

# GENETIC VARIATION AMONG OIL PALM PARENT GENOTYPES AND THEIR PROGENIES BASED ON MICROSATELLITE MARKERS

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## ABSTRACT

Knowledge on genetic distances and relationships among breeding materials has a significant impact on crop improvement. Molecular markers are being used increasingly to determine the genetic distance between individuals. In this study, microsatellite markers were used to estimate the genetic distances between selected oil palm parent lines. Microsatellite markers are highly reliable, inherited in codominant fashion whereby heterozygotes and homozygotes are distinguishable, easy to score and can be rapidly produced using PCR technology. Nine microsatellite markers were used to screen selected parent palms (15 duras and 4 pisiferas) and their progenies (16 DxP crosses). Data were scored and analysed using the Biosys-1 software to calculate the genetic distance values. A total of 29 polymorphic bands were generated. The genetic distances between progenies ranged from 0.089 to 0.313. These results indicate that microsatellite markers are powerful tools for studying genetic relationships among DxP progenies. These markers should be further explored to assist oil palm breeding.

**Keywords:** oil palm, microsatellite markers, genetic distance.

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## INTRODUCTION

*Elaeis guineensis* Jacq., which is the name given to oil palm by Jacquin in 1763, originated from the tropical rain forest region of West Africa. As it is a perennial crop, a long period of time is needed for the selection of the best parents which give potentially high yields. The theoretical potential yield for oil palm has been estimated at 17 t oil ha<sup>-1</sup> yr<sup>-1</sup> (Corley, 1983). Under favourable environments, oil palm yields achieve about 6 t oil ha<sup>-1</sup> yr<sup>-1</sup> which is considerably higher than the yields of other oil crops. By the year 2020, Malaysia is expected to attain a production level of 18.81 million tonnes of oil per year (Jalani *et al.*, 2002).

In oil palm, selection is based on phenotypic characters; thus, the breeding programme ends up with many crosses and many progeny palms. Being a perennial crop, the oil palm breeding cycle is very long, that is 10 to 15 years. As only 148 palms can be planted per hectare, oil palm progeny trials require large areas and high costs in maintenance. Also, because economically important traits are controlled by many genes, a long period of time is required to produce planting materials with the combination of traits of interest.

Genetic distance is a measure of the dissimilarity of genetic material between different species, or between individuals of the same species. There are many factors that influence the amount and pattern of genetic variation between populations. Some of these factors include isolation, genetic drift and types of selection pressure imposed on the populations. Microsatellites are also known as Simple Sequence Repeats (SSRs). They have short tandem repeats of DNA sequences (2-6 bp) and are highly polymorphic due to the variation in the number of repeat units. They are inherited in codominant fashion and are highly heritable. Besides, they are easy to score and can be produced rapidly using PCR technology.

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Many studies have been reported on the use of microsatellite markers. The microsatellites are widely used for DNA fingerprinting, linkage map construction and population genetic studies (Bindu *et al.*, 2004). Billotte *et al.* (2005) in collaboration with MPOB have identified a total of 390 microsatellites in oil palm.

The objectives of this study were to optimize the protocols for DNA extraction, PCR reaction and PCR amplification, to select microsatellite primers which show polymorphism, and to estimate the genetic distance between parent palms and between their progenies.

**MATERIALS AND METHODS**

**Materials**

Nineteen parent palms (15 Serdang *duras* and 4 AVROS *pisiferas*) and their progenies (16 DxP) were used in this study (Table 1). Young leaves of selected parent palms were sampled at the MPOB Research Station, Kluang, Johor, while samples from the 16 DxP progenies were collected from the MPOB Research Station Keratong, Pahang. Twenty palms were randomly selected per progeny. The total

number of DxP palms included in the study was 339. Samples were cut into small pieces, packed in plastic bags, labelled and stored in liquid nitrogen during transportation to MPOB Head Office. These samples were stored at -80°C until needed.

