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RAPD markers for identifying oil palm (*Elaeis guineensis* Jacq.) parental varieties (*dura* & *pisifera*) and the hybrid *tenera*

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Random amplification of polymorphic DNA (RAPD) analysis was done by arbitrary primers for determining the DNA polymorphism among the oil palm (*Elaeis guineensis*) varieties *dura*, *pisifera* and *tenera*, and monitoring the specificity of the primers for identifying each genotype. The three varieties were evaluated using thirty, 10-mer primers. Of the 30 primers, 26 yielded significant polymorphic DNA bands. A total of 185 bands were determined (300-1500 bp), of which 59 were representing *dura*, 65 for *pisifera* and 61 bands for *tenera.* The dendrogram obtained, using UPGMA, grouped all the three varieties into two distinct clusters. In one cluster, the shelled ones (*dura* and *tenera*) were grouped, while the other comprised the shell-less one (*pisifera*). The data also provide sufficient evidence for identifying each variety, *dura*, *pisifera* and *tenera* separately as well as the parentals *dura* and *pisifera* together. This is the first report of DNA based polymorphism assay to assess the level of variability in oil palm varieties.

Keywords: *Elaeis guineensis*, hybrid, parental varieties, random primers, RAPD
IPC Code: Int. Cl.[8] C12N 15/10

Introduction

Oil palm (*Elaeis guineensis* Jacq.) has got greater acceptance than the other oleaginous crops of the tropical belt due to its high yielding potential[1]. Unlike traditional oilseeds, the fruit mesocarp of oil palm has a good reserve of storage lipids[2]. Lipid composition, fatty acid profile and vitamin content (A & E) of palm oil have given the crop a distinct nutritional status commercially[3]. Within the species *E. guineensis*, three varieties, viz. *dura, pisifera* and *tenera* are recognized. However, intra-specific identification of these varieties remains cumbersome, since they have very similar morphological features at vegetative phase. The only possible way to identify them is the presence or absence of endocarp or shell of the fruits. The shelled ones are *dura* and *tenera* varieties, while the *pisifera* is the shell-less variety. The thin-shelled nature and the fiber ring distinguish *tenera* from the thick-shelled *dura.* Hence, the varieties are otherwise called as 'fruit forms'[4]. Detection of *tenera*, an accepted commercial cultivar, at the vegetative phase from the t x t population has been puzzling to oil palm breeders and growers. Previous reports on identification of oil palm varieties at vegetative phase by comparing the activity of marker enzymes of lignin synthesis reveal the possibility of distinguishing the varieties biochemically under ideal conditions[5, 6]. Advent of molecular marker techniques has provided a different and sensitive approach in the study of genetic diversity of plants that cannot be identified by morphological characters.[7-12]. The PCR amplification of genomic DNA using random primers detected DNA polymorphism and genetic relatedness between cultivars and varieties.

Moreover, the data can be further used for identifying the better cultivars and to solve the ambiguity of their phylogenetic status. So, the present study has been undertaken to assess the DNA polymorphism and genetic relationship among oil palm varieties *dura, pisifera* and *tenera* using RAPD markers for distinguishing them at the vegetative phase.

Materials and Methods

Plant Material

The healthy palms were identified from the experimental garden of National Research Center for Oil Palm, Palode, Thiruvananthapuram. Palms of the

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three varieties *dura, pisifera* and *tenera* were identified randomly. Uniformity was maintained in age, size and position of the leaf and leaflets during collection. The middle leaflets from the tender leaves of *dura, pisifera* and *tenera* were collected for the molecular experiments. The leaf samples were surface sterilized and stored at –20°C.

Extraction of DNA

Genomic DNA was extracted and purified from the frozen leaf samples of the three varieties by CTAB method with some modifications[13]. An aliquot of the purified DNA was dissolved in 3 mL of TE buffer. DNA was quantitated spectrophotometrically at 260 nm.

Primers

Thirty decamer primers were obtained from the Operon Technologies, USA (Table 1) and were used for the amplification reactions.

PCR Amplification

The PCR amplification was carried out at varying concentrations of $MgCl_2$ and DNA template for optimizing the PCR conditions[14]. The PCR mixture (25µL) contained 1× PCR buffer, 100 µ*M* dNTP, 2µ*M* $MgCl_2$, 3 units of *Taq* DNA polymerase (Bangalore Genie), 75 ng DNA and 25 pico moles of primers. The amplification reaction was performed in a Minicycler™ (MJ Research, USA), programmed for 40 cycles of 30 sec at 94°C, 30 sec at 36°C, 2 min at 72°C and a final extension of 5 min at 72°C.

