Determination of phylogenetic relationship among oil palm (*Elaeis guineensis*) varieties with random amplified polymorphic DNA

Odenore, V. D.[1], Eke, C. R.[1], Asemota, O.[1] and Shittu H. O.[2*]

[1]Physiology Division, Nigerian Institute for Oil palm Research, Benin City, Nigeria
[2]Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria

***Corresponding Author E-mail:** olalekan.shittu@uniben.edu

Abstract
Discrimination among Oil palm (Elaeis guineensis) fruit forms: Dura, Pisifera and Tenera (accepted commercial cultivar with higher palm oil content), at the vegetative phase have been puzzling to Oilpalm breeders and growers in Nigeria. The aim of the study was to develop a Random Amplified Polymorphic DNA (RAPD)-based assay, using six primers of arbitrary nucleotide sequence for identifying the various fruit forms at the seedling stage. Also to determine the genetic relatedness between them and another variety of Oil palm, the Virescens fruit type. The DNA bands generated by each primer were counted and the presence or absence of each band was treated as a binary character in a data matrix (coded 1 or 0, respectively). A dendrogram was constructed, grouping the four varieties into three clusters. The findings showed close similarities between Dura and Virescens, and provided evidence for identifying the four types of fruits separately as well as the parentals (Dura and Pisifera) linked by the hybrid Tenera.

Key words: *Elaeis guineensis,* Fruit form, Dura, Pisifera, Tenera, Virescens, RAPD

1.0 Introduction

Oil palm (*Elaeis guineensis*) is one of the most important plantation crops in the world (Corley and Tinker, 2003). It is of high economic value more than the other oleaginous crops of the tropical belt due to its high yielding potential (Paranjothy, *et al*., 1989). Three fruit forms are distinguished in oil palm: Dura, Pisifera and Tenera. These differ in thickness of their seed shells, mesocarp and lower oil contents. The shell thickness is controlled by a gene called the SHELL gene, a homologue of seedstick responsible for Oil palm fruit forms (Rajinder,*et al.,* 2014). The Pisifera fruits have no shell and naturally, most are sterile and have no economic importance (Hartley, 2000).There are variations in the internal morphology of the oil palm fruits which consist of the outer exocarp or skin; the mesocarp or the pulp, which is fleshy and oily; and the endocarp that has a thick shell. The kernel or seed is found within the thick shell (Bassey, 2006).The most

cultivated high yielding Oil palm variety, the thin shellTenera (oil bunch[O/B] >20%) is produced when the thick shellDura (O/B = 17%) is crossed with the shell-less Pisifera. The Pisifera which is female sterile is used as the pollen source.

Oil palm produces four fruit types: Nigrescens, Virescens, Albescens and Mantled which are distributed among the three fruit forms (Corley and Tinker, 2003). The Virescens is emerald green unripe, ripening to bright orange, reflecting degradation of chlorophyll and accumulation of carotenoid (Sambanthamurthi*et al.,* 2000). It has received little breeding attention since it does not appear to confer any advantage. However, the colour differences between ripe and unripe Virescens fruits can also be used to improve milling with camera based segregation of different ripeness fruit (Seng*et al*., 2007). Virescens fruit type is controlled by the VIR gene responsible for Oil palm fruit exocarp pigmentation (Rajinder,*et al.,* 2014). Shell thickness, fruit skin colour and the mantled fruit form are known to be monofactorially inherited and economically important in oil palm or potentially so (Hartley, 2000).The identification of Dura, Tenera, Pisifera and Virescens palms in the nursery stage without needing to wait 3-4 years to verify the kind of fruit, will save time, reduce research cost, meet preferred needs and increase palm oil production for farmers.

