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ORIGINAL PAPER

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Genetic diversity of oil palm (*Elaeis guineensis* Jacq.) germplasm collections from Africa: implications for improvement and conservation of genetic resources

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Abstract A total of 723 accessions of oil palm (*Elaeis guineensis* Jacq.) from 26 populations representing ten countries in Africa and one Deli *dura* family were screened for allelic variation at seven enzyme loci from six enzyme systems using starch gel electrophoresis. On average, 54.5% of the loci were polymorphic (0.99 criterion). The average and effective number of alleles per locus was 1.80 and 1.35, respectively. Mean expected heterozygosity was 0.184, with values ranging from 0.109 (population 8, Senegal) to 0.261 (population 29, Cameroon). The genetic differentiation among populations was high (F_{ST}=0.301), indicating high genetic divergence. The calculation of F_{ST} by geographic zones revealed that the high F_{ST} was largely due to F_{ST} among populations in West Africa, suggesting diversifying selection in this region. The mean genetic distance across populations was 0.113. The lowest genetic distance (D) was observed between population 5 from Tanzania and population 7 from the Democratic Republic of the Congo (0.000) and the highest was found between population 4 from Madagascar and population 13 from Sierra Leone (0.568). The total gene flow across oil palm populations was low, with an Nm of 0.576, enhancing genetic structuring, as evident from the high F_{ST} values. UPGMA cluster analysis revealed three main clusters; the western outlying populations from Senegal and Sierra Leone were in one cluster but separated into two distinct sub-clusters; the eastern outlying populations from Madagascar were in one cluster; the populations from Angola, Cameroon, The Democratic Republic of the Congo, Ghana, Tanzania, Nigeria and Guinea were in one cluster. The Deli *dura* family seems to be closely related to population 6 from Guinea. Oil palm populations with high genetic diversity—i.e. all of the populations from Nigeria, Cameroon and Sierra Leone, population 6 of Guinea, population 1 of Madagascar and population 2 of Senegal should be used in improvement programmes, whereas for conservation purposes, oil palm populations with high allelic diversity (A_e), which include populations 22 and 29 from Cameroon, populations 39 and 45 from Nigeria, population 6 from Guinea, populations 5 and 13 from Sierra Leone and population 1 from Madagascar should be selected for capturing as much genetic variation as possible.

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Introduction

The oil palm, *Elaeis guineensis*, grows in the wild, semi-wild and cultivated parts of the tropics, within ±10° latitude of the equator, in Africa, South East Asia and South and Central America. However, it is endemic to the tropical lowlands of West and Central Africa, spreading from 16°N in Senegal to 15°S in Angola (Hartley 1988). The oil palm is also found in East Africa, including the island of Madagascar where it is believed to have been introduced by Arab slave traders in the tenth century (Hartley 1988).

Palm oil has long played an important role in the diet and economy of the village communities found in the African rainforest. The use of oil palm by man and also their activities in the African rainforest, such as forest clearing for shifting cultivation, may have disturbed the oil palm habitats and consequently reduced the genetic diversity of oil palm natural populations. Man is thought to be the most important vector for oil palm seed dispersal in the tropical rainforest of Africa, with other major means of seed dispersal including mammals, rodents and birds. Gravity and water are other less important agents of oil palm seed dispersal. In the African forest, the oil palm

is mainly pollinated by the weevil, *Elaeidobius kamerunicus* (Hartley 1988).

Oil palm is currently the most important plantation crop in Malaysia, and since 1971 Malaysia has been the world's largest producer and exporter of palm oil. Deli *dura*, which is the breeding population used in most of the commercial programmes for hybrid production for oil palm plantations in Malaysia and South East Asia, is descended from only four palms that were introduced to Java, Indonesia in 1848 (Hartley 1988). Since the gene pool of current oil palm planting materials is very limited, the Malaysian Palm Oil Board (MPOB), formerly known as Palm Oil Research Institute of Malaysia (PORIM), initiated searches to collect oil palm genetic materials at its centre of origin—Africa—in order to broaden the genetic base of oil palm breeding materials. Most of the countries of Africa in which the oil palm can be found, including Nigeria, Cameroon, The Democratic Republic of the Congo, Tanzania, Madagascar, Angola, Senegal, Gambia, Sierra Leone, Guinea and Ghana were explored for oil palm populations (Rajanaidu and Jalani 1994). An evaluation of oil palm germplasm collections is very important for gaining insight into the genetic structure of natural oil palm populations in Africa. The information gathered will be useful for the effective utilization of oil palm genetic resources in breeding programmes and also for the development of guidelines for ex-situ conservation of germplasm and planning for future sampling.

Oil palm germplasm materials maintained at MPOB have been evaluated in the field for morphological and physiological traits (Rajanaidu 1980), fatty acid composition (Arasu 1985) and yield parameters (Rao 1987). Genetic variation for these quantitative traits in the Nigerian population has also been investigated (Rajanaidu et al. 1989). Some molecular markers and protein markers have also been used to investigate the genetic diversity in the oil palm germplasm; these include restriction fragment length polymorphisms (RFLPs) of ribosomal DNA (Rajanaidu et al. 1989, 1993; Shah et al. 1993), random amplified polymorphic DNA (RAPD) (Shah et al. 1994; Rajanaidu et al. 2000), inter-microsatellite DNA variation (Shah and Lim 1996), RFLP using cDNA probes (Maizura 1999), amplified fragment length polymorphism (AFLP) (Kularatne 2000) and isozyme markers (Sapurah 1990; Choong et al. 1996). Additional studies that evaluated genetic diversity in oil palm populations using isozyme and molecular markers have been conducted in many countries with different oil palm populations (Ghesquiere 1985; Purba et al 2000).

