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The utility of RAPD markers for the determination of genetic variation in oil palm (*Elaeis guineensis*)

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Abstract The genetic variation among different accessions of oil-palm germplasm collected from Africa was estimated using random primers and the polymerase chain reaction. The present study revealed high levels of genetic variation in these accessions. Electrophoresis of the amplification products indicated that nine out of 20 primers were able to generate polymorphic products ranging in length from 0.2 kb to 2.3 kb. No individual palm or population-specific products were observed. Greatest diversity was seen in Zaire population 5 and the least in Zaire population 2.

Key words Oil palm germplasm · RAPD
Genetic diversity

Introduction

The oil palm (*Elaeis guineensis*) is a major plantation crop throughout the humid and subhumid tropics. In its centre of origin in West Africa, however, it occurs in wild groves which are harvested for local use but have also provided exports since the early 1800s. The present distribution of oil palm extends from 16°N in Senegal to 15°S in Angola although it generally follows a 20° latitudinal band from 10°N to 10°S. Populations in East Africa, however, are believed to be derived from plants taken there by Arab slave-traders. Hartley (1977) distinguishes between wild, semi-wild and domesticated groves in west Africa, where semi-wild, groves refer to ones which owe their existence to the disturbance of the habitat by man. Given the long history of habitat disturbance and use of oil palm by man, he considers it impossible to determine the difference between many wild and semi-wild groves. In some areas of the Congo, the semi-wild groves have been replaced as high forest has been re-established, indicating that mans disturbance is important in the maintenance of many groves.

Oil palm was grown outside Africa as early as 1730, yet its greatest appreciation as an exotic arose when four palms (*dura* variety) were sent to Indonesia in 1848 (Purseglove 1988). These four palms were for many years to form the basis of the plantation industry in Southeast Asia. The success of the Southeast Asian plantations is shown by the superior quality and volume of production compared to West Africa (Purseglove 1988).

Deliberate breeding through mass selection began in Indonesia in the 1920s. The selections, however, were all descended from the four palms, introduced to Java in 1848, of the largely-inferior variety *dura*. This population of palms was particularly uniform and the original parents were themselves believed to have been derived from palms in Mauritius where it is not native (Hartley 1977). The existence of little variation in bunch characters in the Indonesian landrace, in contrast to Africa where large variation was evident, led to subsequent introductions of *dura*, and the superior variety *tenera*, from Africa (Hartley 1977). The discovery that *tenera* was a hybrid between *dura* and *pisifera* varieties led to further improvements through controlled pollinations. Large gains in fruit characters have been achieved although many are strongly influenced by environment (Hartley 1977). In West Africa, plantation breeding concentrated on the *tenera* variety although there was some selection and deliberate planting of desired types in forest clearings (Hartley 1977).

Concerns over the narrow breadth of the genetic base of oil palm in Malaysia led the Palm Oil Research Institute of Malaysia (PORIM) to collect a number of new accessions from Africa. The collections spanned Nigeria, Cameroun, Zaire and Tanzania (Rajanaidu 1987). The morphological variation in a number of quantitative traits is

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currently being assessed although no investigations into the genetic control of diversity or pattern variation are underway. In oil palm, fruiting does not occur until 3–4 years of age. Given that improved fruiting is the objective of most breeding programmes, a more rapid assessment of variation should be useful. Newer molecular techniques, such as Random Amplified Polymorphic DNA (RAPD), suggest themselves as suitable methods. Oil yield per palm is a composite character made up of many traits including sex ratio, fruit to bunch ratio, weight per bunch, bunch number and oil per fruit. These characters vary continuously although some of them are highly heritable (Purseglove 1988) and may be amenable to QTL analysis using molecular markers. The present study sought 1 to determine the utility of RAPD markers in estimating genetic diversity in oil-palm accessions; and 2 to uncover any geographical pattern of genetic variation in the oil palm.

