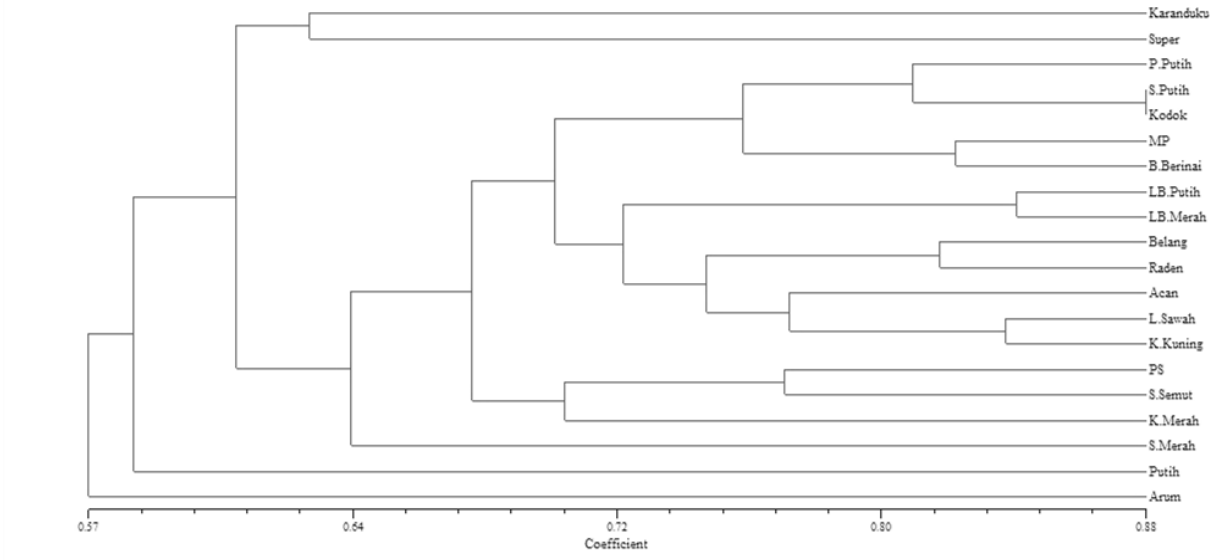

Fig. 2. Dendogram 20 Local Rice Access in Tidal Area of Indragiri Hilir Regency.

The results of the grouping of 20 tidal rice accessions can be seen in Figure 1. Analysis of genetic diversity was used to determine the kinship relationship among 20 tidal rice accessions in Indragiri Hilir Regency. The basis of grouping is the level of genetic similarity of each genotype. Characterization based on RAPD markers shows a similarity coefficient between 0.57-1 or genetic distance 0 - 0.43 (Figure 2). The greater the genetic similarity coefficient value between two or more accessions, the genotype will be in one group.

Cluster analysis of tidal rice Indragiri Hilir have used 10 RAPD markers. They produced an UPGMA dendogram from 20 tidal rice accessions in the Indragiri Hilir Regency. At the level of similarity 60% formed 4 clusters. Cluster I consists of Arum. Cluster II consists of Putih. cluster III consists of: K. Merah, S. Merah, S. Semut, PS, K. Kuning, L. Sawah, Acan, Raden, Belang, LB Merah, LB Putih, MP, B.Berinai, Kodok, Putih, P. Putih. Cluster IV consists of my Super and Karanduku.

The combined correlation coefficient of 10 primers addresses values that include very good fit (r> 0.9). This means that the primers were fairly reliable to be used in this molecular analysis. However this dendogram also shows that in group III Dendogram (Fig 2), there are several accessions that are not in the group of species such as Acan, P. Putih and S. Semut which are groups of glutinous rice. This is possible because rice is a cross-pollinating plant so there is a possibility of recombination of new genes from other species. Acan, P.Putih and S.Semut are groups of glutinous rice. Acan is black gloutinous rice and P. White and S. Semut are included in the group of white glutinous rice. On the Dendogram, it can also be seen that the tested accessions included in one glutinous rice species are not 100% genetically the same. *Oriza sativa* var glutinous which consists of 3 accessions have a different genetic makeup. They have a level of genetic similarity 68-80%. These rices not only differ in their genetic makeup but also differ in their phenotypic properties. Suhartini 2003 states that rice in the same species has differences in agronomic characters such as : plant height, number of tillers, flowering age, grain size, aleuoron color, fur length, 1000 grain weight.

IV. Conclusion

Allele variations of 20 rice accessions with 10 RAPD primary markers were moderate (mean 6.8). PIC values had variations from 0.255 - 0.833. Dendograms that had been formed in 4 groups with phenotypic correlation are very good fit (r> 0.9).

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