**Methods**

**DNA extraction.** Twenty palms were randomly selected per progeny. The total number of DxP palms included in the study was 339. DNA extraction was done using the GeneTACG (Maxi) Kit provided by Amersham Bioscience. One gram of leaf sample was ground in liquid nitrogen to a fine powder and transferred into a sterile 15-ml falcon tube. Four millilitres of GPB1 Buffer and 40 µl of RNase solution (100 mg ml<sup>-1</sup>) were added to the powdered tissue and vigorously vortexed. The mixture was incubated at 65°C for 10 min. The samples were inverted two to three times during the incubation. After that, 1.3 ml of GPB2 Buffer were added to the lysate, vortexed and incubated on ice for 5 min. The lysate was applied to a shearing tube sitting in a collection tube, and centrifuged at 3000 rpm for 2 min. The solution harvested in the collection tube was transferred to another 15-ml falcon tube. Then, 0.5 volume of GPB3 Buffer and one volume of absolute ethanol were

TABLE 1. NUMBERS OF PALMS SAMPLED FOR EACH PROGENY CODE AND THEIR PEDIGREE INFORMATION

Progeny	Progeny code	No. of sampled palms	Parents				Grandparents			
			(A x B)		(C x D)		A		B	
			<i>Dura</i>	<i>Dura</i> code	<i>Pisifera</i>	<i>Pisifera</i> code	<i>Dura</i> parents	<i>Dura</i> parents	<i>Pisifera</i> parents	<i>Pisifera</i> parents
PK1179	DP1	19	0.212/ 41	D1	0.174/498	P1	0.82/2360	0.82/2360	0.79/577	0.79/45
PK1269	DP2	20	0.212/515	D2	0.174/498	P1	0.85/4338	0.85/4338	0.79/577	0.79/45
PK1291	DP3	20	0.212/ 70	D3	0.174/663	P2	0.82/2054	0.102/8544	0.79/575	0.79/213
PK1379	DP4	20	0.212/369	D4	0.174/663	P2	0.102/8453	0.3/55	0.79/575	0.79/213
PK1380	DP5	20	0.212/424	D5	0.174/663	P2	0.102/8453	0.3/55	0.79/575	0.79/213
PK1381	DP6	20	0.212/482	D6	0.174/663	P2	0.85/4338	0.85/4338	0.79/575	0.79/213
PK1384	DP7	19	0.212/272	D7	0.174/348	P3	0.102/8453	0.3/55	0.79/22	0.79/213
PK1387	DP8	20	0.212/648	D8	0.174/348	P3	0.102/8428	0.102/8539	0.79/22	0.79/213
PK1388	DP9	20	0.212/515	D2	0.174/348	P3	0.85/4338	0.85/4338	0.79/22	0.79/213
PK1389	DP10	20	0.212/265	D9	0.174/247	P4	0.102/8453	0.3/55	0.79/21	0.79/32.7
PK1396	DP11	20	0.212/268	D10	0.174/247	P4	0.102/8453	0.3/55	0.79/21	0.79/32.7
PK1397	DP12	19	0.212/ 26	D11	0.174/247	P4	0.102/8453	0.3/703	0.79/21	0.79/32.7
PK1399	DP13	19	0.212/645	D12	0.174/498	P1	0.102/8428	0.102/8539	0.79/577	0.79/45
PK1400	DP14	17	0.212/438	D13	0.174/498	P1	0.82/2360	0.82/2360	0.79/577	0.79/45
PK1401	DP15	20	0.212/134	D14	0.174/498	P1	0.85/4264	0.85/4264	0.79/577	0.79/45
PK1403	DP16	16	0.212/481	D15	0.174/498	P1	0.85/4338	0.85/4338	0.79/577	0.79/45

added to the clear lysate and inverted three to five times. Five millilitres of the mixture were applied to a column that was placed in a collection tube. The tubes were centrifuged at 3000 rpm and the filtrate was discarded. The column was washed twice with 5 ml of W2B Buffer by centrifuging at 3000 rpm for 3 min. The column was then centrifuged again to remove any leftover W2B buffer. Two millilitres of 65°C TE were added to the column and centrifuged at 3000 rpm for 5 min to elute the DNA. The DNA was then stored at -20°C prior to analysis.

**Digestibility test and determination of oil palm DNA concentration.** A digestibility test of the DNA was carried out using two restriction enzymes, *EcoR*I (6 base cutter) and *Hae*III (4 base cutter). Both digested and undigested DNA were loaded into 0.8% agarose gel and electrophoresed at 100V in 1X TAE buffer. The gel was stained in ethidium bromide and viewed under UV light. The image was captured using a Polaroid camera.