Table1—List of primers and their sequences used for RAPD analysis of oil palm varieties

Primer	Sequence
P1	AACCGACGGG
P2	GGGGGTCGTT
P3	TGCCCTGCCT
P4	CCAGACCCTG
P5	AAGCTCCCCG
P6	TACCACCCCG
P7	GGCGGACTGT
P8	GTCACTCCCC
P9	ACCGCGAAGG
P10	GGACCCAACC
P11	GTCGCCGTCA
P12	TCTGGTGAGG
P13	TGAGCGGACA
P14	ACCTGAACGG
P15	TTGGCACGGG
P16	GTGTGCCCCA
P17	CTCTGGAGAC
P18	GGTCTACACC
P19	AGCGCCATTG
P20	CACCGTATCC
P21	GGGGTGACGA
P22	CTTCCCCAAG
P23	CATCCGTGCT
P24	AGGGCGTAAG
P25	TTTCCCACGG
P26	GAGAGCCAAC
P27	CTGGGGACTT
P28	ACCCGGTCAC
P29	CTTGCCTCCC
P30	CGCGGACGAT

Agarose Gel Electrophoresis

The amplification products were separated on 1.5% Agarose gel by electrophoresis in 1X TAE buffer at 80 to 90 volts for 2 h. Gels were stained with 0.5 µg/L ethidium bromide and photographed by UV Gel Documentation system. The band pattern was analyzed by cluster analysis.

Data Analysis

Polymorphic DNA bands that showed consistency in repeated experiments were screened according to their presence (1) or absence (0) in each of the sample. Percentage genetic similarity between the three varieties was estimated according to Sneath and Sokal, Nei and Li, Jaccard, and Rogers[7] and the similarity coefficient was determined. A similarity matrix was computed using the similarity coefficient. A dendrogram was constructed by UPGMA cluster analysis.

Results and Discussion

RAPD Data and Dendrogram

The DNA polymorphism at the level of genetic variability in *E. guineensis* varieties or fruit forms was analyzed by RAPD method. Fig. 1a and b represent the RAPD profile for the three varieties generated with 28 primers out of the total 30. The presence or absence of bands and the total number of bands amplified were the possible means used for selecting better and specific primers. A total of 185 bands were generated using the chosen 28 primers and the sizes of the amplified products varied between 300 and 1500 bp. Table 2 demonstrates the comparison of amplified fragments in the 3 varieties *dura* (D), *pisifera* (P) and *tenera* (T). The RAPD profile of the varieties showed more matches between *dura* and *tenera* than *pisifera*, suggesting the genetic relatedness of *dura* parent with the hybrid *tenera.* The genetic distance between the varieties was calculated from the pair wise comparison of shared and unique bands (Table 3). The pair wise genetic distance among the varieties was detected by the methods of Sneath and Sokal, Nei and Li, Jaccard, and Rogers, which are

