RAPD Amplification on Cocoa (*Theobroma cacao L.*) From East Kolaka, Southeast Sulawesi Province

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RAPD Amplification on Cocoa (*Theobroma cacao L.*) From East Kolaka, Southeast Sulawesi Province

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Abstract. Kolaka is one of the best quality cocoa bean producing regions in Indonesia. Plant breeding to maintain and improve crop quality needs to be done so that genetic diversity information is needed. This study aims to obtain RAPD markers that can be used to analyze cocoa genetic diversity from kolaka. A total of 4 primers from 15 selected primers produced polymorphic, bright and clear bands so that they could be used to analyze cocoa genetic diversity. The primers are OPA15, OPD3, M29, and OPP08. The highest aneling temperature was 43°C at the OPD03 primer, and the lowest aneling temperature was 36°C in the OPA15 primer.

1. Introduction

Kolaka is one of the districts in Southeast Sulawesi province that produces the highest quality cocoa beans. According to Kolaka Plantation Office Chief Baharun Hanise, Antara News [1] said that the quality of cocoa beans from the region was ranked third after Bali and Papua were the most favored Belgian producers of chocolate. Southeast Sulawesi has superior local clones with the production of 1.5 to 2 tons Ha-1. The superior clones include cacao species and have a resistant and moderate character to the attack of cocoa fruit rot [2]

The development of the type of cocoa in the future does not only pay attention to the level of production and resistance to pest attacks but also the resistance to global climate change which has an impact on temperature rise, drought or stagnant water due to the increased intensity of rainfall with a shorter period. Information on genetic diversity is needed to assemble cocoa that is adaptive to these environmental changes. Genetic diversity can be measured using molecular markers. Genetic diversity is essential for breeding programs. Bahian cacao showed low genetic diversity and 30 simple sequence repeat (SSR) markers were used to genotype 279 cacao plants from germplasm and local farms [3].

One molecular marker commonly used to obtain information on genetic diversity is RAPD (Random Amplified Polymorphic DNA). Some 50 RAPD primers were randomly selected in Gambier ((Uncaria Gambir) [4]. RAPD markers have been widely used for genetic analysis of various plants such as hybrid sugar cane [5], soybeans [6], and babassu palm [3]. This molecular marker is widely used because it is easy, fast, and inexpensive to apply. This marker can be applied to plants whose DNA sequence is unknown. A RAPD primer is needed that is able to distinguish the individual from one another by producing polymorphic, bright, and clear bands. Primary selection needs to be done to obtain polymorphic primers. This study aims to obtain primers that produce polymorphic bands, bright and clear. The primers obtained from the results of this study can then be used to analyze the genetic diversity of cocoa from Kolaka.

2. Material and Methods

This research was conducted from March to May 2018. Sampling was carried out in the polipolia village, Southeast Sulawesi Province. Primary screening is carried out at the Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Hasanuddin University, Makassar.

The sample used was cocoa leaves. Twelve samples were used for primary selection. The primary selection was carried out on 20 RAPD primers. DNA extraction was carried out using the CTAB method [7] with modification [8]. DNA was then made DNA working with a ratio of 1µL master DNA with 9µL ddH_2O.

The DNA amplification process was carried out using a PCR machine sensoquest thermal cycler. One PCR reaction consisted of 2µL DNA molds; Mix Hotstar (Qiagen) PCR 0.625µL; ddH2O 3µL, RAPD primer as much as 1.25 µL. The PCR process follows the following procedure: preheat at 95°C for 5 minutes, denaturation at 94 ° C for 60 seconds, attaching the primer to the DNA template (aneling temperature) specific to each primer, extension of 72 ° C for 60 seconds, final extension 72 ° C for 10 minutes. The process of denaturation until the extension is repeated 35 times. In the process of attaching the primer to the DNA mold a temperature gradient of ± 5 °, C is carried out from the primary reference temperature. This is done to obtain a temperature that produces a clear and clear ribbon.

The results of DNA amplification were repaired using a horizontal electrophoresis method. The separation was carried out using 2% agrosa dissolved in TAE 1× at a 100 volt voltage for 90 minutes. The results of the separation were given UV light and documented using doc gel.

3. Results and discussion

The results of the primary election conducted on 16 RAPD primers showed that as many as 4 primers were able to amplify cocoa DNA and produce bright and polymorphic bands (Figure 1). One primer produced a monomorphic band, OPA-02. The OPZ-05 primer does not produce amplification products. A total of 10 primers produce opaque ribbons. The quality of the tape produced by each primer can be seen in table 1.

