Genetic diversity of local rice varieties in Kampung Naga- Tasikmalaya West Java based on RAPD

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Abstract. Kampung Naga is a traditional village for the Sundanese tribe located near Tasikmalaya, West Java Province, Indonesia. Agriculture serves as the primary economic activity for the people of this community, both inside and outside of the cultivated lands. They continue to use conventional agricultural practices to preserve local wisdom passed down by their ancestors to ensure food security in the village. A total of eleven local rice varieties widely cultivated in Kampung Naga used in traditional ceremonies were tested in this study. This study aimed to investigate the genetic diversity and relationship among the local rice varieties cultivated in Kampung Naga. We used RAPD markers in the testing process to measure genetic diversity more precisely and efficiently. The data was analyzed using NTSys and grouped based on their genetic similarity. Cluster analysis to form a phylogenetic tree in a dendrogram was constructed using UPGMA. Based on the genetic relationship analysis, the eleventh rice varieties were clustered in two groups with genetic similarity ranging from 25.53%-88.89%. Saptinah and Cere were clustered in one group, while the others in the other cluster. The genetic distance between these varieties was quite high (above 0.5). The genetic diversity and relationship observed in this study emphasize the importance of determining of the genetic identity of local varieties for sustainable use and support rice breeding initiatives.

1 Introduction

Kampung Naga is one of the traditional villages for the Sundanese tribe located in the Tasikmalaya, West Java Province, Indonesia, with an area of about 10 hectares, and its preservation is currently maintained [1]. The primary source of income for the residents of Kampung Naga is farming. Rice is important to the people of Kampung Naga because it is the primary food supply, a source of local income, and a representation of culture and tradition. Currently, Kampung Naga farmers cultivate fifteen (15) local and some new varieties. According to [2], this number of local rice are much lower now than it was in the

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1980s, before the start of the green revolution era, when they limited their cultivation to local varieties. The locals documented 24 local varieties known as "pare ageung" [2, 3]. If not preserved and utilized, this quantity may continue to decline.

Local rice varieties frequently provide a number of benefits that are still being discovered. Investigating the potential use of local rice is a critical step in ensuring future sustainability and food security, as well as enhancing farmer welfare and safeguarding the environment. Previous studies revealed that the local rice variety is rich in genetic diversity [4-7]. Those varieties may have useful genes to help build new rice varieties that are more productive and resistant to a number of environmental issues, such as pests, diseases, and challenging environmental conditions in rice production. Therefore, it is important to study genetic diversity at the morphological and molecular levels. To further facilitate its use in plant breeding, how resistant or tolerable plants are to biotic and abiotic environmental stresses needs to be assessed [4, 8]. To the best of our knowledge, there has been limited research conducted on the local rice varieties of Kampung Naga.

The study of genetic variety has been extremely beneficial for sustainable agriculture and conservation. Molecular markers offer a more precise and efficient method to measure genetic diversity since they are not impacted by environmental influences. Numerous molecular marker approaches, including RFLP, SSR, AFLP, SNP, RAPD, and ISSR, have been developed to date [9]. With all of this in mind, RAPD is a useful and efficient technique for a wide range of genetic applications, especially when original genomic data are not yet easily accessible. One additional benefit of utilizing RAPD is its minimal DNA requirement and simplified, cost-effective deployment technique.

In this manuscript, we report the genetic variation and genetic relationships of eleventh local varieties from the Kampung Naga Tasikmalaya using the RAPD-based marker approach. The genetic diversity and relationships of each variety were discussed and successfully revealed.

2 Materials and Methods

2.1 Plant materials

Eleven local rice varieties of Kampung Naga are presented in Table 1. Ten RAPD markers were used, including OPA 02, OPA 07, OPA 09, OPA 18, OPB 17, OPB 18, OPC 05, OPC 08, OPC 11, and OPE 20 (Table 2).

