RAPD Markers on Genetic Diversity in Three Populations of *Pisifera* Type of Oil Palm (*Elaeis guineensis*)

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Abstract. Palm oil (*E. guineensis*) is one of the major commodity and contributing largely to non-petroleum oil of Indonesian foreign exchange. *E. guineensis* has three fruit types, dura (female), pisifera (male), and tenera —a hybrid between dura and pisifera. *Pisifera* plays an important function in the production of seed oil palm. The purpose of this research is to analyze genetic diversity of pisifera type of *E. guineensis* from three populations, Yangambi, Lame and Lame further cross in Bangun Bandar, North Sumatra, Indonesia. Eighteen samples for each population were analyzed using six RAPD markers. Results showed that RAPD markers were low polymorphic with 1.49, 1.39, and 1.00 average number alleles detected for Yagambi, Lame, and Lame further cross, respectively. The level of genetic diversity detected for each population was 0.28, 0.22, and 0.21 for Yagambi, Lame, and Lame further cross, respectively, indicating that the populations had little genetic variation. The highest of polymorphic information content (PIC) was found on the P11 primer of Yangambi (0.49) and P10 primer for lame further cross (0.49). By contrast, the lowest PIC belongs to P21 for Lame population (0.01). This data is likely to contributing oil palm breeding.

Keywords: Breeding, conservation, female sterile, genetic variation, North Sumatra

1. Introduction

Palm oil is one of the major commodities of Indonesia, and current world's largest producer and exporter of palm oil. The oil palm (*Elaeis guineensis* Jacq, Arecaceae), a tropical perennial plant originated from West Africa. *E. guineensis* categorized based on the presence or absence of the shell in their fruits as *dura* (thick-shelled/sh^+sh^+), *pisifera* (shell-less/sh^-sh^-), and *tener*a (thin-shelled/ (sh^+sh^-)—a cross between the *dura* and *pisifera* [1]. *Pisifera* (male parent) has an important function and high economic value in the production of oil palm seeds. In the oil palm seed industry, conservation of the *pisifera* pollen will be able to support a sustainable artificial pollination program in seed production and breeding. Therefore the population of *pisifera* is one important factor to be managed and developed [2].

Given the importance of *pisifera* as pollen sources are selected for commercial palm oil production of D×P planting materials. This economic important character of *pisifera* palms, which is female sterile due to premature rotting fruits thus unable to be fruits [3]. Information on genetic diversity of *pisifera,* therefore, is very important for survival and adaptation to environmental changes. However, a few studies on genetic diversity on the *pisifera* population in *E. guineensis* from North Sumatra. The aim of this research is to analyze genetic diversity of *pisifera* type of *E. guineensis* from three populations, Yangambi, Lame and Lame further cross in Bangun Bandar, North Sumatra, Indonesia.

2. Materials and Methods

2.1. Plant sample and DNA extraction

A total of 54 young leaves of individual *E. guineensis* were collected at Bangun Bandar Seed Production, Serdang Badagai, North Sumatra from three origins of *pisifera* as three populations: Lame (18 samples), Yangambi (18 samples), and Lame further cross (18 samples) and used for DNA extraction. Total genomic DNA was extracted from *E. guineensis* leaves using modified cetyl trimethyl ammonium bromide (CTAB) procedure [4]. The quality of the DNA was evaluated using 1% agarose gels and quantified using UV-Spectrophotometer. The DNA extraction was stored at -20 ºC.

Six RAPD primers, namely P6 (5'-TACCACCCCG-3'), P10 (5'- GGACCCAACC -3'), P11 (5'-GTCGCCGTCA-3'), P15 (5'-TTGGCACGGG-3'), P19 (5'-AGCGCCATTG-3'), and P21 (5'-GGGGTGACGA-3') was used in this study as previously reported [5]. PCR amplification was performed using GoTaq green master mix 2X (Promega), primer 10 μM, DNA template, and nuclease-free water. PCR reaction was carried out in PCR PC-806 (Astec) for 1 cycle at 95 ºC for 4 min; 40 cycles at 95 ºC for 45 s, 35 ºC for 45 s, 72 ºC for 1 min and a final extension 72 ºC for 7 min.

