

Amplification Of Rapd Primers Of Sugar Palm (*Arenga Pinnata Merr*) From South Sulawesi

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INTRODUCTION

Sugar palm (*Arenga pinnata Merr*) is a type of palm with high economic value. It can be found in various regions in Indonesia (Nirawati et al., 2020). Superior palm products can be used as a source of food and energy, including brown sugar, granular brown sugar, fresh sap, sugar palm fruit, and alcoholic drink. Besides that, sugar palm products are widely used for craft materials, as well as building materials (Manambangtua et al., 2018).

Based on data from the Central Java Forestry Service (2010), the main production centers of sugar palm can be found in 14 provinces, which are Maluku, North Maluku, Papua, North Sumatra, West Sumatra, West Java, Central Java, Banten, North Sulawesi, South Sulawesi, Southeast Sulawesi, Bengkulu, South Kalimantan, and Nanggroe Aceh Darussalam. The total area of sugar palm plant centers in 14 provinces in Indonesia is around 70,000 Ha (Wulantika, 2019). While the area of sugar palm plantations in South Sulawesi is around 6,060 ha, and the average sugar production is 4,121 tons/year. It can be found growing in several districts, including Maros district with an area of 251 ha and Sinjai district with an area of 35 ha (BPS, 2017). The natural distribution (provenance) at the location is assumed to have high diversity and categorized as a “tree of life” for farmers.

The vast area of sugar palm in Indonesia has not been matched with the application of the right cultivation technology. Therefore results obtained are not optimal. Due to a lack of proper cultivation techniques, then crop productivity is still relatively low (Indasary, et al., 2019). If this continues, it is feared that the sugar palm genetic sources which have high sap potential will be gone. Furthermore, one of the essential things that should be done in sugar palm cultivation is the availability of technology, for example, using superior sugar palm seeds from selected parent trees with high sap production (Tenda and Mahayu, 2015).

Due to its risky characteristic and population, sugar palm must be optimized by using a tree breeding program. A study by Asmono et al. (2005) reported that molecular information produced can be used as a basis for maintaining genetic material for conservation and instruction of tree breeding. A molecular marker is a tool to identify genetic potential between each species, species and population, and their relationship with the special nature of plants. Molecular analysis is an approach that can be done to determine genetic factors. One of the DNA-based molecular markers that have been widely applied as a marker of plant genetics is Random Amplified Polymorphic DNA (RAPD) (Sumiyati et al., 2009).

Research on genetic diversity through the RAPD molecular approach for forestry plants has begun to be carried out, for instance, in white Jabon population (Nurtjahjaningsih,

et al., 2014), analysis genetic diversity of *Pinus merkusii* in UNHAS education forest (Gusmiaty, et al., 2016), Analysis of Genetic Diversity of Palm Oil (*Elaeis Guineensis Jacq.*) Moderate Resistant to Ganoderma (Melati, 2019), cocoa from east Kolaka (Yolanda Fitria Syahri et al., 2020). However, research on the evaluation of sugar palm genetic diversity in Sulawesi using RAPD markers has not been conducted. Therefore, this research is conducted to initiate steps for plant breeding on sugar palm based on RAPD markers.

The RAPD method is a molecular marker based on PCR. PCR is a synthesis and amplification of DNA with in vitro techniques (Prayogi et al., 2015). RAPD can be used to identify plant genotypes because of the advantages in implementation and analysis. RAPD requires DNA extraction, optimum amplification conditions, and data analysis, all of which can be done in relatively rapid time (Poerba and Yuzammi, 2008). RAPD has particular advantages and can be applied to reveal sugar palm genetic diversity in Maros and Sinjai provenances. RAPD marker is a reliable method for identifying varieties by analyzing DNA polymorphisms (Sathish and Mohankumar, 2007).

RESEARCH METHODOLOGY

A. Research Time and Place

This research was conducted from October 2019 to February 2020. The research was carried out in two stages: research sampling and primers selection using RAPD markers. Samples were taken from two locations, first in the Bonto-Bonto Village, Maros, South Sulawesi, and second in the Bonto Sinala Village, Sinjai, South Sulawesi. Primers selection was carried out at the Laboratory of Biotechnology and Tree Breeding, Faculty of Forestry, Universitas Hasanuddin, Makassar.