The previous assessments of genetic diversity in the germplasm collections at MPOB were not comprehensive, with the exception of the screening done by Kularatne (2000) and Rajanaidu et al. (2000) using AFLP and RAPD markers, respectively, as they did not cover most of the collections of African oil palm natural populations. The evaluation of oil palm germplasm collections reported here includes 26 populations from ten African countries and is the first comprehensive study using isozyme analysis. Isozyme analysis has been used to estimate the genetic diversity in natural plant populations (Cottrell and White 1995; Wickneswari and Norwati 1993; Granger et al. 1993). Although DNA markers have many advantages over isozyme markers, isozymes are still being used in genetic diversity studies of plant populations due to their cost effectiveness when screening large number of samples and ease of handling. As long as the isozyme polymorphism in the plant species investigated is high, this marker adequately reveals the level of genetic diversity and genetic structure of plant populations. Indeed, the patterns of population genetic structure revealed might be similar to that observed using expensive nuclear DNA markers (Byrne et al. 1998). In the investigation reported here, isozyme analysis was used to determine: (1) the genetic structure of natural oil palm populations from different geographic regions in Africa and (2) the genetic relatedness between oil palm populations in Africa.

Materials and methods

Plant materials

Twenty-six populations of oil palm (*Elaeis guineensis* Jacq.) germplasm collections representing ten countries in Africa were used in this study. One Deli *dura* family (DD232), a breeding population used in the production of commercial hybrid oil palm planting materials, was used to compare genetic diversity with that of the natural African oil palms. Approximately 30 progenies were sampled per population, yielding a total of 723 individuals. Two to three populations were chosen from each country, based on their size, and three families, on average, were selected to represent a population. Table 1 shows the samples used for isozyme analysis. A more detailed description of the populations and families used in this study are given in Hayati (2002). The locations of the oil palm populations in Africa are shown in Fig. 1. Seed samples of these palms had been previously collected from their countries of origin and grown at the MPOB Research Station, Kluang, Johor.

Enzyme extraction

Enzymes were extracted from unopened spear leaves by grinding approximately 0.5 g of leaf tissue to a fine, dry powder in liquid nitrogen using a mortar and pestle. The powdered tissue was then transferred to a 2.5×2.5-cm weighing boat containing 1 ml of cold extraction buffer (Wickneswari and Norwati 1991) and mixed to create a slurry. The crude extract was then filter-centrifuged using an Eppendorf tube with a hole at the bottom plugged with a layer of cotton wool layer for 20 min at 7,000 *g* and 4°C into another Eppendorf tube. The filtered extract was divided into two portions and immediately frozen at −70°C until required for isozyme analysis.

Isozyme electrophoresis and staining

Starch gel electrophoresis with different buffer systems was used to assay the different enzyme systems. The concentration of starch (Sigma soluble potato starch; Sigma, St. Louis, Mo.) used for gel preparation was 10–12% (w/v). Separation of the isozymes was performed at 45–60 mA constant current at a voltage not exceeding 300 V. Details of the electrophoretic procedures and references for staining each enzyme system are summarized in Table 2. Due to lack of consistent activity, only 6 out of the 20 enzyme systems screened were used for the genetic diversity study. These six enzyme systems were alcohol dehydrogenase (ADH), glucosephos-

Table 1 Summary of the populations and number of samples used for isozyme analysis

Country	Population	Number of Families	Number of palms
Angola	5	1	30
	6	3	30
	7	3	30
Cameroon	22	1	30
	29	3	28
Ghana	3	1	30
	9	2	25
	13	2	32
Guinea	2	2	30
	6	3	30
Madagascar	1	6	17
	4	1	8
Nigeria	12	5	28
	35	5	25
	39	5	28
	45	5	31
Senegal	2	5	30
	8	3	13
	12	3	30
Sierra Leone	5	3	30
	13	3	28
Tanzania	1	3	28
	5	3	29
	6	3	29
The Democratic Republic of the Congo	7	3	21
	36	3	24
Deli *dura*	-	-	29

Fig. 1 The location (*filled circles*) of African oil palm populations used in this study

phate isomerase (GPI), phosphoglucomutase (PGM), malic enzyme (ME), isocitrate dehydrogenase (IDH) and uridine diphosphogluconate pyrophosphatase (UGP).

Data analysis

The genetic basis of complex polymorphic patterns (IDH and GPI) was inferred from full-sib progeny arrays. For each enzyme system, the assignment of a locus was based on the zones of isozyme activity and segregation of bands in the gels. Each locus was numbered sequentially, beginning with the most anodal migrating locus being designated as locus 1, the next fastest as locus 2 and so on (e.g. *Gpi-1*, *Gpi-2*, etc.). Likewise, within each locus, the fastest migrating band was designated as allele 1, with each successively slower band numbered as allele 2, 3, etc. Only loci that exhibited consistent activity and distinct bands were considered in the analysis.

The isozyme data were analysed using the computer programme POPGENE version 1.32 (Yeh and Boyle 1999). The genetic diversity parameters estimated were allelic frequencies, mean number of alleles per locus (A), effective number of alleles per locus (A_e), which was calculated according to Crow and Kimura (1970),

Table 2 Enzyme and electrophoresis systems

Buffer system	Electrode buffer	Gel buffer	Gel concentration	Running conditions	Enzyme system[a] and reference for staining
TEMM (adapted from Spencer et al. 1964)	0.100 *M* Tris, 0.065 *M* maleic acid, 0.01 *M* EDTA Na_2, 0.01 *M* $MgC_2l.6H_2O$, pH 7.0	1:10 dilution of electrode buffer	10% starch + 3% sucrose	65 mA, 5 h	ME (Soltis et al. 1983)
Sodium borate (Poulik 1957)	0.30 *M* Boric acid, 0.06 *M* NaOH, pH 8.2	0.076 *M* Tris, 0.005 *M* citric acid, pH 8.7	12% starch	50 mA, 3 h	ADH (Tanksley 1979) GPI (Delorenzo and Ruddle 1969) PGM (Tanksley 1979)
Tris citrate (adapted from Poulik 1957)	0.3 *M* Boric acid, 0.1 *M* NaOH, pH 8.6	0.1000 *M* Tris, 0.0069 *M* citric acid, pH 8.6	10.5% starch	60 mA, 4 h	UGP (Wickneswari and Norwati 1991)
Morpholine citrate (Clayton and Tretiak 1972)	0.04 *M* Citric acid [adjusted to pH 6.1 with *N*-(3-amino-propyl)-morpholine]	1:20 dilution of electrode buffer	10% starch + 2% sucrose	60 mA, 4 h	IDH (Fine and Costello 1963)

