# Genetic Diversity Of Locally Rice Germplasm From Tana Toraja And Enrekang Based On RAPD (Random Amplified Polymorphism DNA) Markers

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**Abstract :** This study aims to determine the genetic diversity of locally rice germplasm based on RAPD analysis. Totally eighten of locally rices were used in this research : Pare Ambo, Pare Bau, Pare Birrang, Pare Bumbungan, Pare Kobo, Pare Lalodo, Pare Lea, Pare Rogon, Pare Tallang, Pare Kamida, Pare Lambau, Pare Solo, Pulu Mandoti, Pare Lotong, Pallan, Mansur, Pare Pinjan and Pare Salle. Extraction of DNA was made according the method of Doley and Doley (1987) with modification. Molecular analysis was performed using six different RAPD primers, there were OPF-14, OPH-14, OPM-05, OPM-12, OPU-15 and OPX-09. PCR products were separated on 1.8% agarose gel and vizualised in EtBr solution. DNA bands were then analyzed with NTSYS program. The results showed a highly variation in the DNA band profiles, the bands number of each primer varied between 4-10 bands with an average of 7 bands from a total of 42 bands. Primers that generated most polymorphic DNA fragments are OPF-14 and OPU-15, as many as 10 fragments/bands. Dendrogram analysis separated 18 local rice accessions in three groups. Pare Pallan, Mansur, Lambau, Solo, Tallang and Lotong has similarity cooeficient of 96 %, while Pare Bumbungan has farther most similarity with other rice genotypes that only 35 %.

**Index Terms:** genetic diversity, locally rice germplasm, RAPD analysis

## 1 INTRODUCTION

Indonesia is known to have a very broad biodiversity. Especially for rice, Indonesia has approximately 17,000 of germplasm accessions and several wild rices with high species diversity. The diversity of this species is very valuable capital base for the assembly and for improvement of rice varieties (Suhartini, 2009)[1]. This crop germplasm is a very important asset that must be preserved. Germplasm contained in the properties necessary for the establishment or improvement of the desired properties of high-yielding varieties. Agricultural development programs require crop varieties of high yielding, resistant to pests and diseases, and specific environmental stress tolerance. To establish the necessary yielding varieties include local varieties and wild relatives as a parent. Local varieties play an important role as a parent that adaptive in specific locations, while the introduction of wild relatives and varieties can be used as parental resistance to pests and diseases (Rais, 2004)[2]. In conventional breeding programs, strategies for genetic diversity evaluation is done through character anatomy, morphology and physiology (Mahapetra *et al*., 1995)[3]. Currently this approach has been equipped with molecular techniques, thus allowing to obtain a marker gene that controls the character of the target quickly and accurately. It is very helpful, effective and efficiency in the implementation of selection in plant breeding programs (Gupta *et al*., 2002)[4].


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Wiesing *et al*., (1995)[5] stated that the development of molecular markers have been shown to facilitate research in the field of sciences such as taxonomy, ecology, genetics and plant breeding. Selection techniques to get the desired character has develop rapidly, various selection methods are also available. Selection can be done at the gametophyte and sporophyte level (Ottaviano and Sari - Gorla,1993)[6], *in vitro* selection (Wenzel and Foroughi-Weber, 1993)[7], and selection in molecular level (Flow and Morino - Gonzales,1993). Utilization of germplasm for improving agronomic traits selected from accessions with genetic determination must be based on more accurate technique, so that the determination of the individual plant as the genetic material in the improving process can be done properly. Molecular markers that commonly used in the analysis of genetic diversity is RAPD (Cheng *et al*., 1998a; 1998b; 1998c; Syafaruddin, 1998a; 1998b; Welsh and Mc Clelland, 1990; Williams *et al*., 1990)[8-14]. RAPD is a PCR-based markers using a random primer with 10 bases. RAPD technique does not require information about the tracer of DNA or DNA sequences tracked. The procedure is simple and straightforward in terms of preparation, may be done to the maximum samples in large quantities, the amount of DNA requirement is relatively small, and the process does not use radioactive compounds. Other molecular markers that can be used for the purposes mentioned above is the use of Sequence Characterized Amplified Regions markers (scars). According to Khush (2002)[15], Scars primers can identify loci that have been identified by PCR based marker systems including RAPD/AP-PCR, DAF, or AFLP. Liu and Furnier (1993 )[16] reported that the use of RAPD analysis always showed higher diversity than isozyme and RFLP. Identification of local rice germplasm based on molecular markers in Indonesia, especially in South Sulawesi is still not widely practiced. Therefore, this activity becomes necessary in the protection and exploitation of varieties of germplasm maximally through the study of genetic diversity and plant identification. Through the use of molecular markers can be identified and characterized certain genetically traits with high accuracy. The information about the genetic diversity of locally rice cultivated by farmers in South Sulawesi is indispensable.