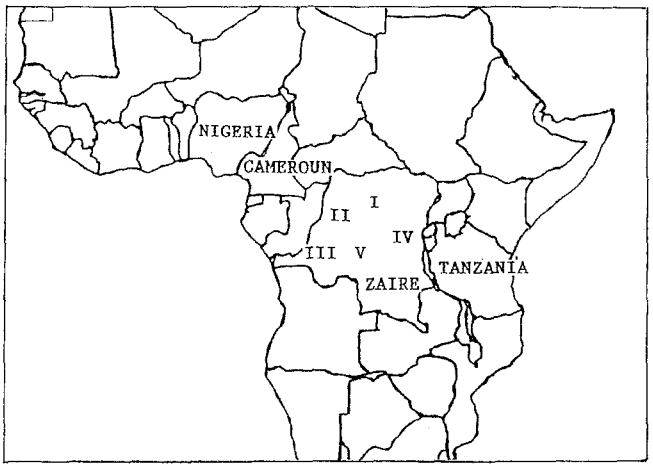

Fig. 1 Map showing areas where oil-palm accession were sampled

Materials and methods

Plant materials

The accessions representing the oil-palm germplasm from Cameroun, Tanzania, Nigeria and Zaire are given in Fig. 1. Seeds from these accessions were collected by PORIM and grown in field stations. Leaf samples were obtained from the first frond. Fourteen samples were selected for each of the five Zairean population and nine samples from each of the Cameroun, Tanzanian and Nigerian populations. Within the Zairean population, 22 different families were sampled.

DNA extraction

DNA from the leaf samples were extracted following the method of Dellaporta et al. (1983)

Primers

Random 10-mer primers with G+C contents of 50–60% were used (Operon Inc, USA); the primers were from kit A and kit C.

DNA amplifications

All experiments were carried out twice for each primer and each acccession, after optimisation of amplification reactions. Twenty 10-mer primers with GC contents of 50–60% were used with 94 oil-palm accessions representing different geographical locations in Zaire, Cameroun, Tanzania and Nigeria.

DNA amplifications were performed according to Williams et al. (1990) with modifications. Genomic DNA (60 ng) was amplified in an optimised reaction mixture of 50 l, containing 80 mM KCl, 10 mM Tris-HCl pH 8.3, 200 M of each dNTP, 1 unit of *Taq* Polymerase enzyme and 0.2 M of primer. Amplifications were carried out in a DNA thermal cycler programmed for 45 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C (Perkin Elmer). Amplified products were analysed on a 1.4% agarose gel, stained with ethidium bromide and visualised under UV.

Data Analysis

For each amplification with a particular primer, the most cathodal product was designated A and the subsequent products B, C, D and so on. Data were scored on absence or presence of amplification products. The scoring was done for each amplification product across all the genotypes sampled. Pair-wise comparisons of genotypes, based on unique and shared polymorphic products, were employed to generate similarity coefficients (Jacquard 1974) which were used to construct phenograms.

Results

From the 20 primers screened, nine were found to give polymorphic amplification products. Some of the products generated are shown in Fig. 2. Only samples that generated polymorphism are shown. The nine primers generated between one and six resolvable product bands per primer with an average of 4.5 bands per primer. The size and frequency of occurrence of each amplification product is given in Table 1. The average frequency of occurrence of product bands was 0.267, although for individual bands this ranged from 0.03 to 0.79. No product bands were found to be population specific although many bands were present in only a few populations albeit generally at low frequencies (for example, 08E). The three bands generated by primer 20 and band 02E, however, were specific to Zaire. The 04E band was found to be a locally common but rare band in only two Zaire provenances. A similar result was seen for band 01D. Four bands absent from the Nigerian provenance were found in the Tanzanian provenance (01B, 08C, 08D and 08F). Thirteen bands were seen to varying extents in all eight provenances and, on average, each band was seen in 5.4 provenances. Zaire 1 showed the highest number of positive primers with 36 out of a possible 41, whereas Cameroun showed the least with only 24 positive primers.

