Analysing South Sumatra red rice polymorphism using random amplified polymorphic DNA (RAPD) markers

by Dr. Laila Hanum, M.si
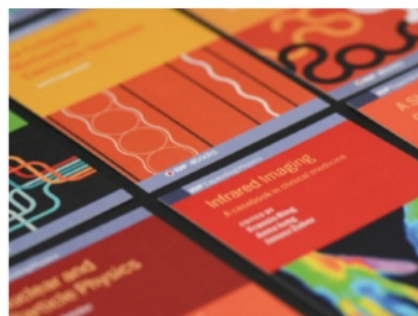
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Analysing South Sumatra red rice polymorphism using random amplified polymorphic DNA (RAPD) markers

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Abstract. this research aims to determine genetic variation based on polymorphism bands and the relationship between red rice in south sumatra based on PCR- RAPD (*Polymerase Chain Reaction- Random Amplified Polymorphic DNA*) tecnique using the mathematical modeling application NTSys 2.1. Collecting data using observation methods where samples are collected genetic variation and presented in quantitative form. Research instruments include tools and laboratory materials that facilitate the research process such as electrophoresis, thermal cycle machines, gel documentation and others. DNA bands obtained electrophoresized PCR sample is converted into binary data which is stated by the presence or absence of DNA bands, if there is a DNA band then marked with (1), whereas if the DNA band does not appear then marked with (0). Data were analysed using the NTSys version program. 2.1. Dendrogram created using unweighted pair-group with arithmetic average with genetic similarity coefficient using Jaccard Coefficient of Similarity and presented in the form of dendrogram. Based on the DNA band profile of RAPD results which 43 DNA bands show polymorphism (93.47%). Based on the analysis using the NTSys 2.1 program, a dendogram was obtained where red rice in South Sumatra was divided into two main clusters at a genetic similarity coefficient level of 0.19, namely cluster A consisting of red rice with CT code (Cahya Tani) and cluster B consisting of red rice SJ code (Sumber Jaya), SP (Sp Padang), and K (Kelirejo). The four red rice individuals taken from different places have a low genetic similarity coefficient of 0.15-0.37, meaning that the fourth genetic variation of red rice is very high.

1. Introduction

Rice *(Oryza sativa L.)* as one of the staple food sources in Indonesia. South Sumatra province has various types of local rice that have the potential as genetic resources for food crops. there are 2 types of rice based on the color of the grains of white rice and pigmented rice. Pigmented rice has phenolic compounds especially anthocyanins which also act as color pigments [1 - 3]. Research on rice in south Sumatra is very limited, especially red rice because red rice has many benefits, so it needs to be researched about its genetic makeup, which can be exploited for the potential of assembling superior varieties and maintaining its existence in order to remain sustainable [4, 5]. Red rice pigment is controlled by the rc-bHLH gene which is a transcription factor for the prothoantocyanidin color pigment protein found in rice seeds [6].

Some researchers have conducted an approach to morphological analysis and genetic variation to determine the relationship of kinship and genetic variation in several types of rice in South Sumatra including research from Hanum *et al* [7], she conducted research on the kinship relationship of local rice varieties in South Sumatra based on PCR-RAPD (Polymerase Chain Reaction-Random Amplified Polymorphic DNA) techniques.

Based on this problem, the role of mathematical modeling is very important in solving problems in determining a decision in DNA analysis. In this research, the mathematical modeling used in the analysis of genetic similarity is the UPGMA model with a genetic similarity coefficient using the Jaccard Similarity Coefficient in the NTSys mathematical modeling program [8]. The PCR-RAPD (Polymerase Chain Reaction-Random Amplified Polymorphic DNA) method is a combination of the PCR method using random sequences in order to amplify random loci from the genome [2, 3, 9]. The RAPD (Random Amplified Polymorphic DNA) technique is one of several DNA-based marking techniques involving the use of a PCR (Polymerase Chain Reaction) machine.

This study aims to determine the DNA band polymorphisms were searched using PCR-RAPD (Polymerase Chain Reaction - Random Amplified Polymorphic DNA) techniques and the kinship relation of red rice in South Sumatra sought a mathematical modeling program in the Ntsys 2.1 application [7, 10].

2. Method

This research was conducted in February 2019 until May 2019. Rice sampling was carried out in several areas. They are red rice from Ogan Komering Ilir districts includes rice from Cahya Tani and SP Padang villages, and East Ogan Komering Ulu districts includes rice from Kelirejo and Sumber Jaya villages. A red rice survey in South Sumatra was conducted using purposive sampling. The location of the presence of red rice has been chosen according to literature studies and interviews with residents. Collecting data using observation methods where samples are collected genetic variation and presented in quantitative form. Research instruments include tools and laboratory materials that facilitate the research process such as electrophoresis, PCR machines, gel documentation and others. Rice samples were tested at the Genetics and Biotechnology Laboratory, Department of Biology, Faculty of Mathematic and Natural Sciences, Sriwijaya University, Indonesia.

