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RAPD primer screening for amplification on Katokkon pepper from Toraja, South Sulawesi, Indonesia

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Abstract. Random amplified polymorphic DNA (RAPD) is a well-known molecular marker that extensively applied to the genetic diversity analysis among species in the populations. Here, this marker was utilized in Katokkon pepper (*Capsicum chinense* Jacq.), a highland pepper and having unique characteristics cultivated in specific regions (Tana Toraja and North Toraja), on preliminary primer screening using 30 RAPD primer pairs. A total of eight primers (OPA-02, OPAE-11, OPG-09, M-29, OPP-08, OPG-19, OPZ-05, and OPD-20) could produce polymorphic bands on twelve randomly selected DNA samples. OPA-02, OPP-08, OPG-19, OPZ-05, and OPD-20 generated polymorphic bands on all twelve evaluated samples, yet OPZ-05 was excluded in further analysis due to producing unclear bands. Clear and polymorphic bands were formed by OPAE-11 on eleven samples, whilst OPG-09 and M-29 only produced bands on ten samples. The primers are suitable to be used for amplifying DNA and determining the genetic diversity of Katokkon pepper in further analyses.

1. Introduction

Pepper is a member of family Solanaceae. It has various variations in characters, e.g., shape, color, and flavor. Its fruits not only contain essential vitamins, such as, vitamin A, vitamin B, vitamin E, but also Oleoresin that is used in numerous food, pharmaceutical and other industries [1].

Katokkon pepper (*Capsicum chinense* Jacq.) is a pepper species that commonly cultivated in Tana Toraja. This species has Paprika-like shape with smaller size and more spicy flavor than Paprika (Figure 1). It thrives in highland areas above 1.000 meters above sea level. [2] reported Katokkon cultivated in 1.000 meters above sea level had higher productivity than that of in lower altitude area (600 meters above sea level).

Katokkon in Tana Toraja has only been grown on small-scale cultivations. The communities plant it on the yard or vacant lands in their neighborhood. The local department of Agriculture has crossed it with other pepper species, yet the hybridizations data are not appropriately recorded. Hybridization is employed to improve the genetics of the plants in order to develop plants with biotic and abiotic stress resistance as well as increase product quality and quantity. Therefore, the purity of parent's genetic and the genetic stabilization of the hybrids are crucial in hybrid seeds production.



Molecular techniques have been widely utilized on genetic diversity of peppers. One of the molecular markers used in those analyses is RAPD marker. It does not require any prior sequences of information of the evaluated target. Moreover, it is easy and relatively inexpensive to apply. [3] applied RAPD marker for identifying interspecific hybrids of Genus *Capsicum*. It was also occupied on germplasm evaluation for Paprika [4]. Shidfar M et al. [5] used RAPD marker for evaluating the genetic diversity of medicinal plant, *Taraxacum* sp and *Cichorium spinosum* L.

Some requisites are necessary to obtain suitable RAPD marker for a species. Not only can the primer amplify DNA samples but also the produced bands must be polymorphic and clear. For that matter, RAPD primer screening is needed to obtain suitable markers.

The objective of this study was to determine the RAPD primers and their annealing temperatures that are suitable for amplifying Katokkon peppers' DNAs. The primers producing polymorphic and clear bands will be used later in genetic diversity analysis of Katokkon.

2. Materials and Methods

2.1. Study time and site

The study was conducted in July up to September 2018. The research field was performed in Buntao and Tikala, North Toraja district, and Rembon To'pao, Tana Toraja district, South Sulawesi, Indonesia. Molecular analysis activities were done in Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Hasanuddin University, Makassar, Indonesia.

2.2. Sampling

Sixty-three individuals of Katokkon pepper were collected from two districts in South Sulawesi, i.e. North Toraja and Tana Toraja. The individuals consisted of nine Katokkon individuals and 10 Bara individuals from Buntao, 20 individuals from Tikala, and 14 Katokkon (4 of 14 individuals are assumed as local Katokkon (K1-K2)) individuals and 10 Bara individuals from Rembon To'pao, respectively. Leaves from each individual were then used as DNA sources. In particular, for this study, only 12 samples that were randomly selected from 63 samples would be analyzed in the RAPD primer screening process because this research is the preliminary study for genetic diversity analysis on Katokkon pepper. The 12 selected samples are presented in Table 1.