2.2. RAPD analysis

The amplicon was separated on 2% agarose gels using electrophoresis in 0.5X TBE buffer at 100 volts for 4-5 h. Gels were stained with GelRed (Biotium) and visualized by UV Gel Documentation system. The band pattern was analyzed by cluster analysis as previously described [5].

2.3. Data analysis

Polymorphic DNA banding patterns were generated by all the different primers were scored according to the presence (1) and absence (0) of the band of a particular molecular size to accumulate a binary matrix which was subjected to cluster analysis. Both faint and intense bands were scored if shown consistency in triplicate experiments.

Genetic variation overall populations were calculated using GenAlEX 6.5 [6] as a number of alleles detected (Na), effective numbers of alleles (Ne), Shannon index (I), unbiased genetic diversity (Uh), the percentage of polymorphism loci (PoP). Polymorphic information content (PIC) and H: heterozygosity was determined by [7].

3. Results and Discussion

Figures 1-2 show RAPD banding pattern using P10 and P11 primers (as representative) for population Yangambi and Lame further cross. The bands showing the different size of DNA from 150 to 4000 bp. The similar banding pattern each sample with different RAPD markers indicating no specific band present in this study.

RAPD markers were low polymorphic with 1.49, 1.39, and 1.00 average number alleles detected for Yagambi, Lame, and Lame further cross, respectively (Table 1). The level of genetic diversity detected for each population was 0.28, 0.22, and 0.21 for Yagambi, Lame, and Lame further cross, respectively, indicating that the populations had little genetic variation. The maximum heterozygosity for RAPD is 0.5 [7]. The Shannon index for each population was 0.42, 0.33, 0.30 for Yagambi, Lame, and Lame further cross, respectively.

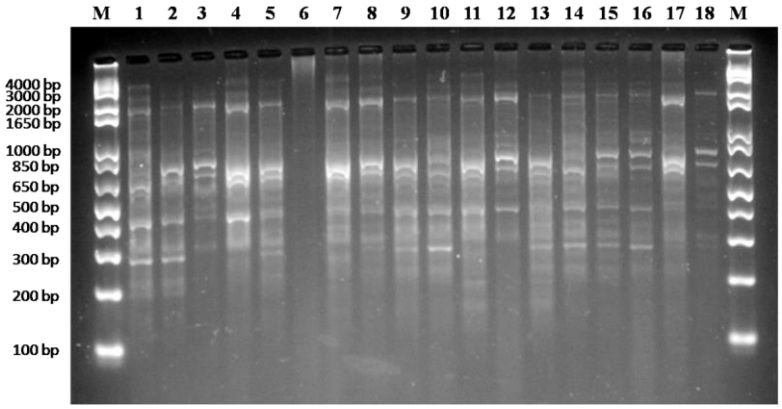

Figure 1. RAPD banding pattern using primer P10. M: marker, lane 1-18: samples from for Yangambi origin

Table 1. Genetic variation on oil palm of *pisifera*-type

Locus	N	Na	Ne	I	H	Uh	PoP (%)
Yangambi							
P6	18	1.85	1.60	0.52	0.35	0.37	92.31
P10	18	0.89	1.24	0.21	0.14	0.15	44.44
P11	18	1.50	1.42	0.38	0.25	0.26	75.00
P15	18	1.33	1.47	0.39	0.26	0.28	66.67
P19	18	1.47	1.52	0.43	0.29	0.31	73.33
P21	18	1.88	1.75	0.57	0.40	0.42	93.75
Mean	18	1.49	1.50	0.42	0.28	0.30	74.25
Lame							
P6	18	0.92	1.13	0.15	0.09	0.10	46.15
P10	18	1.56	1.52	0.45	0.31	0.32	77.78
P11	18	1.62	1.60	0.48	0.33	0.35	81.25
P15	18	2.00	1.54	0.52	0.34	0.36	100.00
P19	18	2.00	1.30	0.37	0.22	0.23	100.00
P21	18	0.25	1.01	0.03	0.01	0.01	12.50
Mean	18	1.39	1.35	0.33	0.22	0.23	69.61
Lame further cross							
P6	18	0.15	1.07	0.05	0.04	0.04	7.69
P10	18	1.44	1.55	0.45	0.31	0.33	72.22
P11	18	1.50	1.54	0.45	0.31	0.32	75.00
P15	18	1.00	1.38	0.29	0.20	0.22	50.00
P19	18	0.67	1.30	0.22	0.16	0.17	33.33
P21	18	1.25	1.42	0.36	0.25	0.26	62.50
Mean	18	1.00	1.38	0.30	0.21	0.22	50.12