2.1. Quantity and quality Test Results DNA Isolation

Isolation of red rice DNA was isolated using a plant genomic DNA kit DP305 in accordance with procedures provided by company (Tiangen). The results of DNA isolation were put into a tube as much as 2 µl, then NFW (Nuclease Free-Water) was added into the tube as much as 198 µl, and the DNA sample is homogenized. Quantitative DNA testing using UV-Vis spectrophotometry, pure DNA can absorb ultraviolet light due to the presence of purine bases and pyrimidines [7, 11]. The double band of DNA can absorb ultraviolet light at a wavelength of 260 nm, while protein or phenol contaminants will absorb light with a wavelength of 280 nm. So that DNA purity can be measured by calculating the absorbance value of wavelength 260 nm divided by the absorbance value of wavelength 280 (A260 / A280) and DNA purity value ranging from 1.8 to 2.0, if the absorbance value is less than 1.8, the DNA sample still contains protein contaminants, proteinase is added to remove it. If the value is more than 2.0, the DNA sample still contains RNA contaminants and ribonuclease is added to remove it [8]. The electrophoresis process is carried out using 1% agarose gel concentration and Electrophoresis results were visualized using gel documentation [7, 12].

2.2. PCR-RAPD Reaction

The composition of the solution for running PCR consists of Go Taq Green mastermix (2x) as much as 12.5 µl, ddH2O as much as 2.5, DNA template used as much as 5 µl and Primer to be used in the PCR-RAPD process including OPA 3, OPA 9, OPA 10, OPA 13, OPA 16, OPA 19, and OPB 8. The primers used were 5 µl. DNA amplification was carried out using a PCR machine

PCR conditions are regulated by temperature and the length of time the cycle lasts. The PCR condition setting is pre-denatured at 94 ° C for 7 minutes, followed by 40 cycles with temperature and the time of each cycle is denatured at 94 ° C for 1 minute, annealing using 37 ° C, for 1 minute, and elongation at 72 ° C for 1 minute 30 seconds. The last cycle is followed by post-elongation at 72 ° C for 2 minutes. Furthermore, PCR products were electrophoretic [7]. The determination of polymorphic and monomorphic bands can be seen in Figure 1. Polymorphic bands are determined by the presence of DNA bands at a certain size and not found in other samples. Meanwhile, to determine the monomorphic band that is the band that appears in all samples of the same size in the electrophoresis results [13].
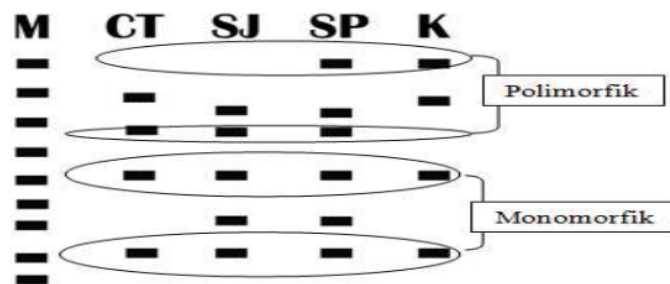
Figure 1. Figure of polymorphic and monomorphic bands.
Information: Marker (M), Samples (CT, SJ, SP, and K)

2.3. Data Analysis

Determination of the size of the amplification of DNA bands is done by measuring the distance of the standard DNA migration (100bp ladder) starting from the well to where the DNA migration. DNA bands obtained electrophoresized PCR sample is then converted into binary data which is stated by the presence or absence of DNA bands, if there is a DNA band then marked with (1), whereas if the DNA band does not appear then marked with (0).

Data were analyzed using the mathematical modeling program of NTSys version 2.1. Dendrogram created using unweighted pair-group method with arithmetic average (UPGMA). Genetic similarity coefficient using Jaccard Coefficient of Similarity and presented in the form of dendrogram [9].

3. Result and Discussion

3.1. DNA isolation results

DNA isolation as an important step in gene analysis using the PCR (Polymerase Chain Reaction) method. DNA isolation is carried out through four stages which include cell lysis, purification or removal of components other than DNA, and DNA concentration. The rice leaves used in this study were young leaves for DNA isolation. Young leaves have a soft texture and contain little fiber and impurities so that it is easy to grind. In addition young leaves contain lots of DNA because it is active in the process of cell division and growth [14].