Table 1. Samples used in the RAPD primer screening

No	Number of samples	Sample	Origin	Sample code
1	2	Katokkon	Buntao	KT 1.2
2	9	Katokkon	Buntao	KT 1.9
3	4	Bara	Buntao	BR 1. 4
4	2	Katokkon	Tikala	KT 2.2
5	20	Katokkon	Tikala	KT 2.20
6	5	Katokkon	Rembon To'pa	KT 3.5
7	2	Bara	Rembon To'pa	BR 3.2
8	10	Bara	Rembon To'pa	BR 3.10
9	1	Katokkon	Rembon To'pa	K 1
10	2	Katokkon	Rembon To'pa	K 2
11	3	Katokkon	Rembon To'pa	K 3
12	4	Katokkon	Rembon To'pa	K 4

2.3. RAPD Primer screening

DNA isolation was performed using the CTAB method [6]. Thirty RAPD were used to generate Katokkon pepper DNA for observing their polymorphism (Table 2). Each PCR reaction consisted of 3 µL of DNA, 1.25 µL of primer, 6.25 µL PCR mix (KAPA 2G Fast), and 3 µL ddH₂O. PCR amplification was conducted by following steps: one cycle of pre-amplification at 95 °C for 3 mins, 35 cycles of amplification that consisted of template denaturation at 95 °C for 60 s, primer annealing for 60 s (at specific temperature for each primer), and primer extension at 72 °C for 60 s, and one cycle of final extension at 72 °C for 5 mins.

PCR products were then separated on Agarose 2% using TAE 1x buffer and stained using Gel Red. The electropherograms were visualized using Geldoc (Biostep) and camera digital.

Table 2. RAPD primer name and sequence

No	Locus name	Primer Sequence (5'-3')	Tm (°C)
1	OPO-14	AGC ATG GCT C	35,1
2	OPK-20	GTG TCG CGA G	38,5
3	OPQ-07	CCC CGA TGG T	38,5
4	OPAC-12	GGC GAGTGT G	38,1
5	OPAE-11	AAG ACC GGG A	35,5
6	OPAA-20	TTG CCT TCG G	35,6
7	OPA-15	TTC CGA ACC C	34,2
8	OPZ-05	TCC CAT GCT G	34,3
9	OPG-19	GTC AGG GCA A	34,7
10	OPA-18	AGG TGA CCG T	36,2
11	OPC-11	AAA GCT GCG G	36,9
12	OPA-09	GGG TAA CGC C	37,4
13	OPP-08	ACA TCG CCC A	37,6
14	OPQ-07	CCC CGA TGG T	38,5
15	OPD-20	ACC CGG TCA C	39,1
16	OPA-02	TGC CGA GTC G	40,7
17	OPD-03	GTC GCC GTC A	40,8
18	OPY-9	AGC AGC GCA C	42,5
19	OPG-06	GTG CCT AAC C	31,8
20	OPAD-11	CAA TCG GGT C	32,1
21	PLC-14	TGC GTG CTT G	32
22	OPG-09	CTG ACG TCA C	32
23	PLR-13	GGA CGA CAA G	32
24	PLW-04	CAG AAG CGG A	32
25	OPA-11	CAA TCG CCG T	32
26	PLB-10	CTG CTG GGA C	34
27	M-33	CCG GCT GGA A	34
28	M-29	CCG GCC TTA C	34
29	PLD-08	GTG TGC CCC A	34
30	M-147	GTG CGT CCT C	34

3. Results and Discussion

Eight of 30 RAPD screened primers were able to amplify Katokkon DNA and produced polymorphic and clear bands. As many as three primer did not generate an amplified band. Smear bands were produced from 18 screened primers. However, those 30 primers have not been able to generate a specific band for distinguishing between Katokkon and Bara Variety individuals. The thirty evaluated primers is presented in Table 3.