[a] ME, Malic acid; ADH, alchohol dehydrogenase; GPI, glucose phosphate isomerase; PGM, phosphoglucomutase; UGP, uridine diphosphogluconate phrophosphatase; IDH, isocitrate dehydrogenase

percentage of polymorphic loci (0.99 criterion) (P), expected and observed proportion of heterozygosities (H_e, H_o), genetic differentiation (F_{st}) and fixation indices (F_{is}) and their variances. Chi-square tests were also performed for each locus to test for deviation of genotypes from the Hardy-Weinberg equilibrium (HWE). The genetic distance (D) between populations was computed according to Nei (1978). These values were then used to generate a dendogram using the unweighted pair-group with arithmetic average (UPGMA) cluster analysis as described by Sneath and Sokal (1973).

Results and discussion

Allele frequencies

For the six enzyme systems investigated, namely GPI, PGM, UGP, ME, IDH and ADH, the progenies were scored for their genotypes at seven loci. *Gpi-1* and *Me* were invariant in all of the 26 populations and one Deli *dura* family of *E. guineensis*. Five loci, *Gpi-2, Ugp, Idh, Adh* and *Pgm*, were polymorphic (0.99 criterion) in at least one population. A total of 21 alleles were detected at the seven loci across the 26 populations and one Deli *dura* family surveyed. Allelic frequencies at these loci for each population examined including the Deli *dura* are given in Table 3. The chi-square analysis of genotype frequencies for each locus in all populations indicated that most loci were in HWE.

The common allele at all the polymorphic loci was not the same in the populations assayed, with the exception of *Adh*, which can be explained by drift and reproductive isolation. The common allele at three of the five polymorphic loci, *Pgm*, *Ugp* and *Idh*, in the Madagascar populations was different from that of the mainland African populations. Allele 3 at *Idh*, which was present at high frequencies in both Madagascar populations, was not detected in any of the other populations assayed, which most likely indicates hybridization and introgression with closely related palm species on the island. A phylogenetic examination of palms in Madagascar could shed some light on this possible speculation. The presence of rare allelic variants at a particular locus in one or two populations could be largely due to drift and, to a lesser extent, to adaptive genetic variants.

Genetic variability measures

The genetic variability measures within populations are presented in Table 4. The mean number of alleles per locus (A) within each population ranged from 1.43 to 2.14 (mean = 1.80), while the effective number of alleles per locus (A_e) ranged from 1.17 to 1.55 (mean = 1.35). The percentage of polymorphic loci (0.99 criterion) ranged from 28.6% to 71.4% (mean = 54.5%). The mean observed (H_o) and expected (H_e) heterozygosities across populations were 0.186 and 0.184, respectively.

Genetic diversity in the oil palm populations examined (H_e=0.184) was less than the mean values reported for common neotropical tree species (H_e=0.211, Hamrick and Murawski 1991) but higher than those reported for rare

Table 3 Allele frequencies in the 26 populations of *Elaeis guineensis* germplasm collections and one Deli *dura* family

Locus	Allele	Country/population																											
		Senegal[a]			Guinea		Sierra Leone		Ghana		Nigeria			Cameroon		Angola			The Democratic Republic of the Congo		Tanzania			Madagascar			Deli *dura*
		12 (30)	8 (13)	2 (30)	2 (30)	6 (30)	13 (28)	5 (30)	3 (30)	9 (25)	13 (32)	39 (28)	45 (31)	12 (28)	35 (25)	29 (28)	22 (30)	7 (30)	5 (30)	6 (30)	36 (24)	7 (21)	6 (29)	1 (28)	5 (29)	1 (17)	4 (8)	(29)
Gpi-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gpi-2	1	-	-	-	-	0.033	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	0.367	0.154	0.283	0.207	0.717	0.411	0.103	-	0.160	-	0.446	0.339	0.125	0.180	0.179	0.233	0.133	0.050	0.133	0.104	0.095	0.130	0.056	0.052	0.118	0.063	0.466
	3	0.633	0.846	0.717	0.069	0.083	0.518	0.552	-	0.220	0.016	0.214	0.129	0.304	0.280	0.268	0.217	0.117	0.200	0.200	0.083	0.286	0.315	0.370	0.259	0.029	-	0.448
	4	-	-	-	0.724	0.167	0.071	0.345	1.000	0.620	0.984	0.339	0.532	0.571	0.540	0.554	0.550	0.750	0.750	0.667	0.813	0.619	0.556	0.574	0.690	0.853	0.938	0.086
Pgm	1	-	-	-	-	-	-	0.100	0.133	0.020	0.172	0.518	0.258	-	0.020	0.161	0.067	0.217	0.217	0.300	0.146	0.119	0.103	0.304	0.121	-	0.063	-
	2	0.783	0.308	0.550	0.983	0.983	0.839	0.850	0.867	0.980	0.828	0.482	0.726	1.000	0.960	0.839	0.933	0.750	0.783	0.700	0.854	0.881	0.897	0.696	0.879	0.382	0.125	1.000
	3	0.217	0.692	0.450	0.017	0.017	0.161	0.050	-	-	-	-	0.016	-	0.020	-	-	0.033	-	-	-	-	-	-	-	0.618	0.813	-
Ugp	1	0.017	-	0.150	0.083	0.233	0.036	0.018	0.017	-	0.031	-	0.145	0.179	0.040	-	-	-	-	-	-	-	0.121	-	0.069	-	-	-
	2	0.983	1.000	0.850	0.917	0.767	0.964	0.982	0.983	1.000	0.969	1.000	0.855	0.804	0.920	0.929	1.000	1.000	1.000	1.000	1.000	1.000	0.793	1.000	0.931	0.353	0.125	1.000
	3	-	-	-	-	-	-	-	-	-	-	-	-	0.018	0.040	0.071	-	-	-	-	-	-	0.086	-	-	0.647	0.875	-
Me	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh	1	-	-	-	0.017	0.183	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	0.233	0.039	0.250	0.150	0.367	0.321	0.333	0.482	0.440	0.532	0.946	0.645	0.732	0.875	0.593	0.400	0.583	0.500	0.733	0.479	0.905	0.776	0.911	0.897	0.235	0.125	0.655
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.647	0.875	-
	4	-	-	-	0.017	0.117	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	0.767	0.962	0.750	0.817	0.333	0.679	0.633	0.519	0.520	0.339	0.054	0.339	0.196	0.083	0.241	0.383	0.317	0.500	0.167	0.479	0.048	0.172	0.089	0.069	0.118	-	0.345
	6	-	-	-	-	-	-	0.033	-	0.040	0.129	-	0.016	0.071	0.042	0.167	0.217	0.100	-	0.100	0.042	0.048	0.052	-	0.035	-	-	-
Adh	1	-	-	-	-	-	-	-	0.204	-	-	0.018	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	1.000	1.000	0.950	1.000	1.000	0.714	0.650	0.796	1.000	1.000	0.982	1.000	1.000	1.000	0.839	0.967	0.967	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	3	-	-	0.050	-	-	0.286	0.350	-	-	-	-	-	-	-	0.161	0.033	0.033	-	0.050	-	-	-	-	-	-	-	-	-