## 2. MATERIALS AND METHODS

### 2.1 Materials

This research was conducted at the Laboratory of Agricultural Biotechnology Research Center, Hasanuddin University in cooperation with the Center for Technology and Genetic Resources, Bogor. The materials used for this study were 18 locally rice germplasm accessions from Tana Toraja and Enrekang, South Sulawesi. PCR analysis (RAPD analysis), used 6 random oligonucleotide primers there were: OPF-14, OPH-14, OPM-05, OPM-12, OPU-15 and OPX-09.

### 2.2 Method

#### 2.2.1 Isolation of DNA

Total genomic DNA was extracted from the rice seeds by miniprep CTAB procedure of Doyle and Doyle (1987) with modification. Grain samples crushed with liquid nitrogen to a powder, inserted into 1.5 ml eppendorf micro tube and added with 500 mL of extraction buffer and then vortexed gently to dissolve the sample. The mixture was added with 25 mL of SDS and incubated at 65$^{o}$C for 10 minutes. After the mixture was added with 85 mL 5M of NaCl, eppendorf tube was inverted until evenly mixed and then added with 10 % CTAB as much as 67.5 mL. The mixture was then incubated at 65$^{o}$C for 10 minutes and added to 675 mL of chloroform, mixed well and centrifuged for 2 min at 12,000 rpm. The supernatant was transferred to a new eppendorf and added to 450 mL of cold isopropanol to precipitate the DNA and then centrifuged at 8000 rpm for 5 minutes. Pellet was washed three times with 70 % EtOH, dried on Biosan Dry Block Heater Termostat(Bio TDB-100) at 40 $^{o}$C then redissolved with 30 mL of TE buffer. A total of 1 µL solution of DNA was analyzed on 1 % agarose gel electrophoresis. Visualization after gel colouring in EtBr was performed via Bio Step Transilluminator (Bio View, USDT-20 SM-8E), to determine the quality of the DNA.

#### 2.2.2 Analysis of PCR

DNA samples were amplified using selected primers from the Operon Kit. Final reaction volume was 40 mL with composition: 1x reaction Buffer (50 mM KCl; 10 mM Tris - HCl pH 9, and 0.1 % Triton X -100), 1 unit of Taq DNA polymerase (Promega), 200 µM dNTP, 0.4 µM of each Primer, and 50 ng of genomic DNA. The amplification process performed for 45 cycles, consisting of : 1 min 94 °C for denaturation process, 1 min 36 °C for annealing and 2 min at 72 °C for extension. DNA amplification product was separated on 1.8 % agarose. Measurement of DNA profiles using 1 kb DNA ladder (Sigma, USA). Visualization of gel was performed using 0.5 mg /ml Ethidium bromide (Sigma, USA) and documented under UV-Bio Step Transilluminator (Bio View, USDT-20 SM-8E).

### 2.3 Data Analysis

Each DNA fragment amplified by a given primer was treated as a unit character and the RAPD fragments were scored as present (1) or absent (0) of the primer-cultivar combinations. Pair-wise comparisons of the cultivars based on the presence or absence of unique and shared amplification products were used to generate similarity coefficients. Estimates of genetic similarity (F) were calculated between all pairs of the cultivars according to Nei and Li (1979)[17] based on following formula:

Similarity (F) = 2Nab/(Na + Nb)

Where Na = the total number of fragments detected in individual ‘a’; Nb = the total number of fragments shown by individual ‘b’ and Nab = the number of fragments shared by individuals ‘a’ and ‘b’. The resulting similarity coefficients were used to evaluate the relationships among traditional varieties and improved cultivars with a cluster analysis using an unweighted pair-group method with arithmetic averages (UPGMA). The analysis was plotted in the form of a dendrogram. All computations were carried out using the NTSYS-pc, Version 2.2 package). (Rohlf, 2005) [18]