The results of genomic DNA isolation of red rice is shown in Figure 2. Based on Figure 2, the DNA band produced is bright and there is no smear, this shows that the isolation process is good enough. Good quality DNA that is not degraded on the electrophoresis results shows a clear band and does not smear [5].
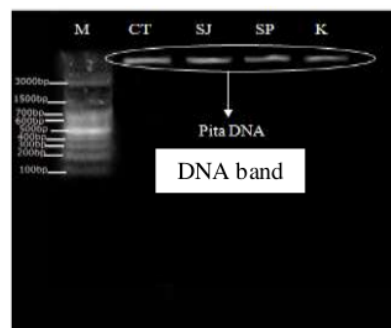

Figure 2. Figure of red rice DNA isolation electropherogram
Information: (M) Marker 100bp, (CT) Cahya Tani, (SJ) Sumber Jaya,
(SP) SP Padang, (K) Kelirejo

Figure 2 shows that the position of DNA not too far from the well. This shows that the isolated DNA has a very long DNA size that is more than 3000bp, so DNA bands migrate very slowly. One factor that affects the speed of migration of a DNA is the size of the DNA [15]. The International Rice Genome Sequencing Project (IRGSP) in 2008 stated that the DNA of the rice genome was 430MB.

The isolated red rice DNA has DNA band sizes above 3000bp. This shows that the isolated sample was good enough, so that a large DNA size was obtained. According to Pangaribuan [16], electrophoresis tests will show molecular weights from large to small of genomic DNA. If the genomic DNA is well isolated it shows a large molecular weight.

3.2. DNA isolation results

Rice DNA testing was quantitatively carried out using a spectrophotometer that included DNA purity. This test is carried out to ensure that there is no contamination of proteins, polysaccharides and other impurities in the DNA to be carried out. The success of a primer in amplifying printed DNA is determined by the quality and quantity of DNA.

Table 1. DNA purity measurement results

Code	Rice Source	A 260/280
CT	Cahya Tani, OKI	1,9
K	Kelirejo, OKUT	1,81
SJ	Sumber Jaya, OKUT	1,87
SP	SP Padang, OKI	1,89

Table 1 shows that the results of quantitative DNA measurements including DNA purity. The results obtained that the DNA purity of the four samples have a good level of DNA purity and meet the standards that range from 1.81 to 1.9. Based on this, the four samples can be used as DNA templates in the PCR process, if the absorbance value is less than 1.8, the DNA sample still contains protein contaminants, proteinase is added to remove it, whereas if the value is more than 2.0 then the DNA sample still contains RNA contaminants and ribonuclease is added to eliminate it [8].

3.3. Monomorphic and polymorphic band profiles

The result of seven primers that have been used are able to show variations between rice genotypes of rice shown by DNA band profiles. Figure 3 shows that DNA bands produced are clear and bright and no smears that significantly affect the visualization of DNA bands. So that the identification and classification of monomorphic and polymorphic DNA bands can be done. DNA bands that have relatively the same thickness show that the primers used are specific to amplifying the target DNA [2, 17, 18].

The SP (Sp Padang) sample does not have DNA bands in OPA 9 primers. This shows that the nucleotide sequences in OPA 9 primers are not found in SP red rice DNA code (Sp Padang). Fragments that do not appear are caused by no amplification. The number of bands produced by each primer depends on the distribution of homologous sites in the genome or primer used that is incompatible with printed DNA [11, 13].

Table 2 shows RAPD DNA band profile results are known that amplified DNA consists of 46 DNA bands, where there are 43 DNA bands showing polymorphic and 3 DNA bands showing monomorphic (Table 2). OPA 16 primers have the most number of DNA bands compared to OPA 3 primers, OPA 9, OPA 10, OPA 13, OPA 19, and OPB 8 which are 14 DNA bands.