Marker Assisted Selection (MAS) is the selection of specific alleles for traits conditioned by a few loci (Ribaut*et al*., 2010; Shittu and Mbeze, 2013).Marker assisted selection is an effective technique for quality traits selection in breeding programs which are impossible by visual observation. These molecular techniques, in particular the use of molecular markers, have been used to monitor DNA sequence variation in and among different crop species. Improvement in marker detection systems and in the techniques used to identify markers linked to useful traits have enabled great advances to be made in recent years. Random Amplified Polymorphic DNA (RAPD) markers have recently caught the fancy of many individuals in the field of applied plant breeding. This molecular marker is based on the PCR amplification of random locations in the genome of the plant. With this technique, a single oligonucleotide is used to prime the amplification of genomic DNA. The goals of RAPD experiments are to compare populations. This requires well to well and run to run consistency, thus, ensuring that each well of the cycler will produce the same result given the same target DNA, primer and dNTP concentration is of upmost importance (Phillip, 1998).

The aim of this study were to develop an assay showing the genetic relatedness of the three Oil palm fruit forms in relation to the Virescens fruit type and also, to be able to identify them at the seedling stage.

2.0 Materials and methods

2.1 Plant material

The materials used for the study were fresh leaves from Dura, Tenera, Pisifera of the Oil palm and a palm expressing the Virescens trait. They were harvested from field sof the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria. Uniformity was maintained in age, size and position of the leaf and leaflet during collection. The leaf samples were surface sterilized and stored at -20^{o}C prior to use.

2.1 Random Amplified Polymorphic DNA Assay

DNA extraction from the Oil palm tissue was performed using DNeasy Mini Kit (QIAGEN).In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, $MgCl_2$ and Taq polymerase. A total of twenty-one random DNA oligonucleotide primers were independently used according to Williams *et al*., (1990) in the PCR reaction. Only six primers succeeded in generating reproducible polymorphic DNA products. Table 1 shows the base sequences of these DNA primers. The PCR amplification was performed in a 25 µl reaction

volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl_2 (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH_2O. Amplification was carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95 ºC for 5 minutes, followed by 35 cycles at 94 ºC for 30 seconds, 37 ºC for 30 seconds and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 12minutes. PCR products alongside with DNA size marker (100 to 1,500 bp) were run at 100 V for 30 min on 1.5 % agarose gels to detect polymorphism between plant species under study. After electrophoresis, the RAPD patterns were visualized with UVTech Documentation system. RAPD markers were scored from the gels as DNA fragments present or absent in all lanes.

Table 1: Nucleotide sequence of primersused for random amplified polymorphic DNA assay

S/N	Name	Nucleotide sequence
1	OP- A02	5´CAA TCG CCG T 3`
2	OP-B01	5`GTT TCG CTC C 3`
3	OP-B11	5`GTA GAC CCG T 3`
4	OP-C**03**	5`GGG GGT CTT T 3`
5	OP- C09	5`CTC ACC GTC C 3`
6	OP- D05	5`TGA GCG GC A 3`

2.3 Statistical analysis

The DNA bands generated by each primer were counted and their fragment sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct a dendrogram among the studied four Oil palm varieties. Calculation was achieved using Dice similarity coefficients (Dice, 1945) as implemented in the computer program SPSS-10.

3.0 Results and discussion

The DNA polymorphism at the level of genetic variability in *Elaeis guineensis* varieties was analyzed by RAPD method. Figure 1(a-f) represent the RAPD assay for the four varieties generated with 6 primers out of a total of 21. Figure 2 shows a dendrogram that was constructed from a data matrix for the presence or absence of band. 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 153 bands were generated using the chosen six primers and the sizes of the amplified products varied between 100-1000bp. Table 2 shows the similarity index between the four varieties of Oil palm.