Table 3. RAPD primer and DNA amplification product of Katokkon pepper

No	Primer Name	Annealing Temperature (°C)	Polymorphic Band	Monomorphic Band	Quality of produced band
1	OPA-02	39.1	3	1	Polymorphic and clear band
2	OPAE-11	39.3	4		Polymorphic and clear band
3	OPG-09	33.7	5		Polymorphic and clear band
4	M-29	38.5	4		Polymorphic and clear band
5	OPG-19	36.4	6		Polymorphic and clear band
6	OPD- 20	40.8	7		Polymorphic and clear band
7	OPP-08	39.3	3		Polymorphic and clear band
8	OPA-18	40.7	5		Polymorphic and clear band
9	OPD-03	-			No band
10	OPO-14	-			No band
11	OPAD-11	-			No band
12	OPA-15	-			Smear band
13	OPZ-05	-			Smear band
14	OPA-11	-			Smear band
15	PLC-14	-			Smear band
16	PLW-4	-			Smear band
17	M-147	-			Smear band
18	M-33	-			Smear band
19	PLD-08	-			Smear band
20	OPAA-20	-			Smear band
21	OPK-20	-			Smear band
22	OPG-06	-			Smear band
23	PLB-10	-			Smear band
24	OPY-09	-			Smear band
25	OPAC-12	-			Smear band
26	OPA-05	-			Smear band
27	OPC-11	-			Smear band
28	PLR-13	-			Smear band
29	OPQ-07	-			Smear band
30	OPA-09	-			Smear band

Primers producing bands on Katokkon DNA generated three up to seven alleles. A number of total alleles were 31 alleles, consisted of 30 polymorphic alleles and one monomorphic allele. Monomorphic alleles were observed on primer OPA-02. The highest number of polymorphic bands from primer OPD-20 was seven alleles ranged between 350bp to 1500bp. Primer OPA-02 and Primer OPP-08 were primers generating the lowest alleles (three alleles) (Figure 1). Utilization of RAPD markers on *Swietenia mahagoni* [7] showed markers also used in this study, (OPA-18 and OPAE-11) could amplify and analyze genetic diversity in Mahogany.

Polymorphic alleles observed by each primer on each sample are different on their sizes and numbers. A polymorphic allele is the allele that can distinguish between individuals. Polymorphism on RAPD

marker can be caused by insertion and deletion (InDel) in the genome sequence of the sample [8]. Figure 1A shows primer OPA-02 on K4 amplified three bands, two polymorphic bands, and one monomorphic band, whilst 11 samples only had one band. The same result is presented in Figure 1D where primer OPG-09 produced one band, yet allele size was different from other samples. K4 on the same primer had different band number and size from other samples.

A number of the band can be different between primers on each sample. It is due to different primer sequences and DNA sample. Each primer sequence has specific annealing site in the genome. More homolog annealing sites of the primer in the sample genome, more bands will be produced. Study by [9] reported primer having more annealing sites produced more amplified bands.

In addition polymorphism, band quality of the product is also a crucial factor in primer selection. Primers producing unclear bands were not used in genetic diversity analysis. It is because unclear bands can misinterpret the data.