B. Research Methods

The materials used were ice gel and 25 sugar palm leaves collected from Maros provenance and 25 sugar palm leaves from Sinjai provenance. The leaves were obtained from sugar palm trees. The part used for DNA isolation was the green leaf. Green leaves contain a lot of DNA because actively carrying out cell division and growth process. Samples were taken from provenance in Bonto-Bonto Village, Maros and Bonto Sinala Village, Sinjai, South Sulawesi, with 25 samples for each provenance. Samples were put into a plastic bag and stored in a cooler box with ice gel to maintain quality and to reduce damage potential. Furthermore, leaves were stored in the freezer until the extraction process was carried out.

Isolation of Sugar Palm DNA

The process of cell walls lysis (isolation) of DNA was carried out using the Cetyl Trimethyl Ammonium Bromide (CTAB) method with modification (Larekeng et al., 2018) Samples of young leaves were weighed as much as 200 mg without leaf venation then placed into a mortar. 500 µl CTAB buffer was added, and the leaves crushed until became powder, poured into a 2 ml tube, and put in the vortex for 15 seconds. The tube containing the solution was incubated in a water bath at 65°C for 90 minutes, the solution was flipped every 30 minutes. A 100 ml of isoamyl alcohol chloroform solution was added to the incubated sample. The tube containing the solution was centrifuged at 10,000 rpm for 10 minutes, the solution in the tube (supernatant) was transferred to a new tube and then poured with 800 ml of isopropanol solution. The supernatant was centrifuged at 1,000 rpm for 5 minutes. The supernatant solution was removed, the tube then dried for one night.

The process of separating DNA from other components was carried out by adding 500 µl of TE buffer to the dried tube and then centrifuged at 10,000 rpm for 10 minutes. The solution was transferred to a new tube, then 100 µl of chloroform was added and centrifuged at 10,000 rpm for 10 minutes. The solution was transferred again to a new tube and added

with 100 µl of sodium acetate and 800 µl of isopropanol. The tube was centrifuged at 10,000 rpm for 10 minutes. The solution was discarded, and the precipitate was taken and dried for one night. A total of 100 µl ddH₂O was added to the dried tube and centrifuged at 10,000 rpm for 1 minute. The solution containing extracted DNA was added as much as 4 µl of RNase and centrifuged at a speed of 10,000 rpm for 1 minute. Last, the DNA solution was stored in a freezer at -20°C.

Primers Selection

Primers selection was done by making several PCR reactions in several different primers under the same conditions and using the same DNA samples for each primer for determining optimum condition and level of band variation produced from each primer. Twelve random samples from each provenance were selected as DNA samples. Primers selection was carried out in 10 RAPD primers. Primers selection was performed to choose polymorphic primers that can well amplify and able to determine the appropriate annealing temperature. Primers that produce clear bands and easily to be scored were categorized as specific (Larekeng, et al., 2015). The selected primers are presented in Table 2.

Table 2. RAPD Primer used for Sugar Palm of Maros and Sinjai Provenances

| No. | Locus Name | Primers Order (5' – 3') | Tm (°C) |
|-----|------------|-------------------------|---------|
| 1 | OPK-20 | GTGTCGCGAG | 38,5 |
| 2 | OPA-15 | TTCCGAACCC | 34,2 |
| 3 | OPD-20 | ACCCGGTCAC | 39,1 |
| 4 | OPY-09 | AGCAGCGCAC | 42,5 |
| 5 | OPA-18 | AGGTGACCGT | 36,2 |
| 6 | OPQ-07 | CCCCGATGGT | 38,5 |
| 7 | OPA-02 | TGCCGAGCTG | 40,7 |
| 8 | OPP-08 | ACATCGCCCA | 37,6 |
| 9 | OPD-03 | GTCGCCGTCA | 40,8 |
| 10 | OPC-11 | AAAGCTGCGG | 36,9 |

Tm : Time malting

The primers selection in the RAPD analysis affected polymorphism of the DNA bands produced because each primer had its attachment location, causing the polymorphic DNA bands produced by each primer to become different, both in terms of the number of base pairs and the number of DNA bands. The intensity of the DNA band amplified from each primer was greatly influenced by the purity and concentration of the DNA template.

Figures 1 and 2 show the electropherograms of DNA amplification of sugar palm from Maros provenance using OPA-15, OPK-20, OPA-02, and OPP-08 primers. Bright, clear, and consistent bands that appeared in each primer can be considered for specific primers.