The number of bands produced by each primer varies from 3 (OPA primary 9) to 14 (OPA 16 primary). The percentage of polymorphic of the seven primers used was 93.47% with the number of polymorphic bands of 43 DNA bands. OPA 9, OPA 10, OPA 13, OPA 16 and OPA 19 primers have a polymorphic percentage of 100%, while OPA 3 shows a polymorphic percentage of 87.5%, and the lowest polymorphic percentage is at the primary OPB 8 of 60%. Polymorphic bands show diversity in

plant genomes. The more primers that can amplify the polymorphic band, the greater the diversity in the plant's genome [5, 18]
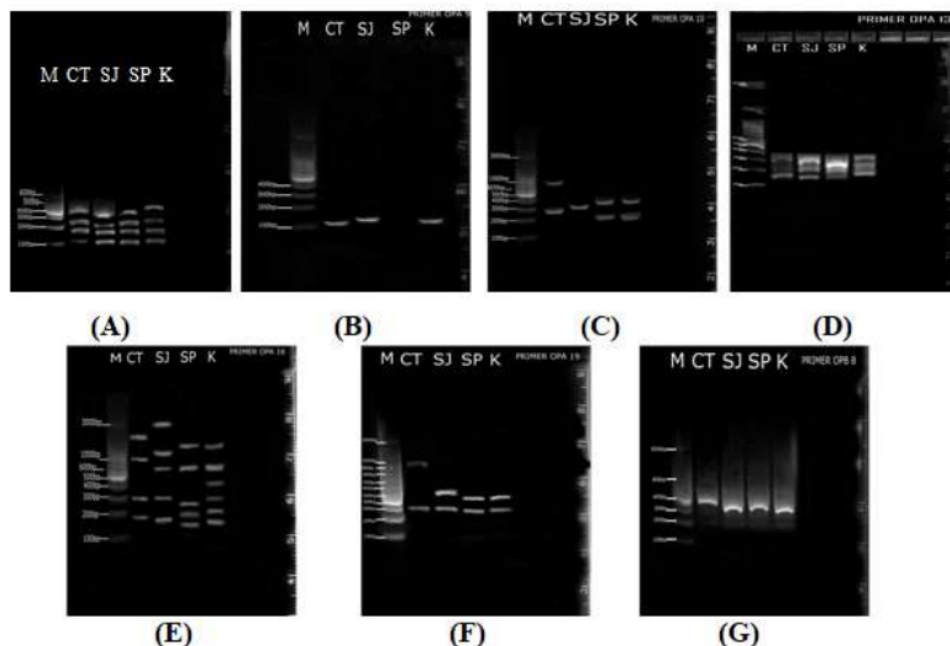

Figure 3. Figure of DNA band profile

Information: (M) Marker 100bp, (CT) Cahya Tani, (SJ) Sumber Jaya, (SP) SP Padang, (K) Kelirejo, (A) OPA 3, (B) OPA 9, (C) OPA 10, (D) OPA 13, (E) OPA 16, (F) OPA 19, (G) OPB 8

Table 2. Polymorphic percentage

No	Primer	Nucleotide	Total of Band		Total	Polymorphic (%)
			Polymorphic	Monomorphic		
1.	OPA 3	AGTCAGCCAC	7	1	8	87.5
2.	OPA 9	GGGTAACGCC	3	0	3	100
3.	OPA 10	GTGATCGCAG	5	0	5	100
4.	OPA 13	CAGCACCCAC	6	0	6	100
5.	OPA 16	AGCCAGCGAA	14	0	14	100
6.	OPA 19	CAAACGTCGG	5	0	5	100
7.	OPB 8	GTCCACACGG	3	2	5	60
Total			43	3	46	93.47

Based on previous studies of local rice in South Sumatra conducted by Adriansyah et al [19], it was found that the highest number of amplified bands in OPA 13 primers was 12 DNA bands, whereas based on this study OPA 16 primers had the most amplified bands, 14 bands. This shows that there are differences in sequence between rice observed. The success of a primer in amplifying printed DNA is determined by the presence or absence of homology of the primary nucleotide sequence with the printed DNA nucleotide sequence [20].

The amplification band pattern shows high genetic variation among red rice in South Sumatra. Genetic variations based on the pattern of DNA band results of this amplification can be used as a basis

in the process of breeding the breeding of plants. Genetic variation is the lowest level in biodiversity. Genetic variation illustrates the diversity within a species. The diversity can be seen from several characteristics, both from the inside (genotype), namely molecular variations related to variations in protein and genetic material (DNA) and from the outside [5, 11].

3.4. Red Rice Cluster Analysis
DNA amplification results from four samples using seven primers were converted into binary data and a similarity matrix was made based on the Unweighted Pair-Group Method Arithmetic (UPGMA) method. Based on the results obtained that the four red rice have a distant kinship relationship.