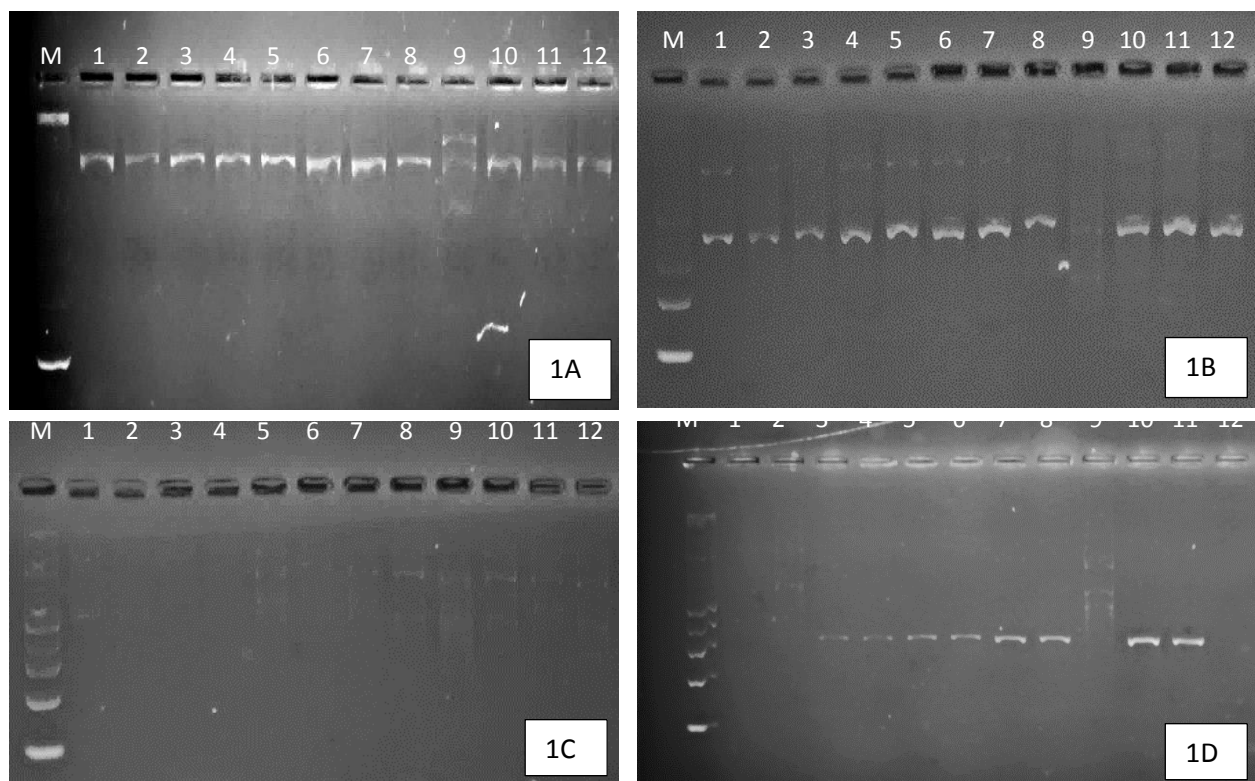


Figure 1. 1A) The amplified DNA of Katokkon using primer OPA-02, 1B) The amplified DNA of Katokkon using primer OPP-08, 1C) The amplified DNA of Katokkon using primer OPG-19 and 1D) The amplified DNA of Katokkon using primer OPG-09. Notes: 1) Katokkon from Buntao (KT 1.2), 2) Kattokkon from Buntao (KT 1.9), 3) Bara variety from Buntao (BR 1.4), 4) Kattokkon from Tikala (KT 2.2), 5) Kattokkon from Tikala (KT 2.20), 6) Kattokkon from Rembon To'pa (KT 3.5), 7) Bara variety from Rembon To'pa (BR 3.2), 8) Bara variety from daeral Rembon To'pa (BR 3.10), 9) Kattokkon from Rembon To'pa (K1), 10) Kattokkon from Rembon To'pa (K2), 11) Kattokkon from Rembon To'pa (K3), 12) Kattokkon from Rembon To'pa (K4)

Band quality is determined by proper annealing temperature. Lower annealing temperature results the primer anneals on incorrect site whereas the higher annealing temperature can cause failure in primer annealing. [4] presented lower annealing temperature produced a number of amplified bands on paprika.

Improvement of plant traits can be done through plant breeding programs. Those activities have resulted in more varied plant types and variety with better adaptability and product (both quality and quantity), inconsequently more alternatives for breeders to manipulate either plant genetic or environmental conditions. Katokkon pepper, locally known as Lada Katokkon, is originated from Tana Toraja and North Toraja. It is known as a flavouring ingredient in numerous traditional Torajan dishes [10]. Genetic conservation using molecular marker approaches are able to amplify local genotypes for analysing genetic diversity. Such studies were also conducted in *Diospyros celebica* [11] *Pericopsis mooniana* THW [12] and *Anthocephalus macrophyllus* [13]. Moreover, the molecular marker could assist in predicting mating system in Ebony from some provenances [14] [15], Kopyor coconuts [16] and study of the maternal effect on generative propagation (Xenia and metaxenia) [17] [18].

4. Conclusion

As many as eight of 30 screened RAPD primers produced polymorphic and clear bands. Those primers were OPA-02, OPAE-11, OPG-09, M-29, OPG-19, OPD- 20, OPP-08 and OPA-18. The annealing temperature of each primer ranged between 39.1 up to 40.3. Those selected primers will be utilized in genetic diversity on Katokkon paper.

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