Jacquard's similarity analysis was used as opposed to other techniques as this examines similarities based upon the shared presence of products and both presence and absence. Following Jacquard's analysis, a similarity matrix was constructed for the eight provenances (Table 2). There was generally a low similarity between palms within prov-

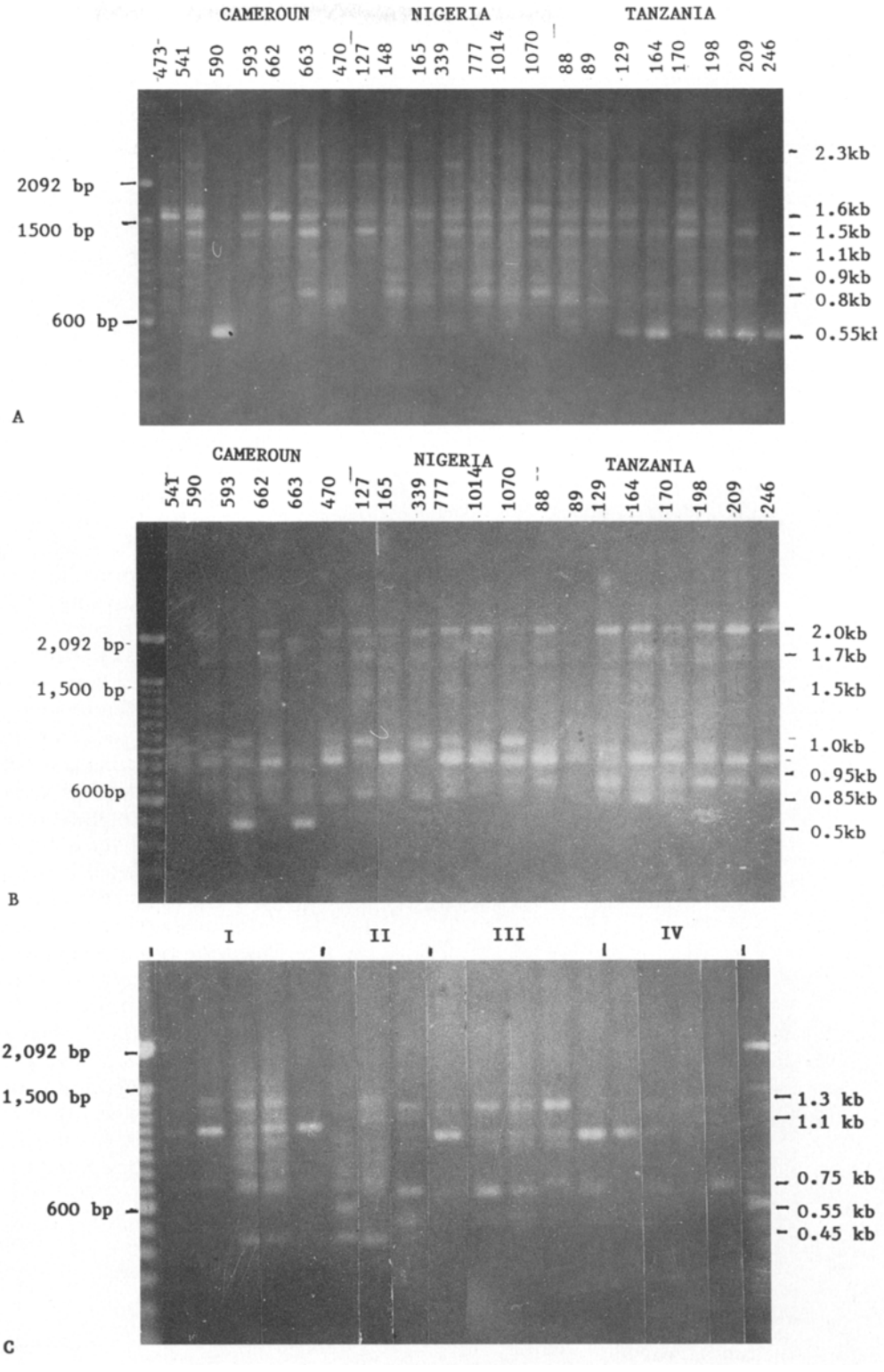

Fig. 2 **A** and **B** represent amplication products of DNA samples from Cameroun, Tanzania and Nigeria using primers 02 and 11 respectively. **C** represents products generated from DNA from Zairean samples using primer 04. The markers ranged from 0.3 kb to 2 kb

enances, with palms from Tanzania showing the greatest degree of similarity to one another. The Zaire 4 population showed least similarity between trees, with a similarity index of 0.173. In addition, there was low similarity between provenances suggesting some degree of population differentiation. The greatest similarity was between Nigerian and Tanzanian provenances (0.324) whilst least similarity was seen between Cameroun and Zaire 5 provenances (0.120).

Considering only the Zaire provenances, Zaire 1 showed the greatest within-provenance similarity. Interestingly, palms from Zaire 3 and Zaire 4 showed greater similarity to palms in Zaire 1 than they did to other palms within their own provenances. This result suggests that

