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The Evaluation of Genetic Diversity of Deli *dura* and African Oil Palm Germplasm Collection by AFLP Technique

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ABSTRACT. *African oil palm Elaeis guineensis Jacq. is an important crop in terms of edible fats and oil production, second only to soybean. The world's biggest palm oil producer, Malaysia, launched an extensive germplasm collection programme from Africa, and South and Central America. The main objective of this study was to investigate the genetic diversity in the African oil palm germplasm collection. A total of 687 accessions belonging to 11 African countries and Deli dura were screened with AFLP markers using eight primer combinations. A total of 377 bands were recorded with an average of 47 bands per primer pair, of which 93.6% were polymorphic. Comparatively, overall genetic diversity of oil palm was the highest in Nigeria and gradually decreased towards Senegal and Gambia, and also towards Angola and Tanzania. However, Ghana oil palm was less diverse, while oil palm from Guinea showed comparatively higher diversity. Cluster analysis separated oil palm into three major groups and those corresponded well to three regions of Africa. Oil palm from Ghana, Nigeria, Cameroon, Congo, Angola and Tanzania made first major cluster where as oil palm from Senegal, Gambia, Guinea and Sierra Leone made the second major cluster. Madagascar oil palm was highly diverse and formed the third major cluster. This study confirmed the low genetic diversity in Deli dura and it was found to be most closely related to Congo oil palm.*

INTRODUCTION

African oil palm (*Elaeis guineensis* Jacq.) belongs to the family Palme (Uhl and Dransfield, 1987) and is considered as originated from West Africa (Zeven, 1967; Hartley, 1988). Palm oil is second only to soybean oil as a contributor to world's total fat and oil production (Rajanaidu *et al.*, 1997). The world's consumption of palm oil from the total edible oil consumption in 1999 was 27.4% whereas soybean oil was 30.0% (Mielke, 2000). Malaysia is the largest producer of palm oil in the world at present, being the leading supplier to the international market, recording 52.1% of the world's palm oil production in 1999 (Mielke, 2000).

It was realized that the genetic base of oil palm in Malaysia was extremely narrow. Therefore, the Malaysian Palm Oil Board (MPOB), previously known as Palm Oil Research Institute of Malaysia (PORIM) made an extensive oil palm germplasm collection from

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natural populations in order to improve future breeding programmes. The collection has been confined mainly to countries in Africa, and to Central and South America (Rajanaidu, 1985a, 1985b, 1986; Rajanaidu and Jalani, 1994). Evaluations of these materials in the field have revealed a large amount of diversity in relation to yield, plant height, and the composition of oil.

The observations based on morphological and agronomic characters are often influenced by environment. Therefore, it is essential to use neutral markers to investigate the genetic diversity accurately to ensure better utilisation of present germplasm materials. In addition to protein markers (Ghesquire *et al.*, 1987; Choong *et al.*, 1996), several DNA marker techniques (Shah *et al.*, 1994; Jack *et al.*, 1995; Shah and Ling, 1996; Mohd *et al.*, 1998; Maizura, 1999) have also proven to be useful in investigating genetic variability in oil palm.

Amplified Fragment Length Polymorphism (AFLP) is a newly developed PCR-based marker technique which is reported to reveal polymorphisms distributed throughout the entire genome (Vos *et al.*, 1995; Barrett and Kidwell, 1998; Barker *et al.*, 1999). It has been proved that the reproducibility of AFLP is extremely high compared to RAPD and SSR (Jones *et al.*, 1997). In this study, natural oil palm (*E. guineensis*) populations representing the entire germplasm collection from Africa and Deli *dura* were evaluated using AFLP markers.

MATERIALS AND METHODS

Plant materials

A total of 687 accessions belonging to 11 countries and Deli *dura* from Malaysia were evaluated in this experiment. Fig. 1 shows the countries from where the oil palm germplasm were collected and the respective numbers of accessions.