## 3. RESULTS AND DISCUSSION

The results show that all six primers are able to amplify all DNA of tested rice samples with a total of 42 DNA bands (Table 1). The number of bands in each primer varied between 4-10 bands with an average of 7 DNA bands. OPM - 12 primer resulted only 4 bands, whereas most bands generated by OPF - 14 and OPU - 15 primer with each of 10 bands. Band size of the amplified DNA ranged from 100-1600 bp. The number of bands produced by each primer depends on the distribution of the site that is homologous to the primer sequences in the genome. Differences in the number and size of bands determine the level of genetic diversity of these local rice accessions. The DNA profiles of six consecutive primers presented in Figures 1 and 2.

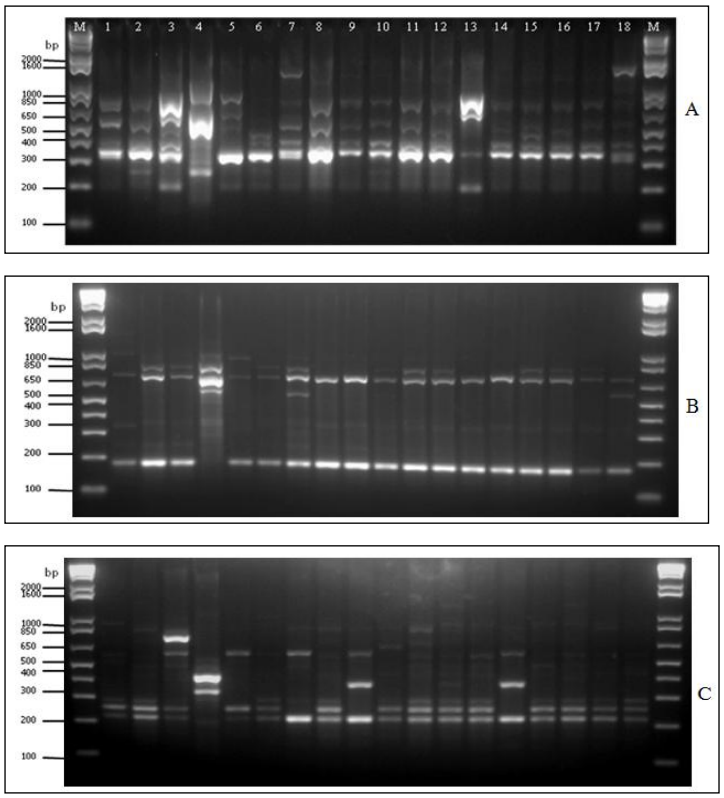


**Figure 1.** PCR products of 18 DNA rice accession using OPF-14 (A), OPH-14 (B), and OPM-05 (C) primers. 1-9 are rice accessions from Enrekang, 10-18 from Toraja. 1. Ambo, 2. Bau, 3. Birrang, 4. Bumbungan, 5. Kobo, 6. Lalodo, 7. Lea, 8. Rogon, 9. Tallang, 10. Kamida, 11. Lambau, 12. Solo, 13. Mandoti, 14. Lotong, 15. Pallan, 16. Mansur, 17. Salle, 18. Pinjan.