Table 3. Matrix of genetic similarity coefficient

Sample	CT	SJ	SP	K
CT	1.00			
SJ	0.15	1.00		
SP	0.15	0.26	1.00	
K	0.25	0.25	0.37	1.00

Table 3 shows that the four sample locations in South Sumatra produced different levels of genetic similarity coefficient between individuals. The matrix level of genetic similarity coefficient of four red rice samples ranged from 0.15 to 0.37. The closest genetic similarity coefficient level is SP individuals (Sp Padang) with K (Kelirejo), which is 0.37, while the farthest level of genetic similarity coefficient is 0.15, namely CT individuals (Cahya Tani), SJ (Sumber Jaya), and SP (Sp Padang). Genetic variation is needed for a population to deal with environmental changes. As a result, if there is no genetic variation in a population or species, extinction is very likely because it cannot survive environmental changes [21].

Based on comparisons of data from the level of genetic similarity coefficient presented in Table 3, it shows that there are genetic variations in individual red rice whose location is different. Differences in environment or geographical location make individuals have to adapt to their environment, both morphologically and genetically. This encourages high genetic variation in an individual. Research conducted by Pratiwi [22] states that there is a significant correlation between genetic distance and geographic distance in *Globba leucantha*, so it is indicated that geographical differences play an important role in genetic diversity.
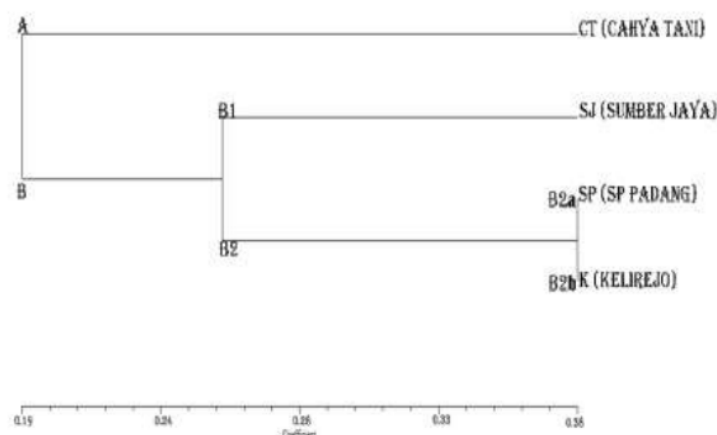

Figure 4. Figure of the genetic similarity

Information: (CT) Cahya Tani, (SJ) Sumber Jaya, (SP) SP Padang, (K) Kelirejo

Figure 4 shows the grouping of four red rice in South Sumatra obtained a genetic similarity coefficient of 0.19 to 0.38. the higher of the genetic similarity coefficient obtained, the closer of kinship between individuals. The genetic similarity coefficient shows information about the level of genetic linkages between individuals.

Based on the dendogram presented in (Figure 4) that there are two main clusters at the level of genetic similarity coefficient 0.19, namely cluster A and cluster B. Individuals included in cluster A are red rice with CT code (Cahya Tani). Whereas cluster B is occupied by SJ (Sumber Jaya), SP (Sp Padang), and K (Kelirejo) code red rice. The similarity value ranges from 0 to 1, so that kinship is said to be close if the similarity value gets closer to 1 [23].

Cluster B presented in (Figure 4) Is divided into 2 sub-clusters at the level of genetic similarity coefficient 0.26, namely B1 and B2 which separates SJ (Sumber Jaya) in subclaster B1, and SP (Sp Padang) and K (Kelirejo) in subcluster B2. Subclass B2 is divided into 2 sub-clusters that have a level of genetic similarity coefficient that is closer to the coefficient value of 0.38, namely subclasses B2a and B2b, which groups SP individuals (Sp Padang) on B2a and K sub-clusters (Kelirejo) on B2b sub-cluster. The higher of the similarity index, it means the smaller of the genetic variation or in other words the smaller genetic distance. Low similarity index between individuals shows high genetic diversity [10, 18].

The four red rice taken from different places have a low level of genetic similarity coefficient so that genetic variation between the four samples is still relatively high. The grouping differences based on genetic similarity are caused by genetic differentiation between populations, which indicates the existence of genetic structure as the beginning of the process of speciation. This is due to external factors such as geographical isolation and habitat fragmentation, as well as internal influences such as mutations, natural selection, and genetic drift [10, 24, 25].

4. Conclusion

The DNA band profile of RAPD results which 43 DNA bands show polymorphism (93.47%). Based on the analysis using the NTSys 2.1 program, a dendogram was obtained where red rice in South Sumatra was divided into two main clusters at a genetic similarity coefficient level of 0.19, namely cluster A consisting of red rice with CT code (Cahya Tani) and cluster B consisting of red rice SJ code (Sumber Jaya), SP (Sp Padang), and K (Kelirejo). The four red rice individuals taken from different places have a low genetic similarity coefficient of 0.15-0.37, meaning that the fourth genetic variation of red rice is very high.

5. Acknowledgement

6. References
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