AFLP analysis

The AFLP procedure was performed with AFLPTM Analysis System 1 from GIBCO BRL, USA, following the recommended protocol by the manufacturer with slight modifications. The digestion mixture contained 300 ng of DNA, 5 µl of 5× reaction buffer, and 3 µl of EcoR1 and Mse1 enzyme mixture (1.25 units/µl). The final volume of the reaction mixture was brought up to 25µl by adding distilled water of AFLP grade. The mixture was incubated for 3 h at 37°C and the enzyme was inactivated by placing samples at 70°C for 15 min followed by immediate immersion of the tube in ice. The digested DNA was ligated to EcoR1 and Mse1 adapters using 24 µl of adapter ligation solution and 1 unit of T4 DNA ligase as recommended by the supplier. Adapter ligation was performed at 20°C for 3 h. The resultant mixture was diluted with TE buffer (pH 8.0) in the proportion of 11:89 and used as template for the pre-amplification reaction.

During pre-amplification, two EcoR1 and Mse1 primers each having one selective nucleotide at the 3' end were used. The reaction mixture contained 5 µl of diluted template from the above, 40 µl of pre-amplification mixture, 5 µl of 10× PCR buffer plus Mg^{2+}, and

1.1 units of Taq DNA polymerase. The total volume was brought to 51 µl for each sample by addition of distilled water. Polymerase chain reaction was performed for 20 cycles using the Perkin-Elmer 9600 Thermocycler, each cycle having the combination of 30 sec at 94°C, 60 sec at 56°C and 60 sec at 72°C. The pre-amplified mixture was diluted to the proportion of 1:29 with TE buffer (pH 8.0) and used as template for subsequent selective amplification.

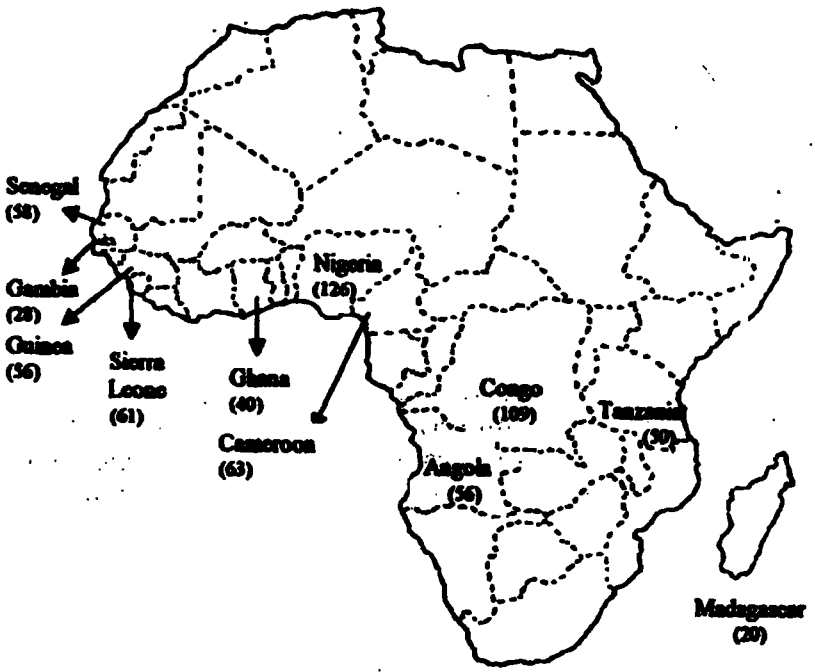

Fig. 1. African countries from which oil palm germplasm materials were collected and the number of plants used in the study.
[Note: 20 samples of Deli *dura* were also collected from Malaysia].

The labelling of EcoR1 primer was carried out with [γ^{33}P]ATP (2000 Ci/mmol). The reaction mixture, for 50 selective AFLP amplifications, contained 9 µl of EcoR1 primer, 5 µl of 5× kinase buffer, 6 µl of [γ^{33}P]ATP and 3 µl of T4 polynucleotide kinase. The total volume was brought to 25 µl by adding distilled water and incubated for 1½ h at 37°C followed by inactivation of enzyme by incubation at 70°C for 10 min followed by immediate immersion in ice.