2.2 DNA extraction

Fresh leaves from every sample were used for DNA extraction utilizing a modified cetyl trimethyl ammonium bromide (CTAB) method. A total of 100 grams of fresh leaves were ground using liquid nitrogen and subsequently combined with preheated (65 °C) DNA extraction buffer in a 50-ml falcon tube. The samples were incubated at a temperature of 65 °C for 1 hour, with the tubes being inverted every 15 minutes for mixing. After incubation, we placed the samples at room temperature and mixed them with equal amounts of chloroform and isoamyl alcohol (24:1). Subsequently, they were put through centrifugation at 12,000 rpm for 10 minutes. Following centrifugation, the liquid phase was collected and transferred into new tubes. The DNA was precipitated by adding an equal amount of cold isopropanol. The mixture was then spun at 12,000 rpm for 10 minutes. The pellet was washed using 70% ethanol, followed by drying and then storage in TE buffer.

Table 1. List of eleven local rice varieties used in this study

No.	Code	Variety name
1	RG	Regol
2	LC	Lokcan
3	PT	Peuteuy
4	JN	Jidah Nangka
5	SK	Seksrek
6	SR	Segon Ranggeui
7	PR	Pare Paray
8	SP	Saptinah
9	JM	Jamlang
10	CR	Cere
11	BG	Badigal

2.3 Polymerase Chain Reaction (PCR) and gel documentation

Genomic DNA samples were subjected to PCR amplification with a set of 10 specifically chosen RAPD primers. The PCR reaction was conducted in a 0.2-ml tube with a total volume of 10 ul. The reaction mixture comprised 100 ng of DNA sample, 2 x My Taq HS Red mix master mix, 5 ul of RAPD primers (0.5 mM concentration), and 3.5 ul of water. The amplification was conducted in *a Bio-Rad T100 PCR Thermal Cycle* machine. The process began with an initial denaturation step at a temperature of 94 °C for 7 minutes. Then it continued with 40 cycles of denaturation at 94 °C for 1 minute, annealing at 37 °C for 1 minute, and extension at 72 °C for 2 minutes. Finally, a final extension step took place at 72 °C for 2 minutes, followed by an indefinite hold at 10 °C. The PCR product was subjected to electrophoresis on a 1.5% agarose gel and then visualized using a UV transilluminator. The identification of the polymorphic and monomorphic bands is displayed in Fig. 1. The existence of DNA bands at a specific size that are absent from other samples characterizes polymorphic bands. Meanwhile, identify the monomorphic band in the electrophoresis data, which consistently appears in each sample of the same size.

2.4 Statistical analysis

The DNA bands were subsequently transformed into binary data, where the presence or absence of DNA bands is indicated by a value of (1) or (0), respectively. The data were evaluated using the NTSys program. The Dice similarity coefficient was utilized for conducting similarity analysis. Furthermore, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was applied to perform clustering analysis and generate a dendrogram.

3 Results

Ten RAPD marker primers were tested on all rice genomic DNA sample used in this study. All the ten RAPD marker primers were successfully amplified and generated DNA bands in all DNA sample investigated. The example of the gel documentation for the amplification results is presented in Figure 1. The image displays different quantities of bands for each local variety tested. This difference demonstrates the existence of polymorphism and then will be used for further analysis.

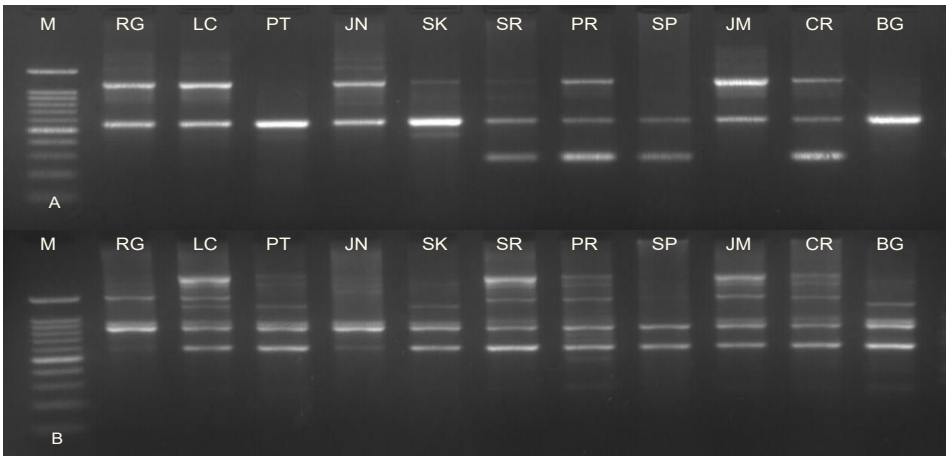

Fig. 1. RAPD markers profile, A : OPA 07 and B : OPA 02. M : Marker (100 bp) ; RG : Regol, LC : Lokcan, PT : Pare Peuteuy, JN : Jidah Nangka, SK : Seksrek, SR : Segon Ranggeui, PR : Pare Paray, SP : Saptinah, JM : Jamlang, CR : Pare Cere, BG : Badigal.