[a] Number not in parenthesis indicates the population; number in parenthesis indicates the sample size

Table 4 Estimates of mean number of alleles per locus (A), effective number of alleles (A_e), percentage of polymorphic loci (0.99 criterion) (*P*), observed (H_o) and expected (H_e) heterozygosities and fixation index (F_{is}) for 26 populations and one Deli *dura* family of *E. guineensis*

Country	Population	*N* [a]	A	A_e	*P*	H_o	H_e	F_{is}
Senegal	12	30	1.57	1.28	57.1	0.171 (0.206)[b]	0.171 (0.207)	0.000
	8	13	1.43	1.17	42.9	0.143 (0.237)	0.109 (0.169)	–0.312*
	2	30	1.71	1.39	71.4	0.224 (0.185)	0.232 (0.203)	0.034
Guinea	2	30	2.00	1.20	57.1	0.145 (0.194)	0.132 (0.174)	–0.098
	6	30	2.14	1.55	57.1	0.224 (0.277)	0.221 (0.286)	–0.014
Sierra Leone	13	28	1.86	1.45	71.4	0.199 (0.175)	0.249 (0.228)	0.201
	5	30	2.14	1.50	71.4	0.255 (0.238)	0.258 (0.248)	0.012
Ghana	3	30	1.57	1.26	57.1	0.175 (0.221)	0.155 (0.200)	–0.129
	9	25	1.71	1.34	42.9	0.160 (0.260)	0.159 (0.259)	–0.006
	13	32	1.71	1.27	57.1	0.150 (0.238)	0.137 (0.222)	–0.095
Nigeria	39	28	1.71	1.42	57.1	0.168 (0.242)	0.182 (0.270)	0.077
	45	31	2.00	1.47	57.1	0.281 (0.295)	0.244 (0.249)	–0.152
	12	28	1.86	1.36	42.9	0.194 (0.249)	0.187 (0.244)	–0.037
	35	25	2.14	1.29	57.1	0.167 (0.245)	0.150 (0.216)	–0.113
Cameroon	29	28	2.00	1.52	71.4	0.279 (0.280)	0.261(0.242)	–0.069
	22	30	1.86	1.50	57.1	0.271 (0.401)	0.204 (0.289)	–0.328*
Angola	7	30	2.00	1.37	57.1	0.233 (0.274)	0.201 (0.238)	–0.159
	5	30	1.57	1.31	42.9	0.229 (0.305)	0.176 (0.225)	–0.301*
	6	30	1.86	1.37	57.1	0.229 (0.261)	0.205 (0.230)	–0.117
The Democratic Republic of the Congo	36	24	1.71	1.28	42.9	0.185 (0.269)	0.159 (0.216)	–0.164
	7	21	1.71	1.23	42.9	0.150 (0.231)	0.130 (0.197)	–0.154
Tanzania	6	29	2.00	1.39	57.1	0.243 (0.270)	0.211 (0.227)	–0.152
	1	28	1.57	1.29	42.9	0.145 (0.199)	0.159 (0.227)	0.088
	5	29	1.86	1.21	57.1	0.148 (0.185)	0.141 (0.166)	–0.050
Madagascar	1	17	1.86	1.45	57.1	0.076 (0.111)	0.243 (0.241)	0.687*
	4	8	1.71	1.17	57.1	0.054 (0.098)	0.125 (0.131)	0.568*
Deli *dura*	-	29	1.43	1.31	28.6	0.123 (0.211)	0.147 (0.253)	0.163
Mean	-	-	1.80	1.35	54.5	0.186	0.184	–0.023