(N: number of individuals, Na: number of alleles detected, Ne: effective number of alleles, I: Shannon index, H: heterozygosity, Uh: unbiased genetic diversity, PoP: percentage of polymorphism loci)

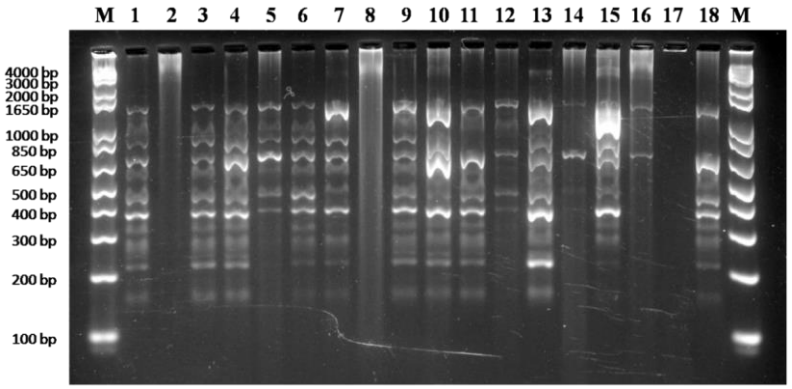

Figure 2. RAPD banding pattern using primer P11. M: marker, lanes 1-18: samples from Lame further cross origin

Table 2. Locus, size band, frequency of band pattern, and polymorphic information content (PIC) on *pisifera* type of *E. guineensis*

Locus	Size band (bp)	Frequency of band	Polymorphic information content (PIC)
Yangambi origin			
P6	300-3000	0.30	0.41
P10	220-4000	0.26	0.38
P11	170-3500	0.53	0.49
P15	200-3000	0.33	0.44
P19	150-4000	0.25	0.37
P21	200-4000	0.39	0.47
Lame origin			
P6	300-3000	0.05	0.10
P10	220-4000	0.22	0.34
P11	170-3500	0.27	0.39
P15	200-3000	0.24	0.36
P19	150-4000	0.13	0.23
P21	200-4000	0.01	0.01
Lame further cross			
P6	300-3000	0.05	0.08
P10	220-4000	0.44	0.49
P11	170-3500	0.41	0.48
P15	200-3000	0.22	0.34
P19	150-4000	0.14	0.24
P21	200-4000	0.27	0.39
Total		4.53	6.01
Mean		0.25	0.33

The population of Yangambi had a high percentage of polymorphism loci (74.25%) compared to other populations, where, Lame further cross contained the lowest one (50.12%). This result parallels with a number of alleles found. The highest of polymorphic information content (PIC) was found on

the P11 primer of Yangambi (0.49) and P10 primer for lame further cross (0.49). By contrast, the lowest PIC belongs to P21 for Lame population (0.01). PIC is important for genetic studies in relation to molecular markers. In this context, the P11 primer is suitable for all populations with PIC value 0.39-0.49. RAPD is a dominant marker, maximum PIC value is 0.5 due to two alleles per locus and affected by a number of alleles [7].

This study demonstrates one way in which DNA markers (RAPD) might be used to assess genetic variation and assist in the development of oil palm breeding [5]. RAPD markers may provide fast and cheap methods for identifying oil palm type, in addition to two-dimensional thin layer chromatography, an alternative approach to identify fruit type of oil palm [8].

4. Conclusions

The level of genetic diversity detected for three populations of *pisifera*: Yagambi, Lame, and Lame further cross was little genetic variation. The conservation of *pisifera* is needed for securing genetic materials. This finding is likely to contributing oil palm breeding.

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