Table 2. List of 10 RAPD primers and genetic variation in 11 local rice varieties

Name	Sequence 5'-3'	Total band	Polymorphic band	Percentage of polymorphic
1. OPA 02	5 TGCCGAGCTG 3	8	6	75
2. OPA 07	5 GAAACGGGTG 3	3	2	66.67
3. OPA 09	5 GGGTAACGCC 3	6	6	100
4. OPA 18	5 AGGTGACCGT 3	3	3	100
5. OPB 17	5 AGGGAACGAG 3	4	4	100
6. OPB 18	5 CCACAGCAGT 3	6	6	100
7. OPC 05	5 GATGACCGCC 3	6	6	100
8. OPC 08	5 TGGACCGGTG 3	5	5	100
9. OPC 11	5 AAAGCTGCGG 3	4	3	75
10. OPE 20	5 AACGGTGACC 3	7	7	100

Total	52	48	
Average	5.2	4.8	91.67

In total, 52 DNA bands were observed, of which 48 were polymorphic with the mean value of the band was 5.2 for each marker primer (Table 2). Seven markers produced 100% polymorphism (OPA 09, OPA 18, OPB 17, OPB 18, OPC 05, OPC 08, dan OPE 20) while OPA 07 yielded the lowest proportion at 66.67%. The OPE 20 is the most efficient markers out of those seven, due to its ability to generate a significant number of polymorphic bands compared to the others. The polymorphic bands mean percentage was approximately 91.67%. The OPA 07 and OPA 18 produced the minimum band of 3, while the OPA 02 produced the maximum band of 8.

The genetic similarity of 11 local rice varieties of Kampung Naga was evaluated according to the Dice coefficient. The genetic similarities among those varieties are ranging from 25.53% to 88.89% (Table 3). This range of genetic similarity values indicates genetic variation in the tested varieties was catagorized as low-moderate to high [10]. The highest genetic similarity of 88.89% (coefficient value 0,8889) was found between Regol and Lokcan. On the other hand, the lowest coefficient value of 0,2553 (25,25% similarity) was observed between Lokcan and Saptinah. Genetic variation represents the most basic form of biodiversity and demonstrates the range of differences within a species [11].

Table 3. Genetic similarity in eleven local rice varieties

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	1,0000										
2	0,8889	1,0000									
3	0,7273	0,7941	1,0000								
4	0,7213	0,7302	0,5965	1,0000							
5	0,7213	0,6984	0,7368	0,6538	1,0000						
6	0,6377	0,6761	0,6154	0,5667	0,6333	1,0000					
7	0,5882	0,6571	0,5938	0,5763	0,5763	0,8060	1,0000				
8	0,2667	0,2553	0,2927	0,3333	0,2778	0,3636	0,4186	1,0000			
9	0,7500	0,7838	0,6471	0,5714	0,6032	0,6479	0,5429	0,2979	1,0000		
10	0,5091	0,5263	0,5098	0,5652	0,5217	0,5185	0,6415	0,6000	0,5965	1,0000	
11	0,6769	0,7164	0,7869	0,5000	0,6071	0,5625	0,5714	0,3500	0,7761	0,5600	1,0000

Note: 1. Regol, 2. Lokcan, 3. Peuteuy, 4. Jidah Nangka, 5. Seksrek, 6. Segon Ranggeui, 7. Pare Paray, 8. Saptinah, 9. Jamlang, 10. Cere, 11. Badigal

The genetic similarities data was, then, applied to construct a dendrogram using the unweighted UPGMA method and to evaluate the relatedness among the samples tested. The result was a distant clustering of the data units in the dendrogram (Fig. 2). The dendrogram indicates the presence of two major clusters with a genetic distance of approximately 0.44.