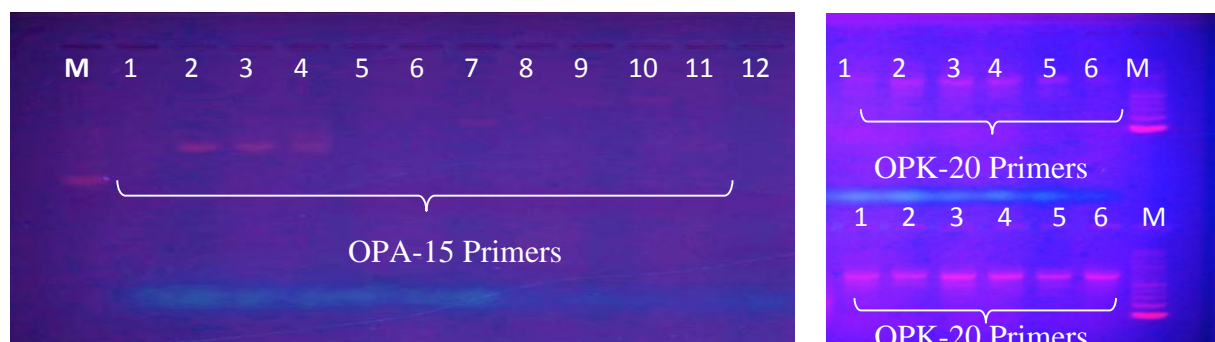


Figure 1. Electropherogram of DNA Amplification Results using RAPD Primers

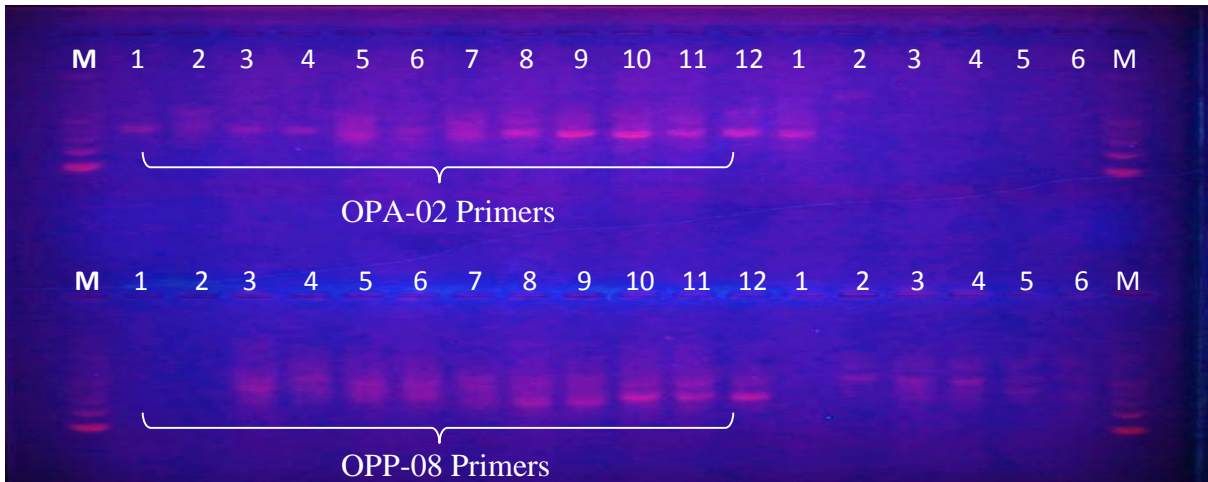


Figure 2. Electropherogram of DNA Amplification using RAPD Primers

Electropherograms of DNA amplification of Maros provenance using OPA-15, OPK-20, OPA-02, and OPP-08 primers produced clear and bright bands. The four primers were used as a reference by researchers to validate 25 samples from Maros provenance. Validation was carried out for further testing of selected primers of sugar palm samples in Maros provenance. After validation was done and the primers showed a polymorphic and clear band, then the primers used to analyze the genetic diversity of Maros provenance.