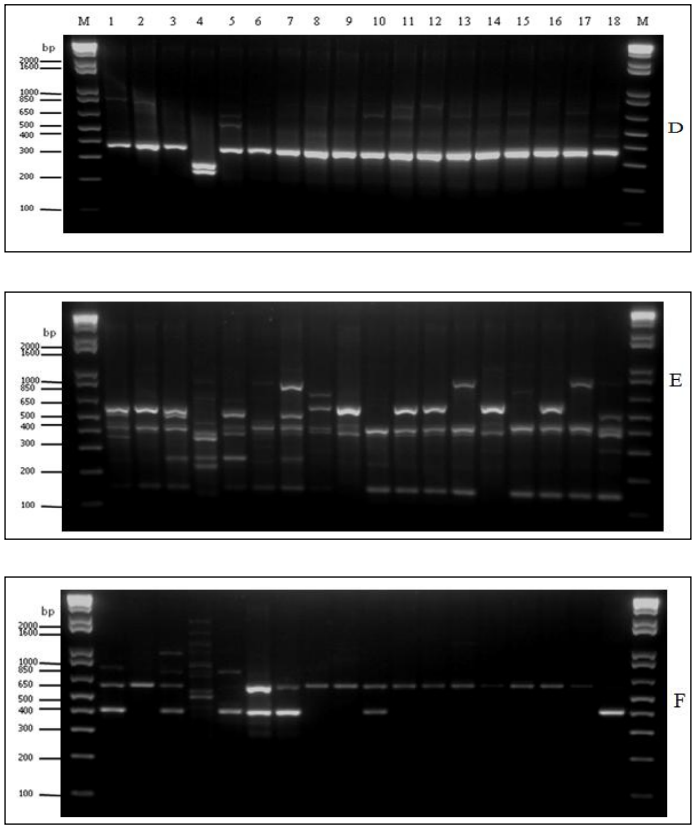


**Figure 2.** PCR products of 18 locally rice accession using OPM-12 (D), OPU-15 (E), and OPX-09 (F) primers. 1-9 are rice accessions from Enrekang, 10-18 from Toraja. 1. Ambo, 2. Bau, 3. Birrang , 4. Bumbungan, 5. Kobo, 6. Lalodo, 7. Lea, 8. Rogon, 9. Tallang, 10. Kamida, 11. Lambau, 12. Solo, 13. Mandoti, 14. Lotong, 15. Pallan, 16. Mansur, 17. Salle, 18. Pinjan.

Weeden *et al*.,(1992)[19] reported that the intensity of each band can not be used to estimate the number of base pairs on each DNA profiles. The intensity of DNA bands in each primers used is strongly influenced by: (1) the purity and concentration of DNA template. DNA template containing compounds such as phenolic compounds and polysaccharides and DNA concentrations were too small often produce DNA amplification bands that vague or unclear, (2) distribution of primer sites in DNA template, (3) the existence of annealing site competition in DNA template that causes one fragment was amplified in large quantities and other fragments was only few amplied.

**Tabel 1. RAPD's Primers, monomorphic and polymorphis of DNA bands after Electrophoresis**

| Primers | Nucleotide base (5'……..3') | Band size (bp) | Monomorphic bands | Polimorphic bands | Amount of bands |
|---|---|---|---|---|---|
| **OPF-14** | TGCTGCAGGT | 200 -1600 | 0 | 10 | 10 |
| **OPH-14** | ACCAGGTTGG | 200 - 850 | 0 | 5 | 5 |
| **OPM-05** | GGGAACGTGT | 250 - 850 | 0 | 7 | 7 |
| **OPM-12** | GGGACGTTGG | 250 - 400 | 0 | 4 | 4 |
| **OPU-15** | ACGGGCCAGT | 100 - 850 | 0 | 10 | 10 |
| **OPX-09** | GGTCTGGTTG | 450 - 650 | 0 | 6 | 6 |
| **Total** | | | | **42** | **42** |

Primers did not produce DNA bands indicates that these primers do not have homologous to the template DNA, due to the formation of DNA bands fragment is dependent on the primer sequence and the genotype of the DNA template. Differences in the number and polymorphism of DNA bands generated from each primer depicts the observed complexity of the plant genomes. According Grattapaglia *et al*., (1992)[20], the number of DNA bands polymorphic will describe the state of the plant genome, while the difference in the number of bands and polymorphism generated by each primer depicts of a plant genome complexity. Weising *et al.,*(1995[5]), states that basically polymorphism in RAPD analysis is the result of several events, namely: 1) deletion of DNA in the genome that undergo annealing, 2) Insertion of DNA between the two primer sites, and 3) substitution at nucleotide annealing sites. Large DNA inserts between the two primer sites led to the inability of DNA polymerase to synthesize DNA, so that the area can not be amplified. Deletions in the genome that contains the primary cause of attachment sites can not stick to the area, so that the area can not be amplified. Deletions between the two sites of attachment cause changes in the length and size of the amplified region. Similarity analysis based on RAPD markers generate 42 DNA profiles with genetic similarity coefficient (SC) values ranging from 0.017 to 0.942 (data not shown). The highest value was observed between PE3 KG-PE2 (0.942), and the lowest similarity coefficient (SC) value was between PT8-PT5 (0.017). Some accessions (PE5-PT9, PE7-PE6) also showed a fairly high degree of similarity (0.938), between PE8-PE6 with SC of 0.869, whereas accession that have low SC value (0.031) is between PE5-PT3, PE2-PT5 and PE4-PE5 (0,067), PE9-PT4 (0.069), PE9-PE5 (0.070). All pairs of locally rice accessions with high SC grades is generally a locally rice accessions with the same category, while the pair of locally rice accessions that have a small SC value is generally a locally rice accessions with different categories. Based on the degree of similarity, it seen that Pare Lotong (PE5) from Enrekang and Pare Tallang (PT9) from Tana Toraja have high levels of similarity with the value of 0.938. According to Lee (1998)[21], closely related individuals will have a close genetic distance, whereas when distantly related will have a far genetic distance. Ahmad *et al.,* (1980), cited by Daradjat *et al.,* (1991)[22] reported that genotypes originating from the same area are not always stand in the same cluster, geographic diversity means not always have anything to do with genetic diversity. Furthermore Erlina (2011)[23], concluded that in the same cluster are characterized almost by the same name of genotype. This suggests that these genotypes formed from the same population, so the level of similarity is closer. In other hand there genotyping with very different names but have very high levels of similarity, cause of the possibility that the genetic material came from the same parent but scattered to different places so that given different names by the collector. Therefore, DNA -based characterization can provide accurate information in determining of genetic diversity.