DNA concentration was determined using a spectrophotometer. Optical density (OD) readings were obtained at wavelengths 260 and 280 nm. DNA concentration was calculated from the OD reading at 260 nm while DNA purity was calculated by the ratio of the readings obtained at 260 and 280 nm. The ratio of good DNA quality ranged from 1.8 to 2.2.

**PCR amplification.** The PCR reaction mixture contained primer mix ( $T_4$  polynucleotide kinase,  $\gamma^{33}P$ -ATP, microsatellite primers and kinase buffer),  $5 U \mu l^{-1}$  Taq DNA polymerase, 10 mM dNTPs, 50 mM  $MgCl_2$ , 10X PCR buffer and 1  $\mu l$  of template DNA. For microsatellite analysis, the DNA template was diluted to a final concentration of  $50 ng \mu l^{-1}$ . The PCR was performed in a Perkin Elmer 9600 thermocycler essentially as described by Billotte *et al.* (2001). The PCR amplification was carried out as follows: denaturation at 95°C for 30 s, annealing at 52°C-65°C (depending on the primer used) for 30 s, and extension at 72°C for 30 s. These steps were repeated for 35 cycles.

**Polyacrylamide gel electrophoresis (PAGE).** A mixture of the final PCR product, bromophenol blue and xylene cyanol were denatured at 90°C. Bromophenol blue and xylene cyanol were used to show migration because they give colour to the PCR product. The samples were electrophoresed in 6% PAGE at 1600V for 2-3 hr. The gels were then vacuum-dried for 1 hr and exposed against X-ray film (Kodak) for three to four days at -80°C, depending on the radioactive signal on the gel.

**Data analysis.** Data were scored and analysed using the Biosys-1 software to calculate the percentage of polymorphic loci, mean number of alleles per locus

and estimates of observed and expected heterozygosities in the progenies studied. The genetic distance values were computed according to Rogers (1972). These values were then used to generate a dendrogram using the unweighted pair-group with arithmetic average (UPGMA) cluster analysis as described by Sneath and Sokal (1973).

## RESULTS AND DISCUSSION

### DNA Extraction

The GeneTACG (Maxi) Kit used for DNA extraction yielded 2 ml of DNA. The DNA purity or the ratio of A260/A280 of the DNA samples showed acceptable protein and polysaccharide contaminations ranged between 1.50 and 2.30. The DNA concentration obtained was in the range between 0.05 and  $2.82 ng \mu l^{-1}$ . As the final DNA concentration was quite low, the DNA solution was further concentrated through ethanol precipitation.

### Microsatellite Primers

The choice of primers used for screening was based on their ability to generate polymorphic bands. A total of nine microsatellite primers with annealing temperatures ranging 52°C to 54°C were selected (Table 2). Figure 1 shows the banding patterns produced by the nine primers. Generally, two to six bands were scored per primer.