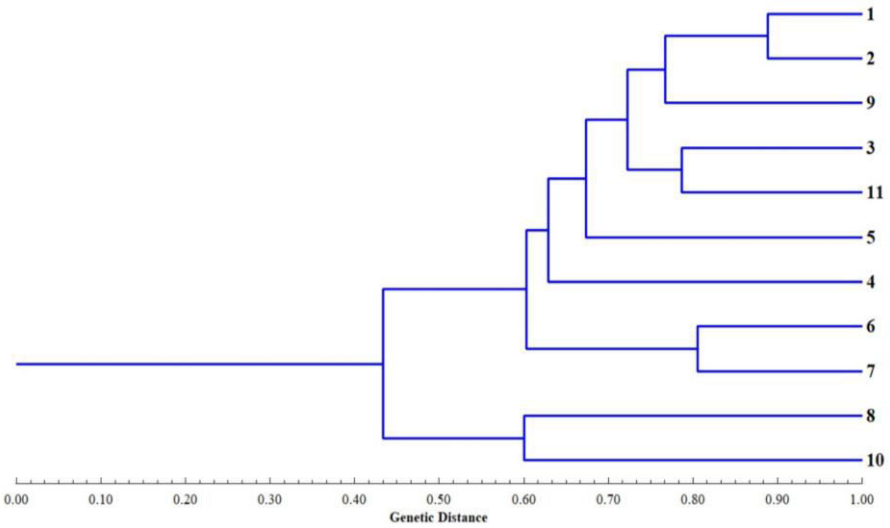

Fig. 2. Dendrogram on eleven local rice varieties based on RAPD markers with UPGMA method.

The first major cluster had only two local varieties, Saptinah and Pare Cere, with a genetic distance of about 0.60. The second major cluster, further divided into two minor clusters, consisted of nine varieties. The first minor cluster had two varieties, Segon Ranggeui and Pare Paray, whose genetic distance is around 0.81, and the second minor cluster consisted of two sub-minor clusters. The first sub-minor cluster had only one variety, Jiddah Nangka, while the second sub-minor cluster had two groups, with Seksrek as the first group and Regol, Lokcan, Jamlang, Pare Peuteuy, and Badigal as the second group. Regol, Lokcan, and Jamlang form a subgroup with a genetic distance of 0.76, while Pare Peuteuy and Badigal form a different group with a genetic distance of 0.78. Regol and Lokcan have the highest genetic distance, approximately 0.89.

4 Discussion

Genetic variation plays an important role in supporting genetic conservation and plant breeding. This study's findings indicate that there is a significant genetic variance in the rice that is cultivated in Kampung Naga, West Java, based on RAPD marker. This data is required in order to support future breeding initiatives because this genetic variation is often used to determine the close genetic relationship between varieties [12]. Based on Dice coefficient value, Regol and Lokcan have the highest coefficient value of 0,89 (Tabel 3), indicating that these varieties have high genetics similarity and are closely related (Fig. 2). Whereas Saptinah and Lokcan have the lowest genetic similarity value (0,26). Furthermore, the genetic similarity values between Saptinah and all others Kampung Naga varieties are low - moderate, with similarity below 50%. This shows that Saptinah has significant genetic differences from the other varieties.

Based on the phylogenetic tree (Fig. 2), the eleven rice varieties evaluated were spreads into two main clusters. One cluster consists of two varieties, Saptinah and Cere, while the other clusters contain nine varieties. The difference might be caused by the rice's origin, which is believed to originate from several regions, and have been planted and grown from generation to generation up until today.

Further characterizations would be required to provide comprehensive data on the potensial used of these varieties in the future breeding initiatives. Those includes the evaluation of morphological, physiological, and agronomical characteristics under certain conditions (abiotic and biotic stressed).

5 Conclusion

Ten RAPD primers have been successfully used to characterize eleven local rice varieties from Kampung Naga, Tasikmalaya. OPE 20 is the most efficient marker for producing polymorphic bands. The genetic relationship analysis clustered the eleventh rice variety into two groups, with genetic similarity ranging from 25.53% to 88.89%. The analysis revealed genetic diversity among those rice varieties. Although the genetic distance between these varieties was quite high (above 0.5), Regol and Lokcan were genetically similar, with a genetic distance of 0.89. Saptinah and Cere were slightly different from the other varieties. We recommended additional assessments with additional markers, such as ISSR, SSR, SNP, and so on, to produce more comprehensive data.

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