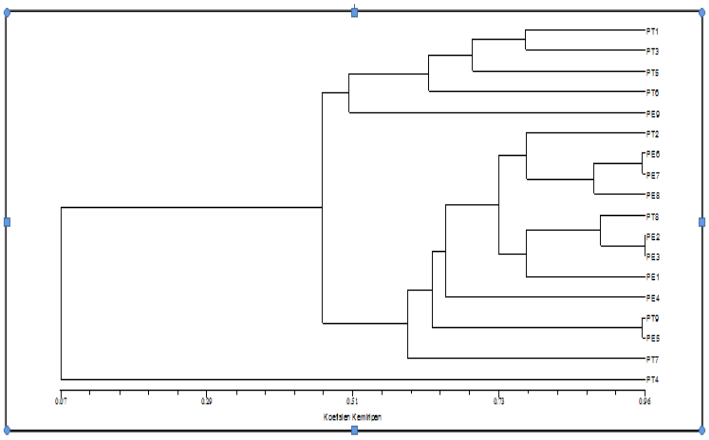


**Figure 3.** Dendogramm of 18 locally rice accessions using UPGM analysis with 6 RAPD primers

The three main components formed at a rate of 0.47 similarity and formed into further 6 sub-groups with the similarity level of 0.51. By similarity coefficient of 0.47, group 1 consisted of 5 local rice accessions, group 2 consisted of 12 accessions and group 3 consisted of one local rice accessions. These three groups can be decomposed into 6 sub-groups on similarity coefficient of 0.51 in which sub-group (a) consists of PT1, PT3, PT5, PT6 and PE9, sub-group (b) consists of PT2, PE6, PE7, PE8, PT8, PE2, PE3 and PE2, the subgroup (c) consists of PE4, sub- group (d) consists of PT9 and PE5 , sub- group (e) consists of PT7, and sub-group (f) consists of PT4. Pare Pallan (PE6) and Pare Mansur (PE7), Pare Lambau (PE2) and Pare Solo (PE3), Pare Tallang (PT9) and Pare Lotong (PE5) has a similarity coefficient of 96 % similarity level, meaning that the rice group has the same genetic make up. While Pare bumbungan (Pt4) has a similarity coefficient of 33 % similarity level, meaning that Pare Bumbungan hasa further level of similarity than other rice. This might be due to the rice comes from a different parent. In accordance with Cahyarini *et al*.,(2004)[24] that if the similarity is said far less than 60 %. Thus the grouping proves that rice accessions that have a significant level of similarity of 96 % derived from the same parent.

## 4. CONCLUSION

The results of RAPD amplification using 6 primer of 18 locally rice accessions from Tana Toraja and Enrekang found diversity in the DNA band profiles, the number of bands per primer was varied between 4-10 bands with an average of 7 DNA bands from a total of 42 bands. OPF-14 and OPU-15 primers generated most polymorphic DNA fragments with 10 fragments of each primer. Bumbungan rice had a furthest alliance with the other of rice accessions.

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