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Preliminary Analysis of Quantitative Trait Loci Associated with Oil Quality in an Interspecific Cross of Oil Palm

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ABSTRACT

Amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) analysis are rapid and efficient techniques for detecting large numbers of DNA markers in oil palm. Both AFLP and RFLP markers were used to generate a genetic linkage map for oil palm. A map was constructed from AFLP and RFLP analysis of the progeny derived from an interspecifc cross involving a Colombian Elaeis oleifera *(UP1026) and a Nigerian* Elaeis guineensis *(T128). This interspecific cross was analysed to map genes associated with oil quality. For this cross, over 4,000 loci were detected using 53 AFLP primer pairs, and 60 informative cDNA probes as RFLP markers. Of these, 435 loci were informative and segregating in a 1:1 ratio, corresponding to DNA polymorphism heterozygous in one parent and null in the other. The results also showed that the male parent (Palm T128, a Nigerian E. guineensis) was more heterozygous than the female parent (Palm UP1026, Colombian* E. oleifera*) in the interspecific cross analysed. A framework map was generated for the male parent, T128, using JoinMap™ ver. 2.0. In the paternal* E. guineensis *map, 297 markers were ordered in 20 linkage groups (866 cM). The* E. guineensis *map was also used in scanning for quantitative trait loci (QTLs) controlling oil quality (measured in terms of carotene content and iodine value). QTLs associated with carotene content and iodine value (IV) were detected.*

INTRODUCTION

The oil palm is a perennial crop which belongs to the genus *Elaeis* and the family Palmae. The crop was originally domesticated in Africa and is now extensively cultivated in Asia and Latin America as well. Within the genus *Elaeis*, two species are distinguished, which are, the economically important oil palm, *Elaeis guineensis*, native to Africa and a South American relative, *Elaeis oleifera*. Fortunately, the *E. guineensis* and *E. oleifera* hybridize readily, producing fertile offspring in spite of their difference in origin.

Oil palm is one of the most important oil bearing crops, being by far the highest oil yielder per unit of planting area. The past 30 years have seen a rapid increase in the production of palm oil in the world, a greater than 7-fold increase from about 3 million tonnes in 1970 to over 23 million tonnes in 2001. Despite the progress, additional gains in agricultural productivity are needed at an ever faster pace due to competition from other vegetable oils and fats. Although traditional breeding continues to play an important role in yield enhancement, it is, however, impeded by the long selection cycle (10-12 years) (Oboh and Fakorede, 1989) and the enormous resources (land, labour and field management) required for oil palm breeding programmes. The ability to select early (at the nursery stage, perhaps) will thus have a great

impact in reducing the time and resources required for varietal improvement in oil palm. This makes marker-assisted selection (MAS) a very attractive proposition as it has the potential to reduce the time to develop new improved varieties.

The burgeoning field of molecular marker technology has provided tools for rapid gathering of genetic information about higher organisms, including agricultural species. In recent years, molecular markers have played a pivotal role in plant research, such as in studying evolutionary relationships among related species (Bennetzen and Freeling, 1993), the cloning of genes (Kazan *et al.*, 1993) and as diagnostic tools.

Perhaps the most widespread application of DNA markers has been in the construction of genetic maps, which can be used to determine the chromosomal locations of genes affecting either simple or complex traits (Paterson *et al.*, 1988). Genetic maps are useful in fundamental genetic research and tree improvement activities that include population management and marker-assisted breeding and selection. Molecular markers associated with selected traits of interest on a genetic map can be assayed in any tissue at any stage of development. This makes them ideal as selection markers in breeding programmes. The application of such DNA based diagnostics has been termed as molecular breeding (Leemans, 1993). The main advantage of this method over the conventional breeding process is its potential for reducing the time required for varietal development (Mazur and Tingey, 1995). The availability of probes of interest will allow breeders to select at the nursery stage thereby reducing the cost and time scale of breeding programmes.

Isozymes, restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers have been applied to oil palm to investigate genetic diversity (Shah *et al.*, 1994), to fingerprint clones (Mayes *et al.*, 1996; Cheah *et al.*, 1996) and in attempts at identifying markers for somaclonal variation (Cheah and Wooi, 1993; Rival *et al.*, 1998). A number of these marker systems have also been applied to genetic mapping in oil palm. RFLP had been applied to oil palm linkage mapping (Mayes *et al.*, 1997; Cheah *et al.*, 1999). The map reported by Mayes *et al.* (1997) harbors 97 RFLP markers in 24 groups of two or more markers and was generated by using the progeny of a selfed *E.guineensis* cross. Recently, Moretzsohn *et al.* (2000) reported genetic linkage mapping for a single controlled cross of oil palm using RAPD markers and the pseudo-testcross mapping strategy. The status of genetic mapping in oil palm, however, is still considered preliminary at this stage. Furthermore, genetic linkage mapping of oil palm is currently carried out only in a limited number of laboratories. Considerable effort is required to generate dense maps and to resolve the number of linkage groups to 16, which is the haploid chromosome number of oil palm (Maria *et al.*, 1995). The ability to generate dense maps and to incorporate molecular marker technologies into existing breeding programmes can significantly accelerate many breeding and selection endeavors in oil palm.