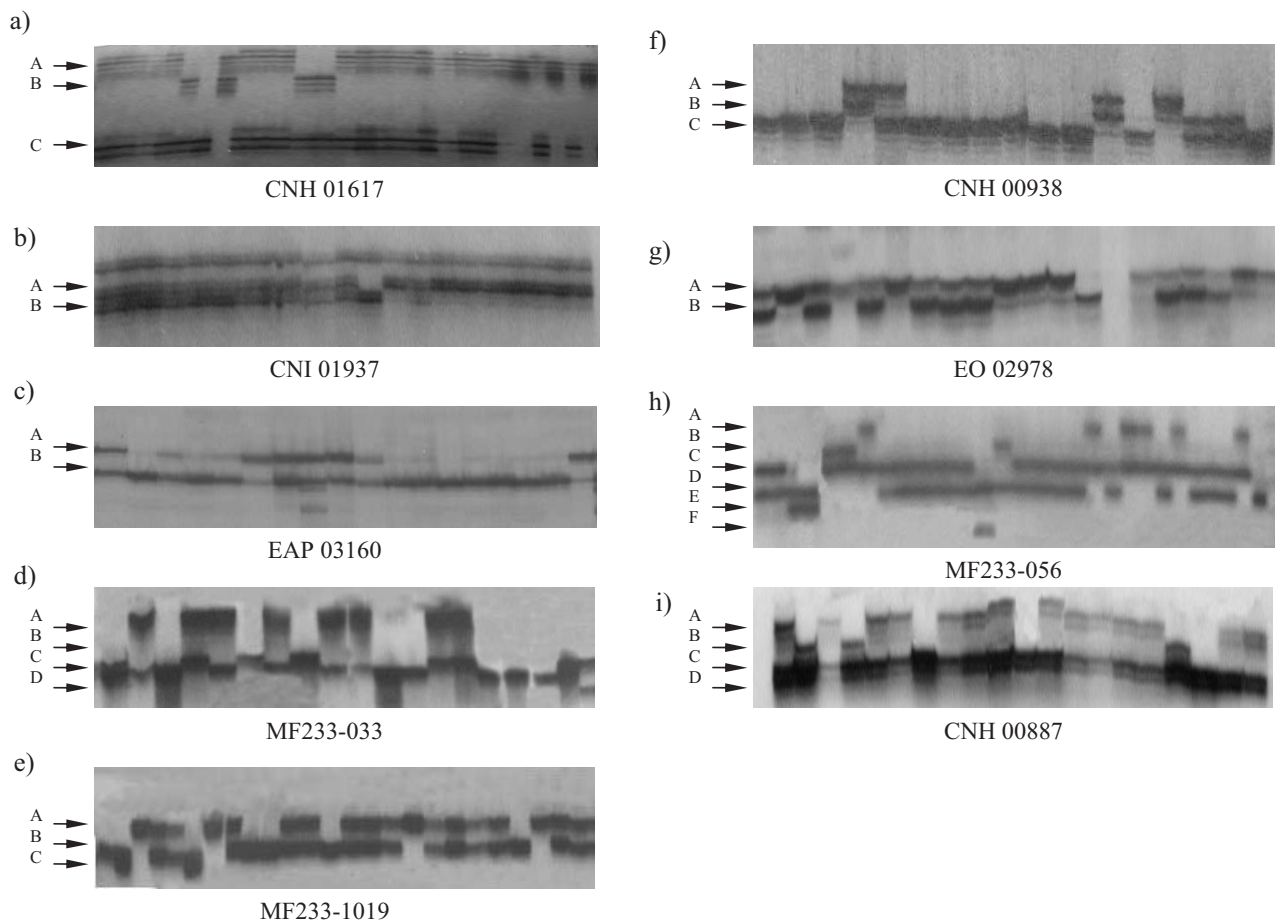
### Microsatellite Polymorphisms

The genetic variability measures for all the DxP progenies included in the study are presented in Table 3. The mean number of alleles per locus within each population ranged from 1.9 to 2.6, with a mean of 2.3. These values were high compared to those obtained from isozyme (mean = 1.6; Hayati *et al.*, 2004) and RFLP (mean = 1.8; Maizura *et al.*, 2006) analyses. However, the values were lower than those reported for other monocot species such as coconut (mean = 4.83; Konan *et al.*, 2006). The percentage of polymorphic loci at 0.95 criterion ranged from 88.9% to 100% (mean = 94.5%). Milbourne *et al.* (1998) found that microsatellites are the better method consistently detected the highest levels of polymorphism in barley (100%) and potato (90.8%).

The mean observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities across populations were 0.621 and 0.455, respectively, implying that the progenies are highly heterozygous. The expected heterozygosity values ranged from 0.387 (DP8) to 0.498 (DP6 and DP1). Meanwhile, mean observed heterozygosity was lowest for DP3 (0.512) while the highest was for DP11 (0.722). The  $H_e$  values obtained in these populations were high compared to other monocot

TABLE 2. LIST OF THE PRIMERS USED IN THE MICROSATELLITE ANALYSES

No.	Primer	5' sequence	3' sequence	Allele size (bp)	Repeat unit
1	CNH00887	TTATTGATTGATGCAAGATACAC	TTGATAAAATACAAGAGATAGCA	165	(AT)9
2	CNH00938	GGACCCTTTTTGTTACTGTTT	AGCCTACCACAACCTTCCTTT	172	(AG)9
3	CNH01617	TCTTTAATTTGTGCGAGGATAATG	ATGCAAGGTTTTGTTGAAACT	130	(CT)20
4	CNI01937	AACTGCAAATGAGACACAGAG	TCCACCAGAGGAGGGTTAGT	170	(AG)9
5	EAP 03160	AACGTGAGAGCCATAGAGATAG	TAATAGAAACTAGACCCGACCA	175	(TATG)6
6	EO 02978	CCGTCTCAAAGCCCTAAAC	TTGTTGTCCCACTCCCTCTT	210	(CGC)7
7	MF233033	GAGGAGGAGGGGAGAAGAGT	AAATACCATTGAGAGAAAGCAC	200	(TC)11
8	MF233056	CCGAATAGAAGAGGAAAGAATA	AGGTTTGGTGGAGAAGTGTT	232	(CT)15
9	MF2331019	TGGGTAAATTGTAATTCTCCT	CCTTTTTCTTCCTCTTTTCCA	195	(AAA)9