Table 1 The size and frequency for 41 randomly-amplified polymorphic bands generated by nine oligonucleotide primers in oil-palm accessions

Primer	Band	Size (kb)	Frequency
01	A	1.8	0.28
	B	1.5	0.18
	C	1.2	0.17
	D	0.8	0.05
	E	0.7	0.04
02	A	2.3	0.38
	B	1.6	0.48
	C	1.5	0.43
	D	1.1	0.14
	E	0.9	0.23
	F	0.8	0.26
	G	0.55	0.38
04	A	1.3	0.24
	B	1.1	0.36
	C	0.75	0.20
	D	0.55	0.09
	E	0.45	0.09
06	A	1.1	0.53
08	A	2.0	0.34
	B	1.7	0.27
	C	1.4	0.13
	D	1.2	0.11
	E	0.8	0.43
	F	0.75	0.15
10	A	2.0	0.29
	B	1.7	0.39
	C	1.4	0.34
	D	1.2	0.13
	E	0.75	0.13
11	A	2.0	0.78
	B	1.7	0.79
	C	1.5	0.12
	D	1.0	0.11
	E	0.95	0.30
	F	0.85	0.54
	G	0.5	0.23
13	A	2.0	0.47
	B	1.6	0.28
20	A	2.5	0.04
	B	2.0	0.03
	C	1.4	0.03
	Mean	1.29	0.267

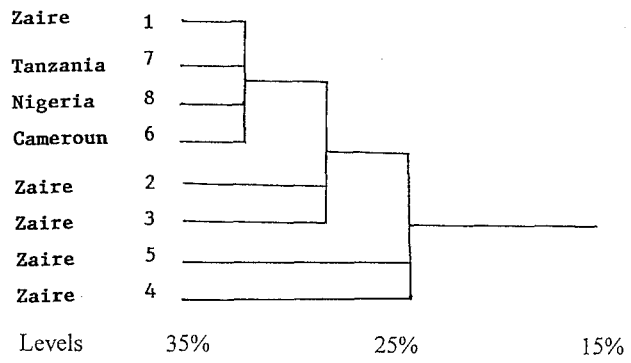

Fig. 3 Dendogram of the eight *E. guineensis* provenances obtained by a group average clustering analysis

these two provenances may be derived from Zaire 1. Hierrachical clustering of the similarity indices was carried out using the group average method (Genstat 1989) and the associated dendrogram is given in Fig. 3. Provenances 1 and 2 show the closest relationship, with provenance 4 being most distinct from the other provenances. A further clustering was done using the 22 families that made up the five Zairean provenances (Fig. 4) which revealed poor separation of families by provenance. The families of provenance 5 were the only ones to cluster out well, indicating poor population differentiation.