* Significantly different from 0.00 ($P<0.05$)
[a] *N* Number of progenies assayed
[b] Values in parentheses are the standard errors

neotropical tree species (H_e=0.142, Hamrick and Murawski 1991). The mean genetic diversity of oil palm was also lower than that reported for widespread tropical woody species (H_e=0.204, Loveless 1992), temperate coniferous species (H_e=0.207, Hamrick et al. 1981) and European angiosperm species (H_e=0.210–0.310, Müller-Starck et al. 1992). However, the H_e value was higher than that reported for monocot species (H_e=0.144; Hamrick and Godt 1989) of which oil palm is a member. The genetic diversity in oil palm was similar to that of Australian angiosperm trees (H_e=0.170; Moran 1992), specifically for eucalyptus species covering all geographic ranges (H_e=0.184; Moran 1992). This result was not surprising as these species share some similar life-history characteristics: both are pollinated by animals, have long generation times and high rates of outcrossing and are widely distributed. The genetic diversity of oil palm populations was higher (H_e=0.184) than that reported for other palm species—*Astrocaryum mexicanum* (H_e=0.153; Eguiarte et al. 1992), *Calamus manan* (H_e=0.060; Wickneswari et al. 2002) and *Ptaychosperma bleeseri* (genetically uniform among all populations, Shapcott 1998). However, the genetic diversity in the Macauba palm, *Acrocomia aculeate*, was much higher (H_e=0.375; Lopez et al. 1992) than that of oil palm in this study.

H_e was the highest in *E. guineensis* population number 29 from Cameroon (mean = 0.261) and lowest in *E. guineensis* population number 8 from Senegal (mean = 0.109). Since population 8 of Senegal may be affected by low sample size (13 samples), population 13 of Ghana can be considered to possess the lowest genetic diversity (H_e=0.137) of the populations surveyed. Generally, H_e values gradually decreased in *E. guineensis* populations found to the west and east of population 29 of Cameroon.

A previous study by Kularatne (2000) using AFLP markers also revealed that Cameroon materials showed the highest genetic diversity among all the populations assayed. However, screening of oil palm germplasm using RFLPs showed Cameroon materials to possess the second highest H_e after Nigeria germplasm (Maizura 1999). In the present study, population 45 of Nigeria also revealed high genetic diversity (H_e=0.244), but other oil palm populations from Nigeria showed only intermediate levels of genetic diversity. The occurrence of a rare allele (allele 3 at *Ugp*) in Cameroon population 29 and Nigeria populations 12 and 35, the high enzyme polymorphisms

Table 5 F_{ST} at all loci for all populations except Deli *dura* (Nei 1978)

Locus	*Gpi-1*	*Gpi-2*	*Pgm*	*Ugp*	*Me*	*Idh*	*Adh*
F_{ST}	0.000	0.267	0.306	0.430	0.000	0.320	0.205
Mean	0.303						

in Cameroon population 29 and the comparatively high genetic diversity of populations from Nigeria suggest that the zone covering Cameroon and Nigeria is likely to be the centre of diversity for *E. guineensis*. In natural plant populations, the highest diversity is expected at the centre of origin, and the diversity tends to decrease with increasing geographical distance from the centre of origin (Maxted et al. 1997). The finding of fossil pollen similar to oil palm pollen in the Miocene and later strata in the Niger delta further supports the premise that the oil palm existed in the Cameroon-Nigeria zone from very early times and that this region is most likely the centre of origin for oil palm (Zeven 1964; Rees 1965).

In addition to the Cameroon and Nigeria populations, other oil palm populations that possess high genetic diversity include those from Sierra Leone, population 6 of Guinea, population 1 of Madagascar and population 2 of Senegal. Similar observations were reported by Kularatne (2000) using AFLP markers. He found that population 6 of Guinea showed a high value for both percentage of polymorphic bands Shannon's index for phenotypic diversity.

Population 1 of Madagascar showed high genetic diversity although only 18 samples were used in this analysis. This population was considered to be unique as it did not follow the natural distribution of oil palm. Rajanaidu and Jalani (1994)reported that oil palm in Madagascar grew very poorly compared to oil palm found in other African countries and concluded that this was most likely a consequence of the poor environment and low rainfall. The high genetic diversity in oil palm populations from Sierra Leone and Madagascar were also reported by Kularatne (2000) using AFLP markers.

Oil palm populations at the extreme ends of its natural distribution demonstrated low levels of genetic diversity within populations. These include populations from Senegal, Tanzania and the Democratic Republic of the Congo. However, screening of the Democratic Republic of the Congo materials using RFLP (Maizura 1999) and AFLP (Kularatne 2000) markers revealed that the Democratic Republic of the Congo populations possessed some of the highest genetic diversity. The inconsistency of the results obtained in this study with those of previous studies could be due to differences in genomic coverage of the markers and sample size used. Populations that occur at the edges of their range are at the outer limits of gene flow, thus reducing the genetic diversity in these populations. These populations may also exhibit a smaller number of alleles due to recent bottlenecks in population size during the colonization of new habitat (Hall et al. 1994).

It was surprising that oil palm populations from Ghana, which are located in the middle of Central and West Africa, also generally showed low levels of genetic diversity within populations. Using AFLP markers, Kularatne (2000) also found that Ghana populations were less diverse than other oil palm populations in Africa. These wild oil palms were cut for the production of 'down-wine', which is an alcoholic beverage extracted from the damaged terminal cabbages of the felled palms (Hartley 1988). It has been postulated that these activities together with the clearance of many forest areas in Ghana for cocoa plantations may have reduced the present oil palm populations in Ghana, thus reducing their diversity (Hartley 1988).

The Deli *dura* family, which had undergone several cycles of selfing, revealed a low level of genetic diversity compared to other oil palm populations. The absence of alleles with intermediate frequency and rare alleles supported the narrow genetic base of Deli *dura*. Some of the alleles might have been eliminated during the selection process or were not present in the founding population.

Based on our estimations of the genetic parameters P, A_e and H_e, we conclude that, in general, the natural African oil palm populations assayed maintained high levels of genetic variation at the population level. Ghesquiere (1985) also showed high enzyme polymorphisms in African oil palm populations using pollen extracts from different geographic regions in West Africa—Ivory Coast, Benin, Nigeria, Cameroon, The Democratic Republic of the Congo, Angola—and from Deli palms from Malaysia-Indonesia.