Table 2: Similarity Index between four cultivars of Palm Oil

	P1	H1	H2	P2
1	1.00			
2	0.40	1.00		
3	0.00	0.26	1.00	
4	0.65	0.64	1.00	1.00

Legend: P1: Dura; H1: Tenera; H2: Virescens; P2: Pisifera

The dendrogram shows groups of three clusters. In one cluster, Dura and Virescens were grouped; in the second cluster, Tenera and Dura were grouped, while in the third cluster, Dura, Tenera and Pisifera were grouped. Among the six primers that showed informative band, none of them showed a band shared only between Pisifera and the Virescens variety. The bands that were common to them were also present in either Dura or Tenera. This indicated a close link between the Virescens and the shelled fruit forms (Dura and Tenera).

The result also showed close similarities between Dura and Virescens variety. The RAPD assay of the varieties showed more matches between Dura and Tenera than Pisifera. From the assay of primer OP-B01, OP-B11 and OP-C03in Figures 1b,c and d, the shelled fruit forms (Duraand Tenera) had similar band patterns. This result is similar to that of Sathish and Mohankumar (2007) in their study to identify Oil palm parental varieties and the hybrid Tenera. Primer OP-B01 showed a common band of 500bp in Dura and Tenera, confirming that they were grouped as shelled fruit forms and it distinguished them from Pisifera. There is also a band of 220bp in the Virescens variety that is present in Dura, but absent in Tenera and Pisifera, which implies that the Virescens was closer to Dura genetically than Tenera and Pisifera. In the case of primer OP-A02, Dura and Tenera showed a common band of 850bp which was absent in pisifera and the Virescens. Dura also showed a common band of 800bp with Virescens which was absent in Pisifera and Tenera. Two unique bands of 250bp and 900bp showed in the Virescens identifying it from Dura, Pisifera and Tenera. The shared bands of 850bp between Dura and Tenera and band of 800bp between Dura and Virescens indicated close relatedness between them. Infigure 1e, the primer OP-C09 showed that the hybrid Tenera(H1) had an intense band of about 600bp that was absent in the parental Dura (P1), Pisifera (P2) and the Virescens (H2), which can serve as an identity for Tenera. For primer OP-B11, A band of 580bp was observed in Dura, Tenera and Pisifera showing the relationship between the parents and the hybrid

The results presented in this paper showed very close similarity between the thick shelled fruit form Dura and the Virescens variety. It also showed that the Virescens fruit type of the Oil palm was closely related with the shelled fruit forms (Dura and Tenera) and not the shell-less one (Pisifera).This relationship may be as a result of the materials used for the early Oil palm breeding programme in Nigeria between 1912-1916. Dura, Tenera, Virescens and Mantled fruits were used to provide seeds (Corley and Tinker 2003), while Pisifera was not used.The study provides evidence that RAPDs have high discriminatory power and can be successfully applied to reveal genetic diversity and relatedness among the four Oil palm varieties. The RAPD markers provide a reliable method for identifying the four varieties separately. This will aid early detection of choice variety for distribution to Oil palm growers.