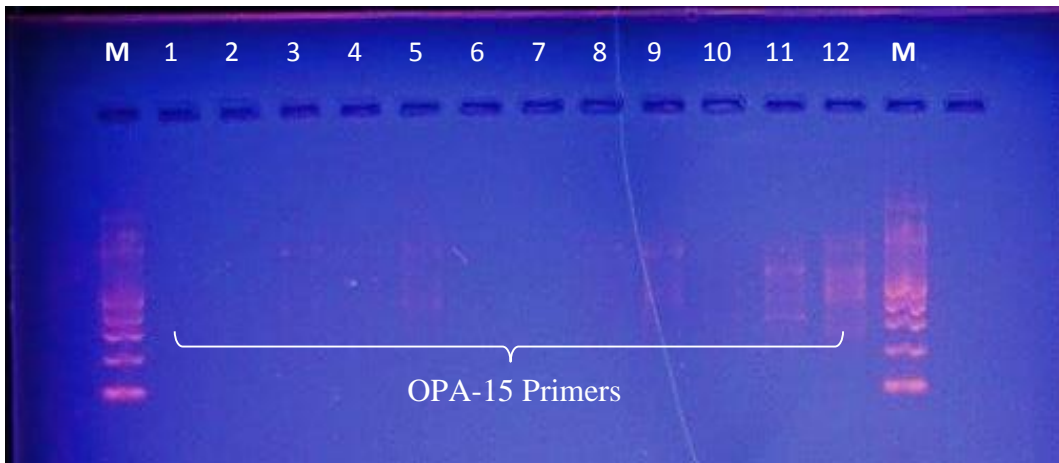


Figure 3. Electropherogram of DNA Amplification using RAPD Primers

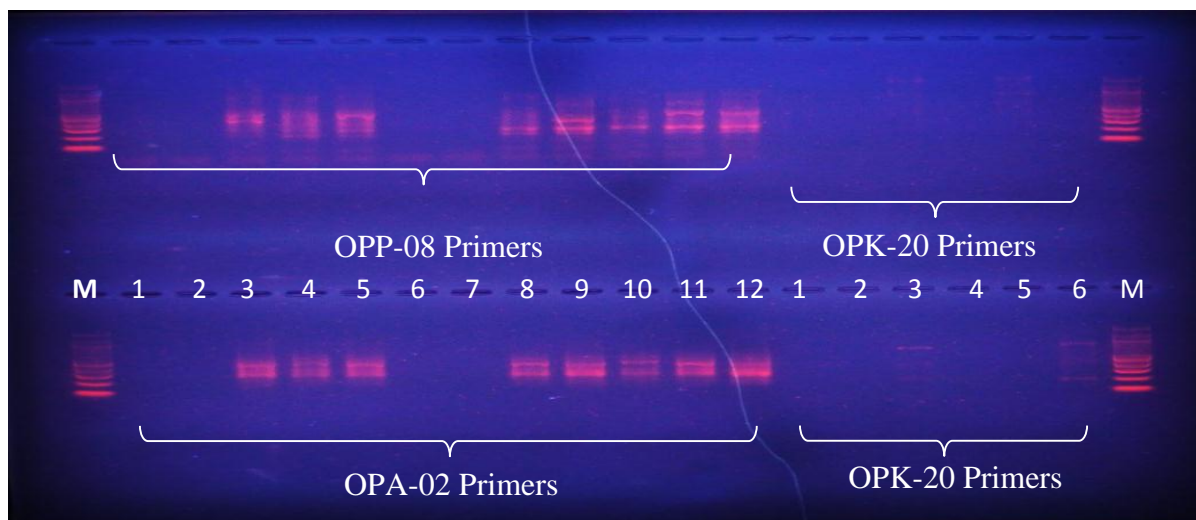


Figure 4. Electropherogram of DNA Amplification using RAPD Primers

Figures 3 and 4 show electropherograms from the amplification result of sugar palm of Sinjai provenance using OPA-15, OPP-08, OPA-02, and OPK-20 primers. The OPA-15, OPP-08, and OPA-02 showed clear and bright bands and can be used as a reference by researchers to validate 25 samples of Sinjai provenance. The OPK-20 primers produced thinner bands compared to the other three primers, but this primer can be considered as a reference to validate the DNA of Sinjai provenance because the DNA electropherogram of sugar palm of Maros provenance using OPK-20 primers produced clear and bright bands.