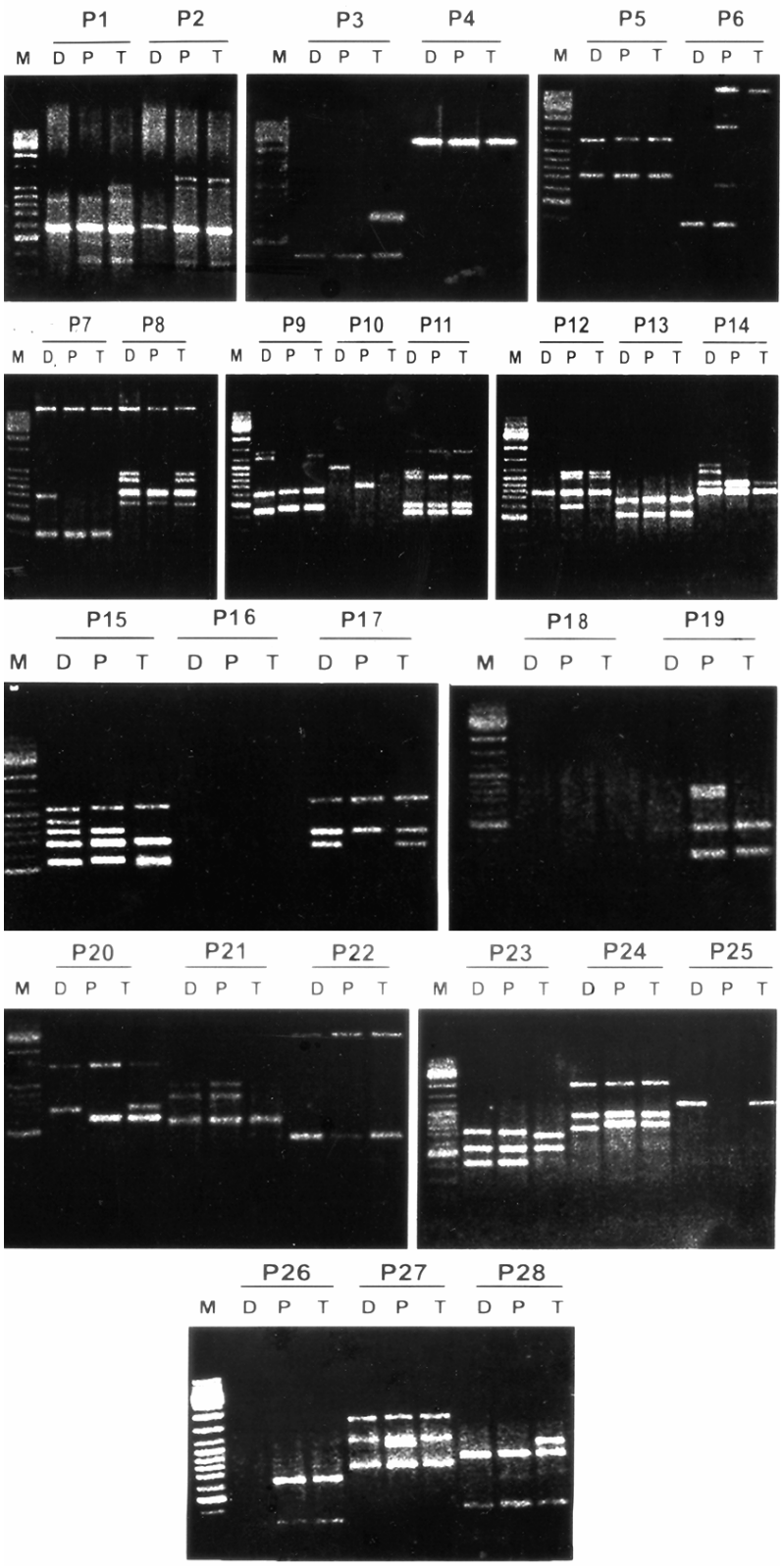

Fig. 1—RAPD profile generated with the primers P1 to P28: Lane 1, marker (1 kb DNA ladder, M); lane 2, *dura* (D); lane 3, *pisifera* (P); & lane 4, *tenera* (T).

Table 2—Comparison of amplified fragments generated by specific primers for identifying the three varieties of oil palm

Sl. No.	RAPD primers	Size of amplified fragments (bp)	Amplification pattern		
			D	P	T
Identification of varieties together					
1.	P12	750	+	+	+
		600	-	+	-
		1100	-	+	+
2.	P15	1000	+	-	-
		800	+	+	-
		700	+	+	+
Identification of *dura*					
3.	P7	700	+	-	-
4.	P10	1000	+	-	-
Identification of *pisifera*					
5.	P10	700	-	+	-
6.	P6	650	-	+	-
7.	P19	800	-	+	-
Identification of *tenera*					
8	P28	1100	-	-	+

D-*dura,* P-*pisifera*, T-*tenera*

Table 3—Pair wise comparison of shared and unique bands among the three varieties of oil palm.

Varieties	a (1,1)	b (1,0)	c (0,1)	d (0,0)
DP	39	19	24	9
DT	37	21	23	9
PT	47	16	14	14

D-*dura,* P-*pisifera*, T-*tenera*

potential genitors for mapping the population. Table 4 demonstrates the pair wise genetic difference among the oil palm varieties. The correlation coefficient was also determined. A dendrogram was prepared by cluster analysis of the RAPD data generated by the 28 random primers. The dendrogram derived indicates that the *dura* and *tenera* were genetically closer than *pisifera* and it appeared as a separate cluster (Fig 2). Thus, the cluster tree reflects a similar grouping of shelled and shell-less nature of the fruit forms.

Identification of Oil Palm Varieties

Based on the specificity of amplification, the primers were categorized into three groups for identifying each variety separately. But, the two primers, viz. P12 and P15 showed an amplification pattern that can be used for identifying all the three varieties (Fig. 3). In the case of primer P12, *dura*, *pisifera* and *tenera* showed a common band of 750 bp, which acts as marker for *dura* variety because other bands shared by *pisifera* and *tenera* are absent

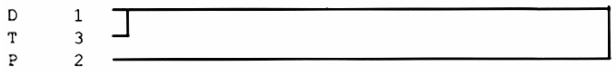

Fig. 2—Dendrogram constructed for the 3 varieties of oil palm using UPGMA cluster analysis of RAPD data generated by 28 primers: 1, *dura* (D); 2, *tenera* (T); & 3, *pisifera* (P).