In this study, the techniques of amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) were applied for construction of a genetic linkage map in oil palm. The map was constructed using progeny palms derived from an interspecific *E. oleifera* x *E. guineensis* cross of oil palm. The AFLP technique which was described by Vos *et al.* (1995) is known to be an efficient PCR based method for the identification of a large number of molecular markers. It has found widespread application in genetic mapping. The technique has been used to generate a large number of markers in the construction of linkage maps in potato (van Eck *et al.*, 1995) and rice (Nandi *et al.*, 1997). The technique is also useful for mapping quantitative traits (Powell *et al.*, 1997). Furthermore, AFLP markers linked to specific traits can be adapted to large scale, locus specific applications (Bradeen and Simon, 1998) and converted into diagnostic tools. In the oil palm, AFLP markers were found to be largely inherited in a Mendelian manner, indicating their suitability for the genetic mapping in this plant (Rajinder *et al.*, 1998).

Co-dominant RFLP markers are unequalled for many applications and have been successfully used in the construction of linkage maps for many plant species including oil palm (Mayes *et al.*, 1997; Cheah *et al.*, 1999). RFLP is often used to place complementary DNAs (cDNAs) on genetic linkage maps. The value of RFLP markers in this study lies in the fact that cDNA clones with known gene identities are used and these markers, being robust, will serve well as landmarks for the eventual integration of

available maps to form an oil palm consensus map. The main objective of this study was to construct dense genetic maps of the oil palm using both AFLP and RFLP markers for locating QTLs associated with oil quality.

MATERIALS AND METHODS

Plant Materials and Preparation of Genomic DNA

A mapping family of 77 F_1 palms derived from the cross UP1026 (*E. oleifera*) x T128 (*E. guineensis*) was analyzed. The palms were planted and evaluated at United Plantations, Teluk Intan, Perak. The female parent, UP1026, is a Colombian *E. oleifera* and the male parent, T128, is a Nigerian *E. guineensis* which produces oil with high iodine value (IV). DNA was prepared from young spear leaves by the method of Doyle and Doyle (1990).

AFLP Procedure

The AFLP assay was carried out by using the GIBCO BRL AFLP Analysis System 1 essentially as described in the manufacturer's manual, with some minor modifications. 350 ng of genomic DNA was digested with 3.2 µl of *Eco*RI and *Mse*I (1.25 units/µl each) at 37°C for 4 hours. After heat inactivation of the enzymes at 70°C, the fragments were ligated to the *Eco*RI and *Mse*I adapters in the presence of T4 DNA ligase at 20°C for 3 hours. A preselective amplification was then carried out by amplifying a 10-fold dilution of the ligation mixture.

For selective amplification, a selected *Eco*RI primer (with three selective nucleotides) was labeled with γ-^{33}P-ATP using T4 polynucleotide kinase. The labeled *Eco*RI primer was mixed with a selected *Mse*I primer (three selective nucleotides containing dNTPs) at a ratio of 1:9 to form a primer master mix. The PCR reaction mixture contained 5µl of a 30 fold diluted pre-amplified DNA, 5µl of primer master mix, 0.5 unit of *Taq* DNA polymerase, 2µl of a 10x PCR buffer in a final volume of 20 µl. PCR conditions as recommended by the manufacturer were adopted for use with the Perkin Elmer 9600 thermocycler.

Aliquots of the post PCR mixture were heated with an equal volume of formamide dye [98% (v/v)], 10 mM EDTA, 0.2% (w/v) bromophenol blue, 0.2% (w/v) xylene cyanol) at 90°C for 3 min. A 5 µl sample was electrophoresed in a 6% (w/v) polyacrylamide sequencing gel with 7.5M urea. The gel was dried and exposed to an X-ray film (Kodak XK-1) at -80°C for 2-3 days.

