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 commodities 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 Yangambi, Lame, and Lame further cross, respectively. The level of genetic diversity detected for each population was 0.28, 0.22, and 0.21 for Yangambi, 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 *tenera* (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].

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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'-TACCACCCCCG-3'), P10 (5'- GGACCCAACC-3'), P11 (5'-GTGCGCGTCA-3'), P15 (5'-TTGGCACCGG-3'), P19 (5'-AGCGCCATTTG-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 Yangambi, 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 Yangambi, 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 Yangambi, 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: Yangambi, 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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References
[1] Basyuni M, Amri N, Putri LAP, Syahputra I, Arifiyanto D (2017) Characterization of fresh bunch yield and the physicochemical qualities of palm oil during storage in North Sumatra, Indonesia Indon. J. Chem. 18 182–190.
[2] Barcelos E, Rios SDA, Cunha RNV, Lopes R, Motoike SY, Babiychuk E, Skirycz A, Kushner S (2015) Oil palm natural diversity and the potential for yield improvement Front Plant Sci. 6 190.
[3] Arolu IW, Rafii M Y, Marjuni M, Hanafi MM, Sulaiman Z, Rahim HA, Kolapo OK, Abidin MIZ, Amiruddin MD, Din AK, Nookiah R (2016) Genetic variability analysis and selection of pisifera palms for commercial production of high yielding and dwarf oil palm planting materials Ind. Crops Prod. 90 135–141.
[4] Basyuni M, Baba S, Oku H (2017) Microsatellite analysis on genetic variation in two populations of red mangrove Rhizophora mangle L. (Rhizophoraceae) and its implication to conservation IOP Conf. Ser.: Mater. Sci. Eng. 180 012243.
[5] Suthish DK, Mohankumar C (2007) RAPD markers for identifying oil palm (Elaeis guineensis Jacq.) parental varieties (dura & pisifera) and the hybrid tenera Indian J. Biotechnol. 6 354–358.
[6] Peakell R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update Bioinformatics 28 2537–2539.
[7] Nagy S, Poczai P, Cernák I, Gorji AM, Hegedûs G, Taller J (2012) PICcalc: an online program to calculate polymorphic information content for molecular genetic studies Biochem. Genet. 50 670–672.
[8] Arifiyanto D, Basyuni M, Sumardi, Putri LAP, Siregar ES, Risnasari I, Syahputra I (2017) Short Communication: Occurrence and cluster analysis of palm oil (Elaeis guineensis) fruit type using two-dimensional thin layer chromatography Biodiversitas 18 1587–1492.