Selective amplification was performed with [γ^{33}P]ATP labelled EcoR1 primer and unlabeled Mse1 primer each having three selective nucleotides at the 3' end. Each reaction mixture contained 5 µl of pre-amplified DNA template, 5 µl of a mixture containing 5 ng of EcoR1 primer labelled with [γ^{33}P]ATP, 30.15 ng Mse1 primer with dNTPs, 2 µl of a mixture of 10× PCR buffer with Mg^{2+}, and 0.6 units of Taq DNA polymerase. The final volume was brought up to 20 µl by adding distilled water. The PCR was performed in three stages. The first amplification consisted of only one-cycle of 30 sec at 94°C; 30 sec at 65°C

and 60 sec at 72°C. During the second stage of amplification, the annealing temperature was lowered by 0.7°C for each cycle for a total of 12 cycles. The third stage consisted of 23 cycles with each cycle having 30 sec at 94°C, 30 sec at 56°C and 60 sec at 72°C.

Once the PCR reaction was completed, a 3.2 µl aliquot of the sample was mixed with 3.2 µl of formamide dye (98% formamide, 10 mM EDTA, 0.002% bromophenol blue and, 0.002% xylene cyanol), heated for 3 min at 90°C and the tube immediately placed in ice. The gel was prepared by mixing 100 ml of 6% polyacrylamide (20:1 acrylamide: bisacrylamide) containing 7.5 M urea and 1 M TBE, 100 µl of 10% ammonium persulphate and 20 µl of N,N,N',N'-tetramethylenediamine (TEMED). Five micro litres each of the heat-treated samples were electrophoresed at 1600 V using BRL Model 2 gel electrophoresis apparatus. Once the slower dye moved to the end of the gel plate, the gel was transferred to a 3 MM chromatography paper, vacuum dried for 1 h at 80°C and exposed to Kodak O-mat X-ray film. Depending on the radioactivity content of the gel, exposed films were kept at −80°C for 2-3 days before the development of film.

Data analysis

The scoring of autoradiograms was performed manually as either band present or absent within the range of 100 bp to 600 bp. The bands present in all the individuals were treated as monomorphic bands, whereas, polymorphic bands were different between at least two samples. The maximum number of bands is the cumulative figure of both polymorphic and monomorphic bands.

Degree of genetic diversity

The degree of genetic diversity was quantified using the Shannon's index of phenotypic diversity (H_0).

$$H_0 = -\sum \pi_i \ln \pi_i$$

Where π_i is the frequency of phenotype i (King and Schaal, 1989). H_0 were calculated for combinations of each primer pair and materials from different countries.

Similarity matrix

R package-CMS (IBM) was used to calculate similarities between each country and Deli *dura* (Legendre and Vaudor, 1991). Cluster analysis was carried out using unweighted pair-group method with arithmetic average (UPGMA). NTSYS package was used to construct the dendrogram (Rohlf, 1993).

RESULTS AND DISCUSSION

A portion of autoradiogram generated by AFLP marker technique is shown in the Fig. 2. The maximum number of scorable bands (maximum bands), monomorphic bands, polymorphic bands and the percentage of polymorphic bands for each primer combination