Fixation indices

Analysis of conformance of a population to HWE can be informative in the evaluation of evolutionary processes within that population. Several factors may contribute to observed departures from HWE, including nonrandom mating, drift, selection, migration and mutation. Of these, nonrandom mating plays a major role; therefore, the mating system of a population or species can be inferred by the distribution of allelic variation into genotypes and by comparison of observed genotypic proportions with those expected at HWE. Deviations of genotype frequencies from HWE within single populations can be represented by the fixation index, F_{is}, which ranges from −1, (indicating an excess of heterozygotes) to 1 (signifying a deficiency of heterozygotes) (Soltis and Soltis 1989). In the present study F_{is} was estimated using the F-statistics of Wright (1965).

F_{is} values for most of the populations were low, with the exception of populations from Madagascar, indicating random mating in most populations and conformance to

Table 6 Estimates of mean genetic distance of Nei (1978) between 26 populations of *E. guineensis* germplasm collections and one Deli

Population identification[a]	AG5	AG6	AG7	CM22	CM29	GH3	GH9	GH13	GV12	GV16	MD1	MD4	NG12
AG5	*												
AG6	0.016	*											
AG7	0.004	0.004	*										
CM22	0.015	0.026	0.013	*									
CM29	0.017	0.008	0.009	0.008	*								
GH3	0.016	0.041	0.020	0.038	0.041	*							
GH9	0.008	0.031	0.014	0.004	0.016	0.027	*						
GH13	0.010	0.020	0.006	0.027	0.027	0.010	0.024	*					
GUI2	0.030	0.086	0.048	0.030	0.062	0.036	0.017	0.046	*				
GUI6	0.100	0.104	0.093	0.055	0.074	0.141	0.064	0.128	0.081	*			
MD1	0.207	0.218	0.196	0.226	0.216	0.215	0.225	0.190	0.232	0.289	*		
MD4	0.391	0.398	0.374	0.428	0.406	0.397	0.429	0.361	0.429	0.504	0.019	*	
NG12	0.030	0.023	0.024	0.022	0.014	0.055	0.022	0.036	0.072	0.070	0.222	0.418	*
NG35	0.038	0.016	0.025	0.030	0.014	0.066	0.033	0.044	0.099	0.079	0.233	0.430	0.004
NG39	0.080	0.031	0.057	0.090	0.055	0.134	0.101	0.102	0.181	0.112	0.293	0.477	0.075
NG45	0.018	0.012	0.011	0.020	0.015	0.048	0.022	0.032	0.057	0.050	0.210	0.393	0.020
SN2	0.128	0.164	0.151	0.109	0.128	0.205	0.107	0.205	0.122	0.120	0.300	0.479	0.141
SN8	0.196	0.258	0.233	0.191	0.223	0.284	0.184	0.290	0.184	0.236	0.344	0.499	0.256
SN12	0.097	0.135	0.119	0.070	0.096	0.166	0.068	0.168	0.083	0.079	0.327	0.544	0.111
SL5	0.053	0.080	0.068	0.043	0.042	0.087	0.040	0.098	0.063	0.110	0.311	0.533	0.073
SL13	0.091	0.117	0.105	0.060	0.070	0.143	0.061	0.153	0.081	0.069	0.337	0.568	0.097
TZ1	0.034	0.006	0.023	0.048	0.021	0.071	0.051	0.046	0.126	0.126	0.246	0.432	0.025
TZ5	0.031	0.009	0.018	0.037	0.016	0.053	0.038	0.030	0.104	0.110	0.227	0.415	0.008
TZ6	0.028	0.014	0.020	0.025	0.010	0.057	0.026	0.036	0.082	0.076	0.208	0.391	0.001
DRC7	0.034	0.008	0.019	0.035	0.014	0.060	0.038	0.036	0.109	0.105	0.240	0.434	0.011
DRC36	0.001	0.020	0.003	0.012	0.019	0.010	0.006	0.005	0.023	0.092	0.201	0.386	0.030
DDR	0.070	0.064	0.068	0.037	0.040	0.127	0.042	0.114	0.095	0.037	0.320	0.561	0.040

[a] AG, Angola; CM, Cameroon; GH, Ghana; GUI Guinea; MD, Madagascar; NG, Nigeria; SN, Senegal; SL, Sierra Leone; TZ Tanzania;

HWE (Table 4). The mean F_{is} values for all populations ranged from –0.328 (Cameroon population 22) to 0.687 (Madagascar population 1). The F_{is} values of populations 1 and 4 of Madagascar were highly positive (0.687 and 0.568, respectively) and significantly different from zero ($P<0.05$), indicating a significant excess of homozygotes, probably due to inbreeding having occurred. The populations of Madagascar Island, which are isolated from the populations of the mainland, could have experienced limited gene flow resulting from restricted pollen and seed dispersal. This might have promoted inbreeding, which may have resulted in the reduced fitness observed in these populations. These high positive values of F_{is} also may be due to the rather small sample size—17 and 8, respectively. The Deli *dura* family showed heterozygote deficiency within population ($F_{is}=0.163$). However, the F_{is} value was not significantly different from zero ($P<0.05$). Zheng and Ennos (1999) reported that the deficiency of heterozygotes in the exotic plantations of Caribbean pine (*Pinus caribaea* Morelet) was substantially higher than that observed in their natural counterparts. They suggested that this may be due to increased levels of self-fertilization in the exotic location where difficulties with flowering may restrict the supply of outcross pollen. The populations with a negative F_{is} value—Senegal 8, Cameroon 22 and Angola 5—showed a significant deviation from HWE ($P<0.05$). An excess of heterozygotes in populations may result from the impact of selection, which generally favours heterozygous individuals. The negative values of F_{is} for most populations also showed that generally high levels of outcrossing prevailed, whereas the positive values in some of the populations indicated high levels of inbreeding.