Notes: A to F refer to allele type. A= Allele A, B= Allele B, C= Allele C, D= Allele D, E= Allele E, F= Allele F. Allele size for each primer is as shown in Table 2.

Figure 1. Autoradiogram obtained from nine different microsatellite primers used in the study.

species such as banana ( $H_e = 0.411$ , Oriero *et al.*, 2006) and *Antirhea aromatica* ( $H_e = 0.185$ , Gonzalez-Astorga and Castillo-Campos, 2004). The high values of observed heterozygosity in the oil palm populations screened in this study might have resulted from the impact of selection which generally favours

heterozygous genotypes. From this study, it seems that the selection pressure applied did not cause inbreeding among the progenies. The average number of alleles per locus for each population based on allele frequencies was also calculated, and was found to differ slightly between populations; for



TABLE 3. GENETIC VARIABILITY MEASURES OF THE 16 DXP PROGENIES IN THE STUDY

Progeny	Mean sample size per locus	Mean No. of alleles per locus	Percentage of polymorphic loci*	Mean heterozygosity	
				Observed (H <sub>o</sub> )	Expected (H <sub>e</sub> )
1. DP1	18.8	2.4	100.0	0.686	0.498
2. DP2	20.0	2.4	100.0	0.656	0.492
3. DP3	19.7	2.4	100.0	0.512	0.414
4. DP4	19.0	2.1	88.9	0.556	0.433
5. DP5	20.0	2.1	88.9	0.544	0.389
6. DP6	20.0	2.3	100.0	0.683	0.498
7. DP7	18.3	2.1	100.0	0.588	0.427
8. DP8	20.0	1.9	88.9	0.556	0.387
9. DP9	20.0	2.1	88.9	0.628	0.435
10. DP10	18.8	2.4	88.9	0.605	0.491
11. DP11	18.0	2.3	88.9	0.722	0.482
12. DP12	19.7	2.6	100.0	0.671	0.484
13. DP13	18.4	2.2	88.9	0.607	0.469
14. DP14	18.8	2.4	100.0	0.634	0.491
15. DP15	19.3	2.4	100.0	0.596	0.417
16. DP16	15.4	2.2	88.9	0.685	0.471
Mean	19.0	2.3	94.5	0.621	0.455

Notes: \*A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

example, DP12 showed 2.6 alleles per locus while DP8 exhibited 1.9 alleles per locus. The results obtained suggest that in spite of extensive selection imposed on the *dura* and *pisifera* parent palms, the DXP progenies showed considerably high levels of genetic variation.

#### Genetic Distance among 19 Parental Palms

Genetic distance values (Rogers, 1972) for *dura* parent palms ranged from 0.050 to 0.573 (Table 4). The lowest genetic distance corresponded to D9 and D10 with a value of 0.050, while the highest genetic distance was between D2 and D14. This means that parent D2 and D14 are 57.3% different in terms of the portion of the genome surveyed by the nine microsatellite markers.

Cluster analysis resulted in a clear separation of the *dura* and *pisifera* parent palms (Figure 2). Within the *dura* cluster, D5 and D7 formed a sub-cluster, probably because they are full-sibs as indicated in Table 1. For the same reason, another two sub-clusters were observed. Palms D9 and D10 were in one sub-cluster, and in the other were Palms D9, D10, D5, D7 and D4, all of which originated from MS 3338 – because the palms in each sub-cluster belong to common parental lines (Table 1).

AVROS *pisifera* performance is good and recognized as being beneficial especially for

increasing oil yield (Lim *et al.*, 2003). These highly bred populations are most extensively used as a source of pollen for DXP production. In general, the *pisiferas* showed genetic distance value of between 0.300 and 0.400. From the dendrogram (Figure 2), all *pisifera* palms formed a single cluster. It can be concluded that these palms were inter-related due to the narrow genetic base of the source population.