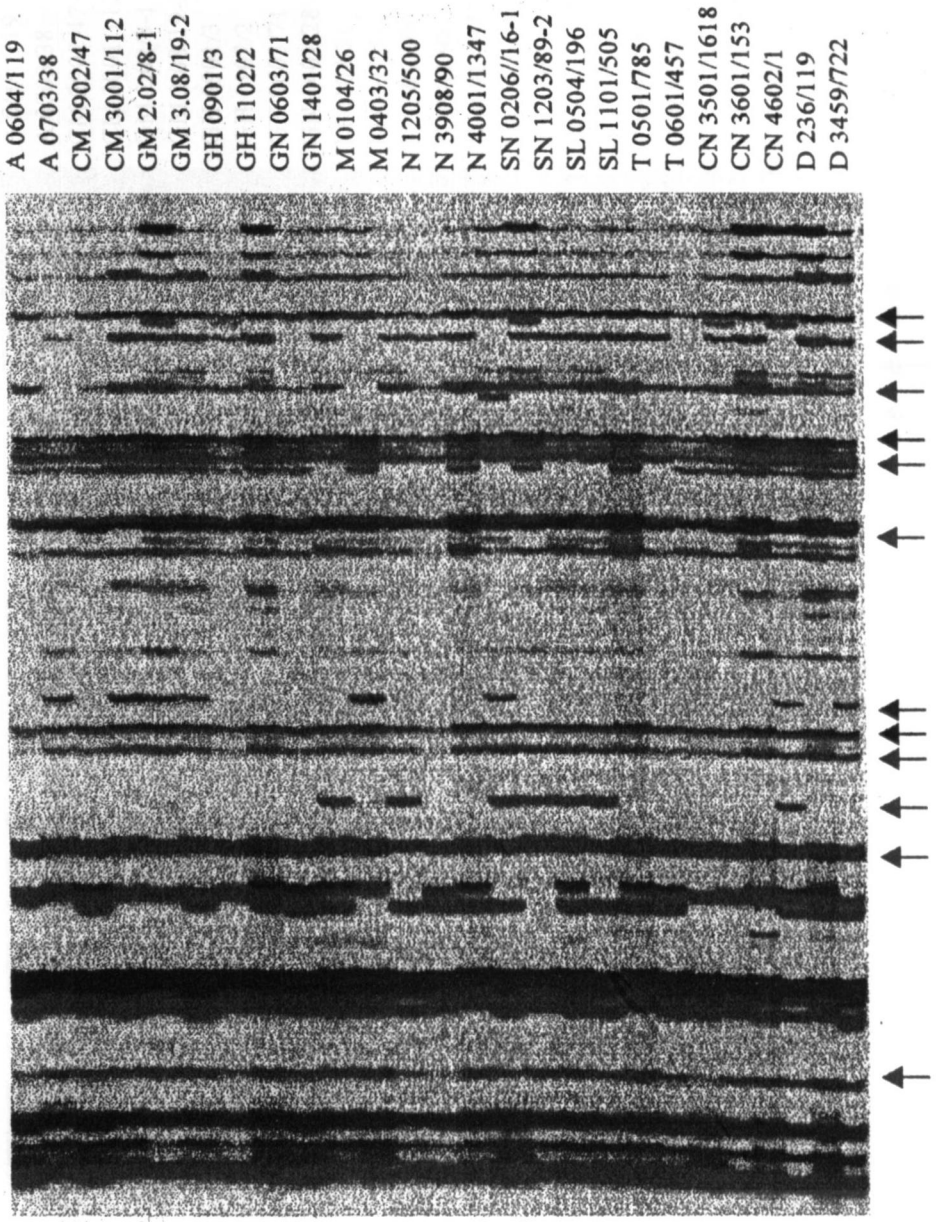

Fig. 2. A representative portion of AFLP autoradiogram of oil palm from different countries and Deli *dura* with E-ACA/M-CTT primer combination.
[Note: Some clear scorable bands are indicated by arrows].

are given in Table 1. The maximum bands produced by each primer pair varied from 17-75 with an average of 47 bands. The number of polymorphic bands also varied widely from 16-70 for different primer combinations, with an average of 44 bands. The percentage of polymorphic bands per primer combination ranged from 87.0- 97.7% for different primer

combinations, with an average of 93.6%. Results indicate the superior power of AFLP by revealing high number of maximum bands and high number of polymorphic bands per single assay when compared to isoenzyme (Choong *et al.*, 1996), RAPD (Shah *et al.*, 1994), RFLP (Maizura, 1999) and microsatellite (Shah and Ling, 1996) marker techniques. In terms of the number of maximum bands and the number of polymorphic bands, primer combination 7 was the most informative, while primer combination 5 was the least informative. Therefore, primer combination 5 may be avoided in future screening of oil palm materials.

Table 1. Distribution of maximum, monomorphic and polymorphic bands and the percentage of polymorphic bands for different primer combinations.