Genetic differentiation among populations

The overall degree of genetic differentiation among oil palm populations was 0.301 (Table 5), indicating high genetic divergence among populations. Significant differences in F_{ST} were observed from locus to locus, with values ranging from 0.207 (*Adh*) to 0.430 (*Ugp*). The high F_{ST} values at *Pgm, Ugp* and *Idh* show that these loci can be used to discriminate *E. guineensis* populations from different geographic regions.

The degree of among-population differentiation in oil palm ($F_{ST}=0.301$) was higher than the G_{ST} values reported in both wind-pollinated conifers (G_{ST}=less than 0.100, Hamrick and Godt 1989) and insect-pollinated eucalypts ($G_{ST}=0.174$, Moran 1992). It was much higher than that reported in biotically dispersed species of tropical trees ($G_{ST}=0.050$, Loveless 1992) and even higher than that of the abiotically dispersed species ($G_{ST}=0.138$, Loveless 1992). However, it was more comparable to the average value of G_{ST} for all categories of plants with animal-mediated seed dispersal, including those that are ingested or that are animal-attached ($G_{ST}=0.223$–0.257, Hamrick and Godt 1989). It was of interest to note that genetic differentiation in oil palm populations was more similar to that of *Acacia* species ($G_{ST}=0.311$ for *A. mangium*,

dura family

NG365	NG39	NG45	SN2	SN8	SN12	SL5	SL13	TZ1	TZ%	TZ6	DRC7	DRC36	DDR
*													
0.052	*												
0.023	0.034	*											
0.160	0.184	0.122	*										
0.277	0.289	0.220	0.020	*									
0.126	0.160	0.095	0.012	0.050	*								
0.088	0.143	0.074	0.063	0.120	0.044	*							
0.108	0.146	0.084	0.033	0.091	0.015	0.017	*						
0.014	0.027	0.025	0.165	0.261	0.141	0.093	0.130	*					
0.003	0.051	0.024	0.186	0.300	0.156	0.098	0.137	0.007	*				
0.002	0.054	0.015	0.141	0.252	0.114	0.076	0.101	0.014	0.004	*			
0.002	0.044	0.025	0.178	0.289	0.144	0.094	0.127	0.005	0.000	0.006	*		
0.038	0.091	0.019	0.149	0.225	0.111	0.064	0.101	0.044	0.033	0.030	0.036	*	
0.036	0.071	0.042	0.079	0.175	0.042	0.058	0.036	0.062	0.063	0.040	0.052	0.076	*

DRC. The Democratic Republic of the Congo; DDR, Deli *dura.* Number indicates the population

Moran 1992; G_{ST}=0.270 for *A. auriculiformis*, Wickneswari and Norwati 1993). This could be due to the similarities of oil palm and *Acacia* species, both of which are insect-pollinated and distributed across large geographic ranges.

F_{ST} analysis based on geographic zones revealed a high genetic differentiation (F_{ST}=0.256) among oil palm populations from West Africa (covering Senegal, Guinea, Sierra Leone, Ghana, Nigeria and Cameroon), whereas populations from the Central Africa zone (The Democratic Republic of the Congo, Angola and Tanzania) and East Africa zone (Madagascar) showed a low genetic differentiation among populations—F_{ST} of 0.073 and 0.055, respectively. However, when populations from West Africa were divided into two zones—populations from Senegal, Guinea and Sierra Leone were separated from the populations of Ghana, Nigeria and Cameroon—those from the more western zone showed a higher genetic differentiation among populations (F_{ST}=0.217) than those from the more eastern zone (F_{ST}=0.135), indicating diversifying selection in this region. Genetic differentiation among oil palm populations decreased along an eastwardly line towards East Africa, indicating that the populations from Central and East Africa are more similar to each other than with the populations from West Africa. This could be explained by gene flow and balanced selective pressure. The differences in F_{ST} values among the three zones showed that total genetic differences among oil palm populations in Africa were mainly due to differences among the three geographic zones and that these could be due to restricted gene flow and ecotypic selection.

Genetic differentiation among populations is principally a function of gene flow among populations via pollen and seeds dispersal (Loveless and Hamrick 1984). Species with discrete or isolated populations experience less gene flow than species with more continuously distributed or contiguous populations and therefore have a relatively lower level of variation within populations and a higher variation among populations. The high levels of population differentiation are supported by low levels of gene migration among populations.

In the present study, the total gene flow across all oil palm populations was low with a Nm of 0.576. Migration rates of more than one migrant per generation can be considered to be high and are thought to be sufficient to prevent population differentiation due to drift (Wright 1931). Nevertheless, the estimate of Nm among oil palm populations was lower than the threshold value of 1.0, which is considered to be insufficient for preventing significant divergence caused by genetic drift. According to Hardon (1974), genetic drift rather than natural selection was responsible for the distribution of variability within and among the oil palm populations in the African rainforest.

Geographic distance and Nm, calculated for pairs of populations, are inversely related (Hall et al. 1994). This indicates that the amount of genetic differentiation existing among populations increases with geographic distance. The high genetic differentiation between oil

palm populations could be mainly due to regional differences as the populations used in this study covered a very large geographic area from the wet west to the dry east of the African continent including populations from the island of Madagascar. The high genetic divergence among the populations may also be due to restricted pollen and seed dispersal. The oil palm is mainly pollinated by insects, and in Africa this role is performed by the weevil, *E. kamerunicus* (Hartley 1988). The weevils are weaker fliers than bees or other insect pollinators, which may result in limited pollen movement and greater population differentiation.

Generally, oil palm seeds are dispersed by animals, humans and water (Hartley 1988). Although the movement of oil palm seeds by gravity and water must be limited because oil palm fruit does not float on water, oil palm seeds could be transported on floating materials. As mentioned earlier, primary populations of oil palm grow in isolated places in its original natural habitat, along river banks, lakes and freshwater swampy areas that are interconnected by watercourses (Zeven 1967). The movement and change in the river courses over time would aid the migration of oil palm seeds to adjacent and comparably distant populations. In the oil palm natural populations of Africa, certain animals are considered to be responsible for the dissemination of seeds (some mammals, rodents and a few birds of tropical Africa, Hartley 1988). It is probable that these animals move over short ranges in the African forest, thus restricting seed dispersal and reduced gene flow, thereby increasing the population differentiation. A previous study on oil palm germplasm collections using AFLP also showed a high genetic differentiation among populations, 45% (Kularatne 2000).