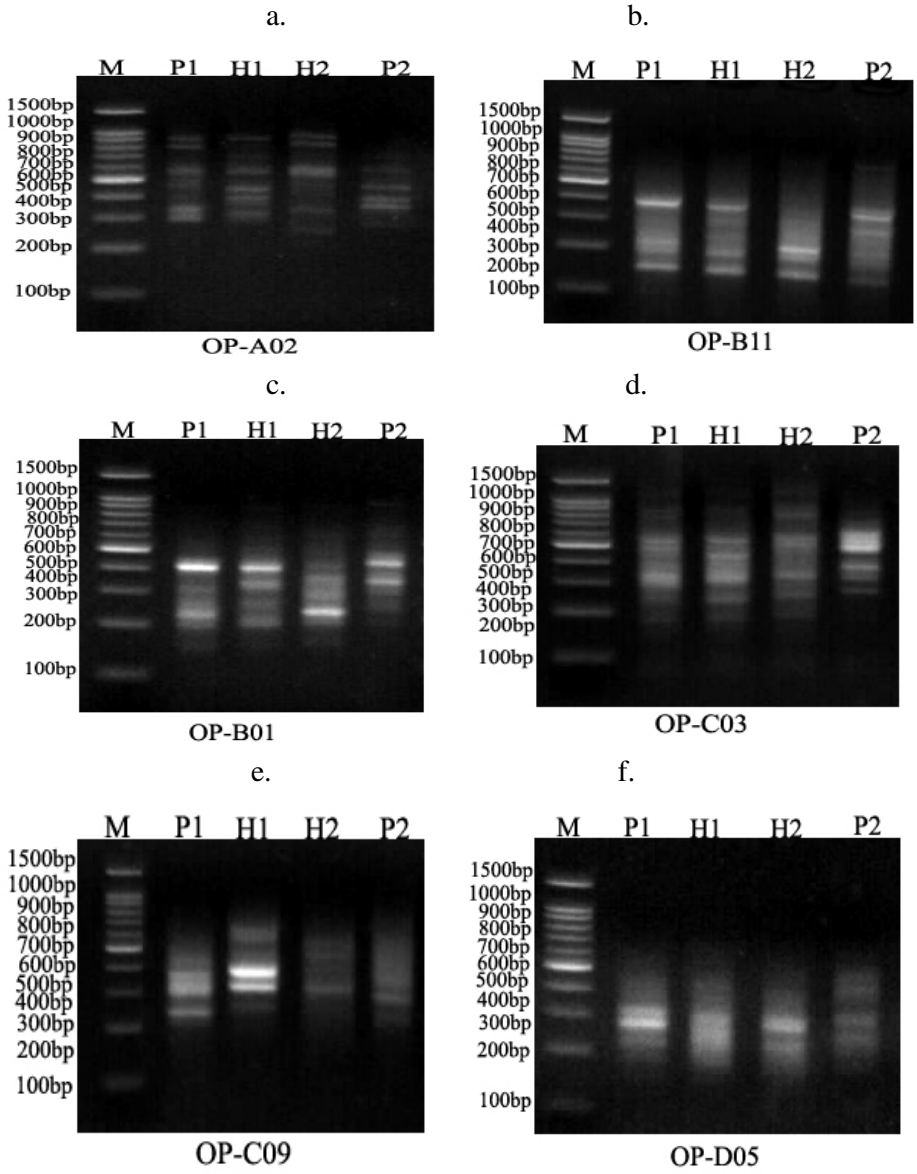

Figure 1: Random amplified polymorphic DNA (RAPD) assay for four varieties of oil palm generated with: (a) OP-A02; (b) OP-B11; (c) OP-B01' (d) OP-C03; (e) OP-C09 and (f) OP-D05 primers. M: 100 bp size marker, P1: Dura; H1: Tenera; H2: Virescens; P2: Pisifera.

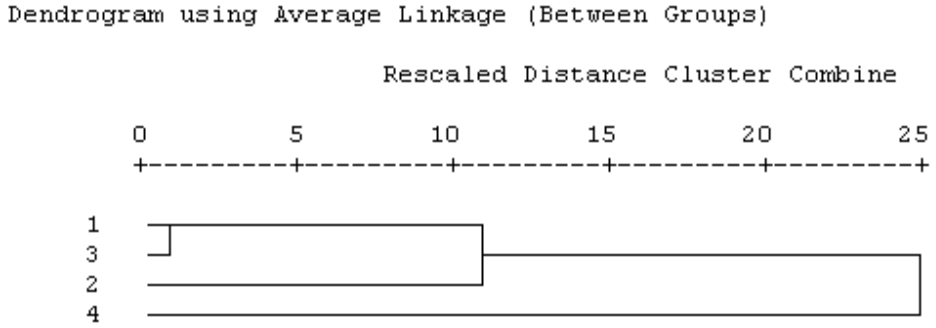

Figure 2: Dendrogram constructed for comparing amplified fragments generated by specific primers for identifying the four varieties of Oil palm.

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