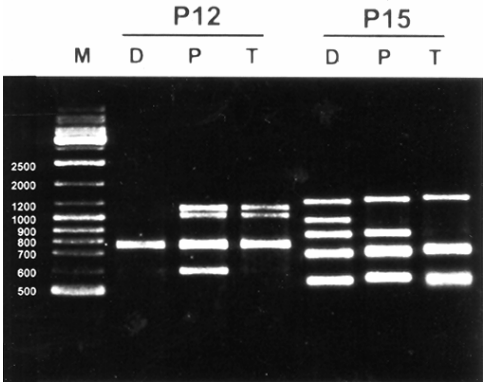

Fig. 3—RAPD products generated with the primers P12 and P15, showing the differentiating ability of the primers for identifying the oil palm varieties *dura* (D), *pisifera* (P) and *tenera* (T).

Table 4—Pair wise genetic distance among the three varieties of oil palm according to four different methods

	Sneath & Sokal	Nei & Li	Jaccard's	Rojers'
DP	0.472527	0.628901	0.277775	0.106119
DT	0.488889	0.598255	0.286785	0.104796
PT	0.329670	0.631631	0.494787	0.062196
Correlations				
Sneath & Sokal	--	-0.63722	-0.99153	0.992807
Nei & Li	--	--	0.531727	-0.99985
Jaccard	--	--	--	-0.54036
Rogers	--	--	--	--

D-*dura*, P-*pisifera*, T-*tenera*

in *dura*. The presence of 600 bp in *pisifera* identifies it from *dura* and *tenera*. Apart from the shared bands between *tenera* and *pisifera*, a specific band was present in *pisifera* that was absent in *tenera*, indicating the identity of *tenera.* Using primer P15, a gradual reduction in band pattern was observed in the order that *dura* has five bands with a unique band with a size of 1000 bp; *pisifera* with four bands, without the band of 1000 bp; and *tenera* with three bands, without the unique bands observed in parentals. Using the primers P7 and P10, it is possible to identify the *dura* variety specifically with a band size of 700 bp and 1000 bp, respectively (Fig. 4a). The primer P10 is also useful in identifying *pisifera* with a band size of 700 bp. The parental variety *pisifera* can be exclusively identified with a band size of 650 and 800 bp for the primers P6 and P19,

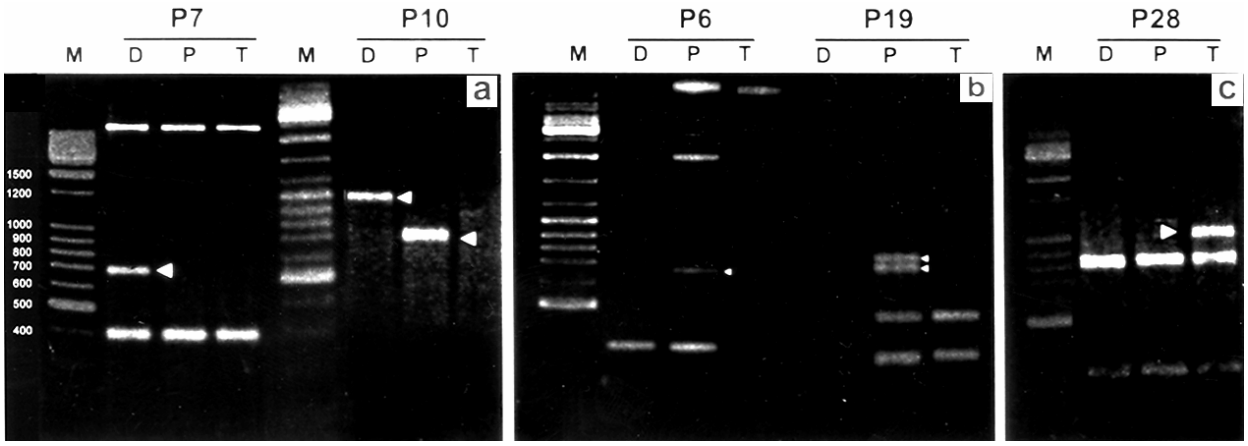

Fig. 4—RAPD profile of the oil palm varieties generated by the primers: a) P7 & P10, b) P6 & P19 and c) P28, revealing the identification of *dura, pisifera* and *tenera*, respectively. Arrows indicate the unique bands that are amplified to each variety (D-*dura*, P-*pisifera* & T-*tenera*).

respectively (Fig. 4b). The 10-mer primer P28 was found specific for identifying the *tenera* variety from the parentals with a 1100 bp band (Fig. 4c).

Thus, the results presented in this paper confirm that RAPDs have high discriminatory power and can be successfully applied to reveal genetic diversity among the three varieties of oil palm. The RAPD markers provide a reliable method for identifying the varieties by the analysis of DNA polymorphism. This is the first report on the use of a DNA based polymorphism assay to assess the level of Variability in oil palm varieties.

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