Primer combination	Maximum number of bands	Number of monomorphic bands	Number of polymorphic bands	Percent polymorphic bands
1 (AAC/CAG)	59	2	57	97.6
2 (AAG/CTA)	54	7	47	87.0
3 (ACA/CTT)	53	4	49	92.5
4 (ACC/CTC)	39	2	37	94.9
5 (ACG/CAA)	17	1	16	94.1
6 (ACT/CTG)	44	1	43	97.7
7 (AGC/CAT)	75	5	70	93.3
8 (AGG/CAG)	36	3	33	91.8
Total	377	25	352	
Mean	47.1	3.1	44.0	93.6

Distribution of bands

All eight primer combinations revealed polymorphism in accessions from different countries and accessions of Deli *dura*. The maximum number of bands, number of monomorphic bands, number of polymorphic bands and the percentage of polymorphic bands for different countries and Deli *dura* are given in Table 2. Nigerian oil palm scored the highest (371) while Deli *dura* recorded the lowest value for maximum bands (272). The highest number of polymorphic bands (284) was also observed in Nigerian accessions, whereas Ghana materials produced the lowest (140). However, comparison of the maximum number of bands and the number of polymorphic bands directly across countries may not be reasonable as sample size for each country was different. Nevertheless, it is interesting to note that Madagascar, which had only 20 samples showed higher number of maximum bands and polymorphic bands than several countries which had more samples. The percentage of polymorphic bands in this study varied from 48% (Ghana) to 80% (Congo). In natural populations, the highest diversity is expected at the centre of origin and the diversity tends to decrease according to the geographical distance from the centre of

origin (Maxted *et al.*, 1997). It is assumed that oil palm originated in West Africa (Hartley, 1988). Therefore, the highest diversity is expected in West Africa. Senegal, Angola and Tanzania are at the extreme ends of natural oil palm distribution in Africa; therefore, low level of diversity is expected in oil palm from these countries.

Table 2. Distribution of maximum, monomorphic and polymorphic bands and the percentage of polymorphic bands for different countries and Deli *dura*.

Source	Maximum number of bands	Number of monomorphic bands	Number of polymorphic bands	Percent polymorphic bands
Angola	311	134	177	56.9
Cameroon	369	118	251	68.0
Congo	339	69	270	79.6
Gambia	288	145	143	49.7
Ghana	289	149	140	48.4
Guinea	318	116	202	63.5
Madagascar	310	115	195	62.9
Nigeria	371	87	284	76.6
Senegal	305	121	233	72.4
Sierra Leone	322	89	233	72.4
Tanzania	296	117	179	60.5
Deli *dura*	272	98	174	64.0

Considering the percentage of polymorphic bands, Congo oil palm had the highest diversity (80%), followed by Nigerian oil palm (77%). The most concentrated groves of oil palm in central Africa were reported to be in Congo, mainly in the south of the country. In some areas, dense groves were comparable to those existing in eastern Nigeria (Hartley, 1988). It has also been reported that until recently oil palm has been confined to West and Central Africa, and Nigeria, Cameroon and Congo are countries within the region. Results showed that these countries had fairly high percentages of polymorphic bands when compared to other countries. Most probably, the high percentage of polymorphic bands in Sierra Leone in this study may be due to the introduced materials from other countries as Sierra Leone is one of the countries that exploited oil palm industry as early as 1910's (Hartley, 1988). Countries like Angola, Tanzania, Senegal and Gambia, which are at the extreme ends of the natural oil palm belt, had comparatively low percentages of polymorphic bands. Ghana oil palm had the lowest percentage of polymorphic bands and is rather unexpected based on natural distribution. Felling of wild oil palm for the production of "down-wine" was mentioned in Ghana and was an entirely a destructive process (Hartley, 1988). Furthermore, cocoa has become dominant economic crop in Ghana and all favourable areas were planted with cocoa (Hartley, 1988). These may have caused significant impacts on the natural oil palm populations in Ghana and the present populations may have been derived from a restricted narrow genetic pool.