#### Genetic Distance among the Progenies

The genetic distances between the 16 DXP progenies are presented in Table 5. The estimates were variable, ranging from 0.089 (between DP10 and DP11) to 0.313 (between DP8 and DP16). Based on pedigree information, DP10 and DP11 share common male and female parent palms (Table 1).

The results of the cluster analysis in the form of a dendrogram are presented in Figure 3. Four clusters were formed. Two clusters were single population clusters (DP4 and DP6) which were isolated from the other populations. It is assumed that the *dura* used to generate DP4 and DP6 had stronger maternal effects over the male parental palms.

The first and biggest cluster can be divided into three sub-clusters. Sub-cluster 1 contained DP2 and DP16 which were related due to a common parent, P1. Sub-cluster 2 consisted of DP12, DP13 and DP14; however, only DP13 and DP14 shared a common

TABLE 4. GENETIC DISTANCES OF THE 19 PARENTAL PALMS IN THE STUDY

Progeny	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
1 D2	*****																				
2 D4	0.371	*****																			
3 D3	0.250	0.400	*****																		
4 D5	0.221	0.250	0.200	*****																	
5 D11	0.250	0.400	0.437	0.387	*****																
6 D8	0.321	0.250	0.300	0.250	0.287	*****															
7 D13	0.337	0.400	0.187	0.337	0.487	0.400	*****														
8 D6	0.300	0.437	0.400	0.450	0.400	0.450	0.423	*****													
9 D14	0.573	0.437	0.500	0.500	0.550	0.450	0.537	0.400	*****												
10 D1	0.350	0.300	0.300	0.321	0.521	0.450	0.250	0.478	0.473	*****											
11 D12	0.437	0.407	0.521	0.350	0.450	0.371	0.487	0.521	0.323	0.421	*****										
12 D7	0.237	0.350	0.300	0.150	0.387	0.350	0.437	0.400	0.450	0.423	0.473	*****									
13 D9	0.300	0.350	0.300	0.300	0.350	0.200	0.373	0.387	0.300	0.387	0.307	0.300	*****								
14 D10	0.250	0.350	0.250	0.250	0.300	0.250	0.323	0.437	0.350	0.337	0.357	0.250	0.050	*****							
15 D15	0.250	0.437	0.300	0.337	0.487	0.337	0.387	0.337	0.437	0.437	0.460	0.350	0.200	0.250	*****						
16 P1	0.444	0.387	0.528	0.437	0.494	0.423	0.507	0.515	0.628	0.557	0.537	0.473	0.494	0.544	0.473	*****					
17 P3	0.687	0.473	0.587	0.587	0.737	0.587	0.550	0.660	0.687	0.473	0.637	0.737	0.637	0.687	0.600	0.400	*****				
18 P2	0.573	0.460	0.687	0.587	0.587	0.587	0.650	0.507	0.473	0.523	0.387	0.623	0.623	0.673	0.623	0.400	0.400	*****			
19 P4	0.710	0.481	0.644	0.607	0.746	0.596	0.607	0.651	0.565	0.494	0.494	0.744	0.633	0.683	0.623	0.350	0.300	0.300	*****		

TABLE 5. GENETIC DISTANCES OF THE 16 DxP PROGENIES

PROGENY	DP1	DP2	DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP10	DP11	DP12	DP13	DP14	DP15	DP16
DP1	****															
DP2	0.179	****														
DP3	0.307	0.300	****													
DP4	0.265	0.284	0.295	****												
DP5	0.292	0.295	0.209	0.189	****											
DP6	0.163	0.193	0.280	0.139	0.196	****										
DP7	0.265	0.228	0.244	0.174	0.132	0.197	****									
DP8	0.105	0.208	0.243	0.191	0.209	0.107	0.210	****								
DP9	0.235	0.264	0.234	0.218	0.234	0.203	0.252	0.203	****							
DP10	0.267	0.261	0.234	0.135	0.111	0.163	0.120	0.192	0.192	****						
DP11	0.189	0.214	0.235	0.203	0.197	0.176	0.161	0.168	0.228	0.137	****					
DP12	0.157	0.242	0.265	0.193	0.185	0.091	0.226	0.090	0.227	0.188	0.196	****				
DP13	0.163	0.178	0.296	0.258	0.223	0.193	0.214	0.199	0.307	0.205	0.173	0.209	****			
DP14	0.198	0.229	0.247	0.209	0.174	0.114	0.147	0.137	0.226	0.144	0.177	0.126	0.210	****		
DP15	0.165	0.230	0.271	0.253	0.193	0.142	0.200	0.147	0.247	0.173	0.178	0.161	0.186	0.089	****	
DP16	0.100	0.212	0.299	0.278	0.307	0.182	0.313	0.137	0.221	0.276	0.235	0.161	0.205	0.221	0.175	****