The DNA amplification results in Maros and Sinjai provenances showed bands with various levels of thickness in each sample, caused by the quality of the extracted DNA that varies in each sample had a crucial role during the PCR stage. A study by Siregar and Diputra (2013) found that each band has a different level of thickness. Level of thickness influenced by the freshness of the samples. Samples with fresh conditions produce thicker bands. Thick bands can also mean higher DNA concentrations (Gusmiaty et al., 2016).

Six RAPD primers consist of OPD-20, OPY-09, OPA-18, OPQ-07, OPD-03, and OPC-11 used during primers selection were taken from 12 random samples from Maros provenance 12 random samples from Sinjai provenance showed smear bands thus it was not used as a reference by researchers for validation of the entire sugar palm samples from Maros and Sinjai provenances.

DNA template containing compounds such as polysaccharides and phenolic compounds, and too small DNA template concentrations often produce smear or unclear DNA amplification bands (Weeden et al. 1992). Primers with smear bands cannot be used for genetic diversity analysis because it can lead to data misinterpretation (Larekeng, et.al., 2019).

The amount and intensity of DNA bands produced after DNA amplification with PCR largely depend on how the primer recognizes its complementary DNA sequence in the DNA template which being used (Tingey et al., 1994). Each primer sequence has a specific annealing site on the genomic of DNA. The annealing temperature phase during the PCR process greatly influenced primers attachment process; every one degree of temperature change can cause primers to fail to attach (Gusmiaty et al., 2012). A study conducted by Wen et al. (2014) reported that primers that had more annealing sites in their complementary genomic DNA samples could amplify more DNA (DNA template).

The annealing temperature of each amplified primer varies. OPA-15 primer had an annealing temperature of 29.7°C for Maros provenance and 38.7°C for Sinjai provenance, the OPK-20 had 41.3°C for Maros and Sinjai provenances, the OPA-02 had 44.4°C for Maros provenance and 43.5°C for Sinjai provenance, and the OPP-08 had 42.6°C for Maros

provenance and 40.4° C for Sinjai provenance. Each primer sequence has a specific annealing site on the genomic of DNA. The annealing phase temperature during the PCR process greatly influenced primers attachment process; every one degree of temperature change can cause primers to fail to attach (Gusmiaty et al., 2012). The right annealing temperature determines the quality of the band. If the annealing temperature is too low, the primer cannot bind specifically to the template. However, if the annealing temperature is too high, the primer fails to bind to the template. A study conducted by Ludyasari (2014) showed that the DNA PCR amplification process in the DNA control area of finger shrimp was not successful because the annealing temperature was too low thus, the primers attachment process was complicated.

The tree breeding program is expected to produce a variety of new superior cultivars that have high productivity and have other characters that can support efforts to improve quality and competitiveness (Ferita, et al., 2015). Tree breeding can improve the genetic quality of seeds and produce high genetic variation. High genetic variation has resistance characteristics to extreme environmental conditions; thus, pests and diseases can be avoided (Boer, 2007). RAPD primers selection for the analysis of sugar palm genetic diversity in South Sulawesi is expected able to maintain the sustainability of sugar palm in the future. It can be developed as a superior product of South Sulawesi, particularly in Maros and Sinjai regencies. In cocoa shows RAPD primers can used to genetic diversity study (Y. F. Syahri et al., 2019; Yolanda Fitria Syahri et al., 2020).

DNA markers based on Polymerase Chain Reaction (PCR) can be the technology chosen because of its efficiency and accuracy during identification, to support genetic conservation and improvement. Molecular markers are not influenced by environmental factors and can be very useful in the analysis of genetic diversity to support tree breeding (Arif et al., 2019). A study conducted by Wicaksono (2017) on genetic diversity in cocoa showed the usefulness of molecular markers in the evaluation of genetic diversity in parental selection for new superior clones.

CONCLUSION

Based on this research, it could be concluded that 4 out of 10 RAPD primers showed polymorphic, bright, and clear bands. The OPA-15 primer had 29.7°C of annealing temperature for Maros provenance and 38.7°C for Sinjai provenance, the OPK-20 primer had 41.3°C for Maros and Sinjai provenances, the OPA-02 primer had 44.4°C for Maros provenance and 43.5°C for Sinjai provenance, the OPP-08 had 42.6°C for Maros provenance and 40.4°C for Sinjai provenance. Researchers will use these four primers for analysis of the genetic diversity of sugar palm in Maros and Sinjai provenances.

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