The percentage of polymorphic bands in Madagascar oil palm was higher than that from Angola or Tanzania and does not correlate with the geographical distribution. It has been reported that oil palm in Madagascar was very poor in growth as compared to oil palms in Nigeria, Cameroon, Congo and Tanzania due to harsh climatic conditions (Rajanaidu, 1986). Fagus sylvatica grown in extreme conditions possess high level of genetic diversity compared to those grown in favourable environments (Muller-Starck, 1985). Therefore, it can be assumed that oil palm must have been introduced to Madagascar at very early stages of evolution and underwent many changes to adapt to harsh natural conditions.

Shannon's index of phenotypic diversity (H_0)

The phenotypic frequencies detected with eight primer combinations were used to calculate the phenotypic diversity within oil palm materials from each country and Deli *dura*. Each primer pair revealed a different level of phenotypic diversity in accessions from each country varying from 0.99 (Deli *dura*) to 13.74 (Madagascar oil palm) (Table 3). The estimated mean phenotypic diversity revealed that Cameroon oil palm had the highest diversity (6.65) while Deli *dura* had the lowest (3.63).

Table 3. Shannon's index of phenotypic diversity (H_0) for oil palm germplasm collection from 11 countries and Deli *dura* with eight primer combinations.

Source	Primer combination									
	1	2	3	4	5	6	7	8	Total	Mean
Angola	4.26	4.25	4.89	4.41	1.33	5.76	8.07	2.79	35.76	4.47
Cameroon	9.00	5.33	6.62	5.76	1.75	7.20	12.09	5.44	53.19	6.65
Congo	5.64	4.96	6.65	5.52	1.66	5.11	10.22	2.90	42.66	5.33
Gambia	3.72	3.17	4.84	3.66	1.43	5.38	7.95	1.72	31.38	3.98
Ghana	3.42	4.62	5 57	5.04	1.66	3.35	5.62	1.44	30.72	3.84
Guinea	5.49	5.99	7.28	4.00	1.80	6.86	7.66	2.22	41.30	5.16
Madagascar	5.18	6.20	8.15	5.17	2.71	6.29	13.74	3.64	51.08	6.39
Nigeria	8.98	5.55	7.45	6.72	1.92	5.88	9.87	5.47	51.84	6.48
Senegal	5.12	3.93	6.56	2.70	1.47	4.33	7.14	2.45	33.70	4.21
Sierra Leone	6.42	4.98	5.82	3.05	1.93	6.83	9.03	2.71	40.77	5.10
Tanzania	5.00	4.93	5.37	4.87	1.72	3.24	3.72	2.20	31.05	3.88
Deli *dura*	4.87	3.50	5.27	3.21	0.99	3.12	6.46	1.62	29.04	3.63

The highest phenotypic diversity within all oil palm accessions was shown in Cameroon followed by Nigeria. The percentage of polymorphic bands was highest in Congo oil palm followed by Nigerian oil palm. It was clear that oil palm from Congo, Cameroon and Nigeria had comparatively high values for both percentage of polymorphic bands and phenotypic diversity. Angola, Tanzania, Senegal and Gambia, the countries at

the extreme ends of the natural oil palm distribution had comparatively low phenotypic diversity values. However, Madagascar oil palm had fairly high phenotypic diversity value and this was shown in the percentage of polymorphic bands as well. Phenotypic diversity values for Ghana oil palm was comparatively low, following the same trend as the percentage of polymorphic bands.

The lowest phenotypic diversity value and lowest number of maximum bands demonstrate the low variability of Deli *dura*. As Deli *dura* originated from four palms introduced to Java, a low level of genetic diversity is expected. Furthermore, Deli *dura* populations studied here were a result of intensive selections through several generations, which reduce the variability. Thus results of this study confirmed the narrow genetic base of Deli *dura*.

Genetic similarity

Table 4 shows the genetic similarity values based on simple matching similarities that varied from 0.9867 (between Nigeria and Congo palms) to 0.8748 (between Deli *dura* and Gambian oil palm). Results showed that Nigerian oil palm was most related to Congo. Similar result was reported in earlier studies of oil palm from Nigeria, Congo, Cameroon and Tanzania (Shah *et al.*, 1994; Shah and Ling, 1996; Kularatne *et al.*, 1998). Deli *dura* showed the least distance to Congo oil palm.