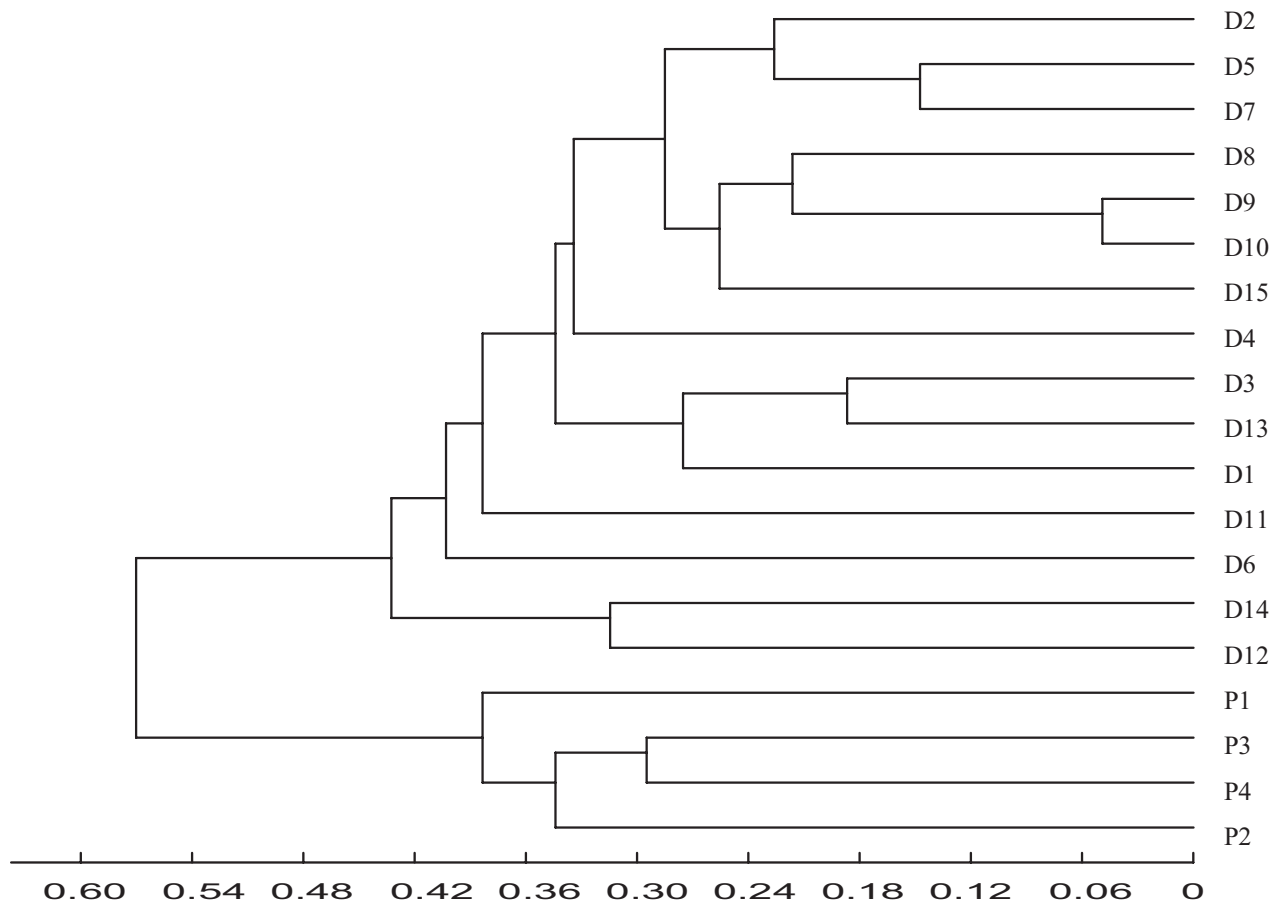


Figure 2. Dendrogram revealed by the UPGMA cluster analysis for 19 parental palms based on Rogers's (1972) genetic distance.

male parent palm which was P1. Meanwhile, in sub-cluster 3, DP9 and DP7 were grouped together as a result of a common male parent, P3. The second cluster consisted of DP3, DP5, DP15, DP8 and DP1 as its components. These DPs were inter-related because some share the same male and female grandparents. Moreover, DP3 and DP5 also had the same *pisifera* parent.