Shannon's index of diversity (Shannon and Weaver 1949) was calculated for each putative locus and summed across loci and palms to give a mean value per provenance (Table 3). The greatest diversity was seen in Zaire 2 (0.46) and the least diversity in Zaire 5 (0.24). Shannon's index, however, is insensitive to differences between classes when the frequency of the band exceeds 0.65 (Dawson et al. in preparation). A second Shannon's index was calculated for each provenance based upon the 31 loci which did not have any provenance frequencies in excess of 0.65. This restriction had no effect on the diversity indices of Zaire 1 and Zaire 2, whereas all other provenances showed reduced diversity levels. These two analyses, identified three similar groups with respect to relative diversity, namely high diversity in Zaire 1 and Zaire 2; medium diversity in Zaire 3, Zaire 4, Tanzania and Nigeria; and low diversity in Zaire 5 and Cameroun.

Table 2 Similarity matrix between eight provenances of *E. guineensis* based upon Jaccard's analysis

1	Zaire	39.6						
2	Zaire	28.7	29.3					
3	Zaire	28.0	23.2	23.1				
4	Zaire	22.6	19.6	19.5	17.3			
5	Zaire	24.6	22.3	18.8	18.4	35.7		
6	Cameroun	19.9	17.9	15.7	13.2	12.0	32.5	
7	Tanzania	32.2	28.5	23.5	16.1	20.0	29.3	44.3
8	Nigeria	26.8	22.0	20.5	17.0	17.4	31.7	32.4	32.5
		1	2	3	4	5	6	7	8

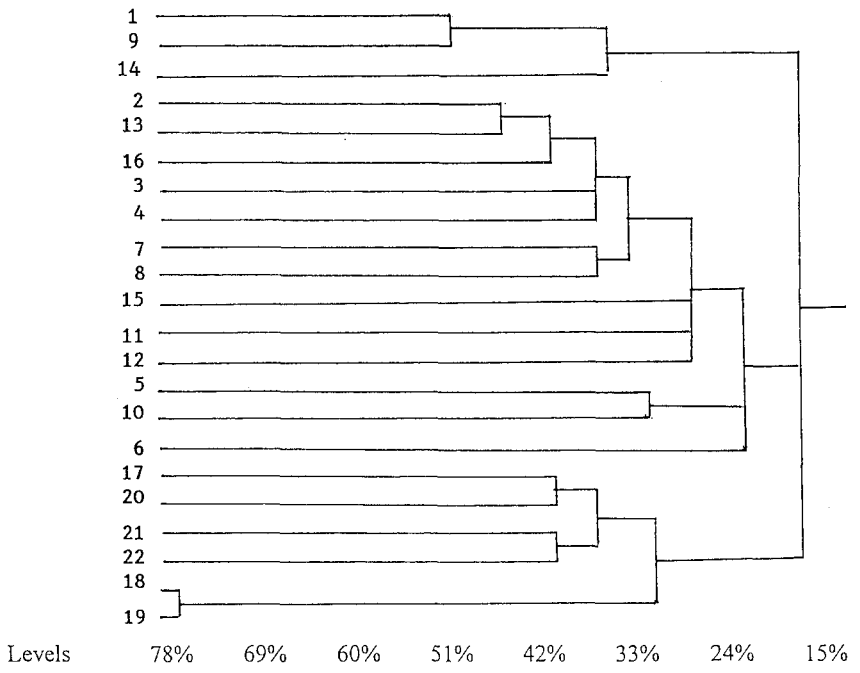

Fig. 4 Dendogram of *E. guineensis* families within the Zaire provenance obtained by a group average clustering analysis. *Families 1–4* represent Zaire population 1; *Families 5–8, 9–13 ,14–17* and *18–22* represent populations *2, 3, 4,* and *5* respectively