Table 4. Simple matching similarities in different countries including Deli *dura*.

	Angola	Cameroon	Congo	Gambia	Ghana	Guinea	Madagascar	Nigeria	Senegal	S. Leone	Tanzania
Angola											
Cameroon	0.9754										
Congo	0.9851	0.9800									
Gambia	0.9079	0.9138	0.9197								
Ghana	0.9601	0.9619	0.9670	0.9124							
Guinea	0.9464	0.9540	0.9553	0.9615	0.9464						
Madagascar	0.9196	0.9215	0.9253	0.8770	0.9063	0.9100					
Nigeria	0.9814	0.9795	0.9867	0.9209	0.9661	0.9579	0.9191				
Senegal	0.9271	0.9300	0.9312	0.9706	0.9230	0.9704	0.8832	0.9329			
S. Leone	0.9232	0.9262	0.9324	0.9641	0.9206	0.9780	0.8869	0.9341	0.9667		
Tanzania	0.9820	0.9685	0.9828	0.9041	0.9640	0.9449	0.9070	0.9855	0.9225	0.9225	
Deli *dura*	0.9367	0.9319	0.9484	0.8748	0.9224	0.9112	0.8925	0.9464	0.8895	0.8920	0.9399

Cluster analysis

The dendrogram showed three major clusters representing three geographical regions (excluding Deli *dura*) in Africa (Fig. 3). Nigeria, Congo, Tanzania, Angola,

Cameroon, Ghana and Deli *dura* formed one cluster. This shows that Deli *dura* is closely related to the oil palm from Nigeria, Congo, Tanzania, Angola, Cameroon and Ghana than to the other countries. The close relationship of oil palm from Nigeria, Congo, Tanzania, Angola and Cameroon in this study was somewhat comparable to the results based on RFLP analysis (Maizura, 1999). Oil palm from Gambia, Senegal, Guinea and Sierra Leone formed the second major cluster separating oil palm according to another geographical region in Africa. Gambia is covered by Senegal in land and oil palm from these two countries clustered together. Similarly, Sierra Leone is largely covered by Guinea and oil palm from the two countries clustered together. Together with Gambia, Senegal, Sierra Leone and Guinea made one cluster representing the northwestern part of Africa. Madagascar oil palm formed the third major cluster away from all the other countries exhibiting the uniqueness of Madagascar oil palm. These findings further confirm the difference of Madagascar oil palm, as was shown before with other genetic diversity measures.

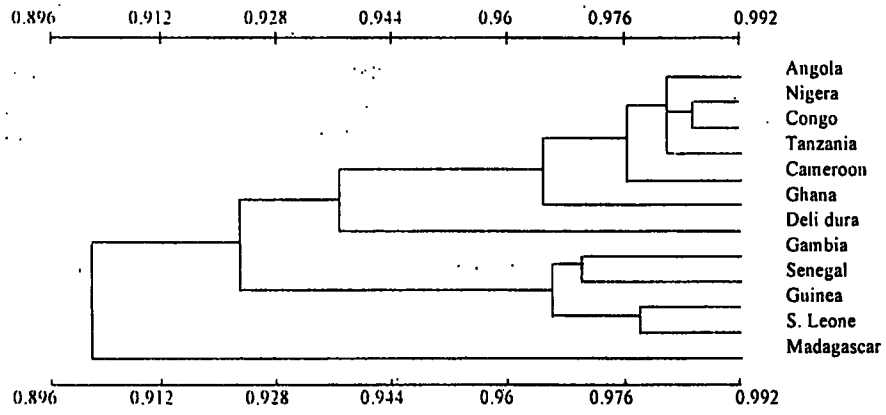

Fig. 3. Dendrogram of oil palm from different countries and Deli *dura* based on UPGMA analysis of simple matching similarities.

CONCLUSIONS

This study confirms that the oil palm germplasm collection established by MPOB is an excellent source of new genetic diversity. These materials, especially high diversity populations from Nigeria, Cameroon, Congo and Madagascar could successfully be used for the introgression of new genetic diversity into existing breeding populations, as well as for the development of new breeding populations.

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