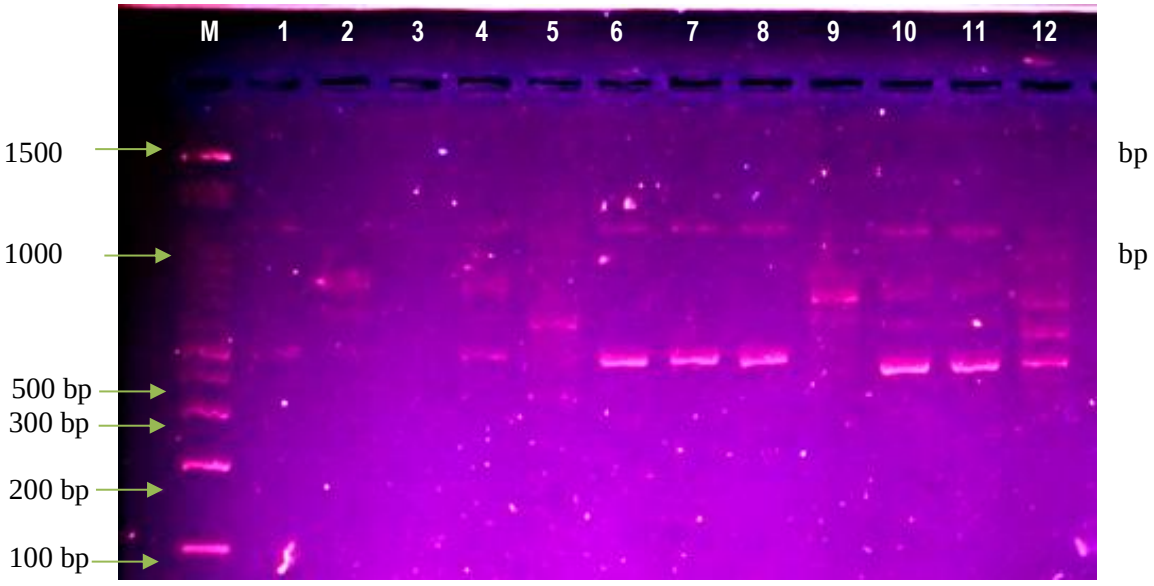

Figure 1. Results of amplification of 12 cacao DNA using primer M29

Table 1. The quality of the tape produced by each primer

No	Primer Name	Sequence	Band	Jumlah pita Polidori	Annealing Temperature (°C)
1	OPQ 07	5'-CCC CGA TGG T-3'	Smear	0	-
2	OPA 15	5'-TTC CGA ACC C-3'	Clear Polymorphic	4	43
3	OPZ 05	5'-TCC CAT GCT G-3'	No bands	0	-
4	OPD 03	5'-GTC GCC GTC A-3'	Clear Polymorphic	3	37
5	OPA 02	5'-TGC CGA GTC G-3'	Clear Monomorphic	0	-
6	OPP 08	5'-ACA TCG CCC A-3'	Clear Polymorphic	6	36
7	PLW 04	5'-CAGAAGCGGA-3'	Smear	0	-
8	PLR 13	5'-GGACGACAAG-3'	Smear	0	-
9	OPA 11	5'-CAATCGCCGT-3'	Smear	0	-
10	OPG 09	5'-CTGACGTCAC-3'	Smear	0	-
11	PLC 14	5'-TGCGTGCTTG-3'	Smear	0	-
12	M 29	5'-TGCGTGCTTG-3'	Clear Polymorphic	5	39,2
13	M 147	5'-GTGCGTCCTC-3'	Smear	0	-
14	M 33	5'-CCGGCTGGAA-3'	Smear	0	-
15	PLB 10	5'-CTGCTGGGAC-3'	Smear	0	-
16	PLD 08	5'-GTGTGCCCCA-3'	Smear		-

The primers who successfully amplified cocoa DNA consisted of OPA 15, OPD 03, OPP 08, and M29. Each primer produces polymorphic bands of 3 to 6 metered bands ranging in size from 150 to 1500 bp. In results of RAPD marker on *Pericoopsis mooniana* showed the primer OPA 15 and OPD 03 very clear and easy to scoring [8]. The polymorphic tape on the RAPD primer is due to differences in the size of the tape produced by each. The existence of mutations in the form of insertions or deletions is a factor that causes polymorphism in RAPD markers [9].

Primers that produce polymorphic but smear / opaque ribbons cannot be used to analyze genetic diversity. Smear ribbons occur because of variations in amplified DNA fractions that have a small met size difference [10]. This causes the ribbon that appears on the agarose gel to overlap so that it is difficult to determine the size of each fragment. A ribbon-like this will complicate the sking process and can cause results of interpretation errors.

RAPD is still used to analyze genetic diversity because of its ability to amplify various species, like Pinus merkusii in Educational Forest Hasanuddin University [11], bamboo [12], Dioscorea sp [13] and Indonesian Edible Canna (*Canna indica* L.) [14]. Other types of molecular markers other than RAPD can be used to identify genetic diversity, namely SSR, ISSR, RFLP, SNAP and much more. SSR used to calculate the genetic diversity in *Anthocephalus macrophyllus* [17], SSR and SNAP marker to calculate the xenia effect in Kopyor coconut [12], and to analysis mating system in Ebony, Jabon Merah and Kopyor coconut [15-17]. This indicated molecular marker very many functions to other studies. In addition to the analysis of genetic diversity, RAPD markers can be used for the development of specific SCAR molecular markers. The study used RAPD markers to develop specific markers to detect *Phytophthora capsica* [18]. Sharma et al [19] reports study used pair-wise RAPD primers for diversity analysis in soybean and demonstrated the usefulness of these markers in the evaluation of genetic variation.

4. Conclusion

a. Primers produce polymorphic band was OPA15, OPD03, M29, and OPP08.
b. The annealing temperature for OPA15, OPD03, M29, and OPP08 were 36°C, 43°C, 37.7°C, and 39.2°C respectively.
c. OPZ05 primer does not produce any band and OPA02 produce clear band but monomorphic.

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