**CONCLUSION**

The microsatellite markers employed in this study provided insight into the genetic relationships between the D x P progenies and between the parent palms. The high level of variability shown in the progenies can be used to advantage in oil palm improvement, and should be further explored towards Marker-Assisted Selection (MAS) in the oil palm breeding programme.

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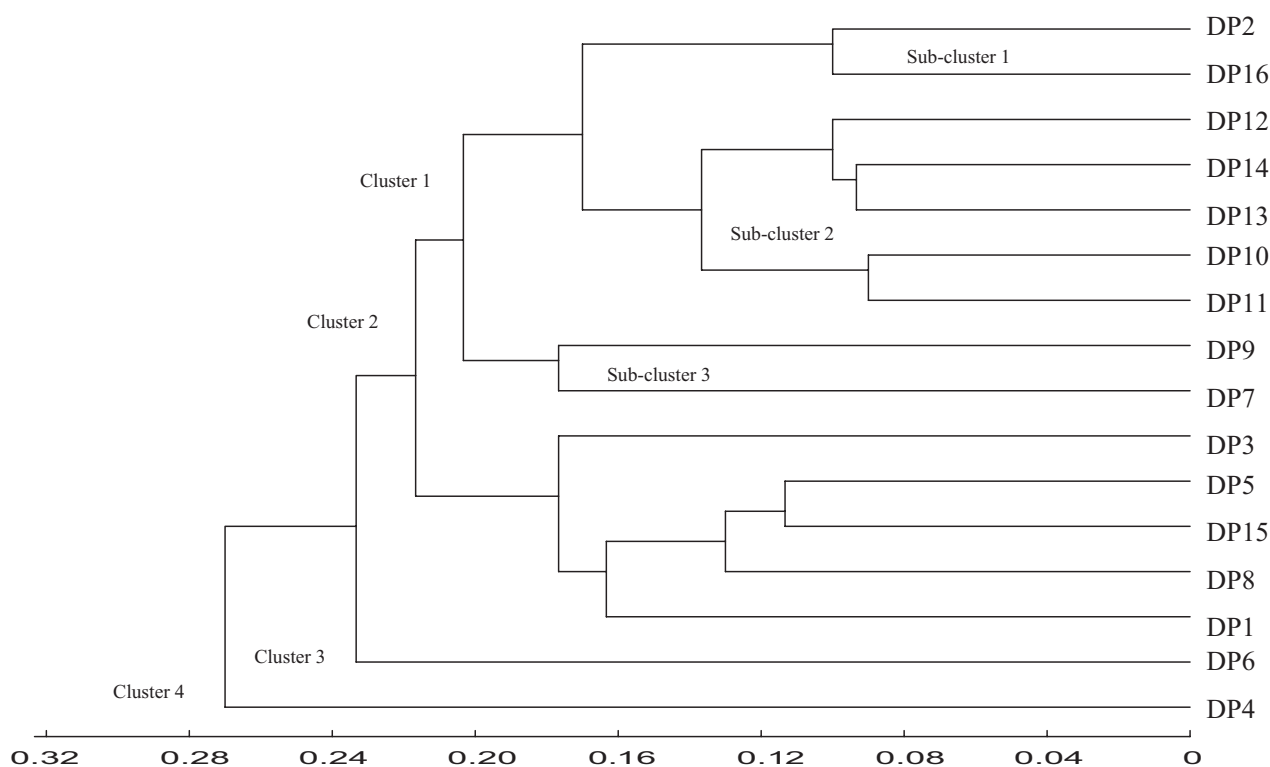


Figure 3. Dendrogram revealed by the UPGMA cluster analysis for 16 DXP progenies based on Roger's (1972) genetic distance.

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