Table 3 Shannon's indices of diversity for eight provenances of *E. guineensis*

Provenance	Country	Shannon's Index	
		(41 primers)	(31 primers)
1	Zaire	0.43	0.43
2	Zaire	0.46	0.46
3	Zaire	0.39	0.33
4	Zaire	0.40	0.35
5	Zaire	0.24	0.21
6	Cameroun	0.32	0.26
7	Tanzania	0.36	0.33
8	Nigeria	0.40	0.34

Discussion

The present study revealed high levels of genetic variation in oil-palm accessions as determined by RAPDs. The large number of polymorphic loci per primer makes RAPDs a cost-effective method of examining variation. The majority of the bands (95%) detected here were below a mean frequency of 0.65. Moreover, within the majority of loci (76%), individual provenances had band frequencies below 0.65 which confirms the suitability of Shannon's analysis (which is insensitive to band frequencies of 0.65–0.99). Previous studies of RAPDs in perennial plant species using Shannon's Index have shown lower levels of variation compared to oil palm (H_0=0.24–0.46). In *Gliricidia sepium*, Dawson et al. (personal communication) scored 28 polymorphic loci from seven primers and obtained H_0 values of 0.15–0.21. Interestingly, the study by Dawson et al. included a population known to be extremely diverse as measured by isoenzymes (P=73.3, H=0.36). Further studies are now planned to examine the degree of diversity in the Malaysian commercial cultivars.

Given the wide native distribution and the uncertain state of domestication of many populations, RAPDs would appear to provide a quick method of screening which germplasm to assess in more costly field trials. Shannon's Index of RAPD amplification frequencies could be used as an initial criterion for narrowing down the number of accessions to test. From the present study it would appear that Zaire 5 and the Cameroun provenance could be eliminated. The success of any breeding programme relies upon the existence of genetic variation and the efficiency with which that variation can be selected. RAPDs are not being suggested for marker-assisted selection but rather as a window on the structure and amount of genetic variation present. Genetic diversity is paramount in oil palm given that it is susceptible to many pests and diseases (Purseglove 1988). Furthermore, since oil palm is an obligate outbreeder (Henderson 1988), and inbreeding in African material has led to depression in growth and yield (Purselove 1988), narrowly-based germplasm should be avoided.

No palm- or population-specific amplification products were observed using the nine polymorphic primers. A valuable role of RAPDs in oil-palm research would be clonal identity both to examine the genetic fidelity of tissue-cultured palms and to monitor controlled-pollination programmes. This function of RAPDs proved successful in examining clones of *Populus* spp. (Castiglione et al. 1993) and it is likely that if more primers were screened unique products may be identified. Certainly, this study has shown the usefulness of RAPDs by identifying which primers give a large amount of information and which can be used to discriminate between accessions from different countries.

Verification of the homology of the bands across different provenances was not carried out and fragments of equal size from one primer are believed to be the same allelic products. Reiseberg et al. (1993) identified this as one of the major problems of RAPDs, although the assumption of homology would appear to be justified given the low levels of intraspecific heterology found for other species investigated to-date, such as the *Brassica* spp.

From the natural distribution of oil palm, with a centre of diversity in West Africa, it was expected that Nigerian and Camerounian provenances would be more variable than the derived Tanzanian provenance. Whilst the Nigerian provenance did show high diversity, the Camerounian provenance was one of the least variable. This could be due to the sample size for these provenances (n=9) although Chalmers et al. (1992) used only n=5 for their study on RAPDs in *Gliricidia sepium*. An alternative explanation is that the Cameroun provenance is itself derived since Hartley (1967) reports that many naturalised groves occur in West Africa. Furthermore, if this was collected in an area where local people cultivate oil palm then there may be reduced genetic variation due to pollen contamination from narrowly-based material. The Tanzanian material, if derived, showed several amplification products that the putative ancestral populations in Nigeria did not show. This may be due to contamination by pollen from palms of different varieties in cultivation nearby.

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