Genetic relatedness

The mean genetic distances D (Nei 1978) between populations are summarized in Table 6. The mean D value was 0.113. The lowest genetic distance was observed between population 5 from Tanzania and population 7 from the Democratic Republic of the Congo (0.000), and the highest between population 4 from Madagascar and population 13 from Sierra Leone (0.568). This result is not surprising as Tanzania and the Democratic Republic of the Congo are neighbouring countries, whereas Madagascar and Sierra Leone are very distant ones. While the overall results show a strong association between genetic distance and geographic location, the former could not always be correlated with geographic location as observed in some of the populations from central Africa. This observation most probably implies that oil palm populations from central Africa may have undergone extensive exploitation and were introduced by man to many other places.

Figure 2 shows the dendogram of genetic relatedness among the 26 populations and one Deli *dura* family assayed. Three main clusters can be discerned. The western outlying populations from Senegal and Sierra Leone are in one cluster but can be separated into two distinct sub-clusters—the eastern outlying populations from Madagascar are in one sub-cluster and populations from Angola, Cameroon, The Democratic Republic of the Congo, Ghana, Tanzania, Nigeria and Guinea are in the second large sub-cluster. The Deli *dura* family seems to be closely related to population 6 from Guinea.

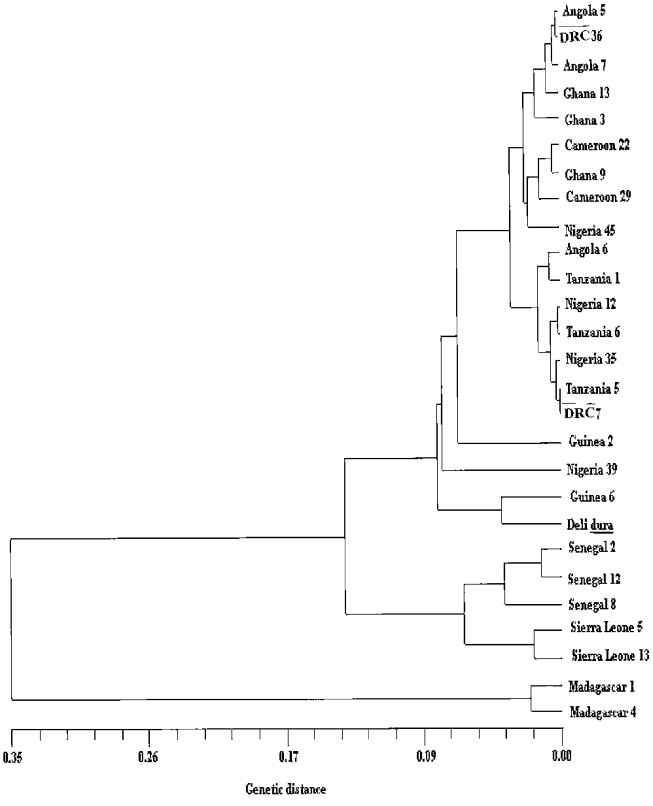

Fig. 2 A dendogram based on UPGMA clustering of *Elaeis guineensis* populations using the genetic distance of Nei (1978). *DRC* The Democratic Republic of the Congo

Cluster analysis clearly separated the Madagascar populations from the rest of the populations from other African countries, indicating that oil palms from Madagascar are unique and different from those of the mainland. The Madagascar palms were also different with respect to morpho-physiological traits in that they have a short trunk and exhibit a slow growth rate (Hartley 1988). Based on these unique characteristics, there have been attempts to classify the oil palm occurring in Madagascar as a different species (Purseglove 1975; Hartley 1988).

The dendogram also shows that populations from the same country tended to group together. This pattern was shown for populations from Sierra Leone (populations 5 and 13), Senegal (populations 2, 8 and 12) and Madagascar (populations 1 and 4). However, for countries such as Angola, Cameroon, Ghana, Guinea, Nigeria, Tanzania and The Democratic Republic of the Congo, this pattern did not hold. For these countries, populations from the same country were not grouped in the same cluster, but

there was a mixture of populations from different countries so that the populations from the same country cannot be differentiated from other countries. Populations from Angola, Cameroon, Tanzania, the Democratic Republic of the Congo and Nigeria were grouped together in the same cluster. This result seems to be similar to that reported by Shah et al. (1994), who used RAPD markers to assay populations from Cameroon, Tanzania, the Democratic Republic of the Congo and Nigeria. A similar study in which microsatellite core sequences were used as primers (Shah and Lim (1996) also showed that the populations from Cameroon and Tanzania are closely related to each other.

In conclusion, the high genetic diversity observed in oil palm natural populations, specifically in the germplasm collections maintained by MPOB, indicates these materials are sufficient-to-good sources of new genes for introgression into the current breeding materials for oil palm and palm oil improvement. In general, all of the populations from Nigeria, Cameroon and Sierra Leone, population 6 of Guinea, population 1 of Madagascar and population 2 of Senegal possess high genetic diversities and should be exploited for oil palm improvement. For conservation purposes, oil palm populations with a high allelic diversity (A_e), which include populations 22 and 29 from Cameroon, populations 39 and 45 from Nigeria, population 6 from Guinea, populations 5 and 13 from Sierra Leone and population 1 from Madagascar may be selected for capturing as much genetic variation as possible. Since the genetic differentiation among oil palm natural populations is high, sampling a few trees from many populations would be effective enough in capturing its natural genetic variation in future collections for conservation.

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