Southern Hybridization

DNA samples (20µg) were digested with restriction enzymes as recommended by the manufacturer. Initially a sample of 10 palms was each digested with 14 restriction enzymes (*Bam*HI, *Bcl*I, *Bgl*II, *Dra*I, *Eco*RI, *Hinc*II, *Hind*III, *Sca*I, *Sst*I, *Xba*I, *Bst*NI, *Hae*III, *Rsa*I and *Taq*I). The restricted DNA fragments were separated by electrophoresis in 0.9% agarose gel in 1xTPE (90mM tris-phosphate buffer, 2mM EDTA pH 8.0) buffer and then transferred onto nylon membranes (Hybond N+, GE Healthcare) by vaccum blotting using 0.4M sodium hydroxide (NaOH) as the transfer buffer.

The set of 140 samples were then hybridized in turn with each candidate probe to identify the probe/restriction enzyme combination that gave a segregation profile. In the case of more than one enzyme showing polymorphism with a particular probe, the probe/enzyme combination that gave a single/low copy clear co-dominant profile was selected for screening the entire mapping family.

DNA probes were labeled with ^{32}P-dCTP (deoxycytidine 5'-[γ-^{32}P] triphosphate) by the method of Feinberg and Vogelstein (1984). *Hind*III and *Hind*III/*Eco*RI digested lambda phage DNA served as molecular weight markers for the estimation of the sizes of the hybridized fragments. Pre-hybridization and hybridization were carried out in glass tubes in a rotisserie oven at 65°C. Membranes were pre-hybridized for 3 hours in a pre-hybridization buffer as follows: 5 X SSPE solution (3M NaCl, 0.2M sodium phosphate, 20mM EDTA pH 8.0), 0.5% SDS, 5X Denhardt's solution (0.1% ficoll, 0.1% polyvinylpyrolidone, 0.1% albumin bovine fraction V) and 100µg/ml denatured herring sperm DNA. The pre-hybridization buffer was removed and replaced with hybridization buffer containing 5 X SSPE solution (3M NaCl, 0.2M sodium phosphate, 20mM EDTA pH 8.0), 0.5% SDS, and 100µg/ml denatured herring sperm DNA. Labeled probes were denatured by heating in a boiling water bath for 10 minutes and plunging into ice before addition to hybridization buffer. The probe was added to a concentration of about 1-3 x 10^6 cpm/ml. Hybridization was carried out overnight at 65°C. Hybridized membranes were washed twice in 2 X SSC (0.3M

NaCl, 30mM trisodium citrate pH 7.0) and 0.1% SDS at 65°C for 15 minutes each time, followed by a single wash in 1 X SSC (0.15M NaCl, 15mM trisodium citrate pH 7.0) and 0.1% SDS at 65°C for 10 minutes. The membranes were then autoradiographed at -80°C using X-ray films with intensifying screens for 7-10 days.

RFLP Probes

The RFLP probes used in this study were cDNA clones obtained from various cDNA libraries (young etiolated seedlings, mesocarp, kernel and root) constructed previously as described by Cheah (1996). cDNA clones from a subtracted flower library (Cheah and Rajinder, 1999) were also used to screen the mapping family.

The cDNA clones were picked at random from the various cDNA libraries. Plasmid DNA was prepared from individual clones by using the QIAGEN tip-20 plasmid prep kit (Qiagen, USA). The presence of the DNA insert was examined by restriction digestion (*Eco*RI) followed by electrophoresis on a 1.5% agarose gel. cDNA clones with insert sizes larger than 500 base-pairs (bp) were selected to screen for their abilities to detect RFLP in the mapping family. Probes for mapping were derived from the selected plasmids as polymerase chain reaction (PCR) amplified DNA fragments. Plasmids of selected probes were maintained as frozen glycerol stocks at -80°C.

Data Analysis

For AFLP and RFLP markers, segregation in the interspecific mapping family was scored on the basis of presence or absence of the band. The parental origin of the markers was also recorded. Two separate data sets were obtained, one for each parent. The pseudo-testcross strategy (Grattapaglia and Sederoff, 1994) was used for analysis of the segregation data. In the pseudo-testcross configuration, each parent is, in turn, considered the heterozygous individual. Segregation is expected to be in the 1:1 ratio for bands present in the heterozygous parent (*Aa*) but absent in the homozygous parent (*aa*). A χ^2 test ($P<0.05$) was performed to test the null hypothesis of 1:1 segregation on all the scored segregating bands. Table 1 illustrates the types of Mendelian segregation of the AFLP and RFLP patterns obtained in the offsprings of the interspecific cross scored as pseudo-testcross.

Map Construction

Map construction was carried out using the JoinMap™ version 2.0 computer programme (Stam and van Ooijen, 1995). The interspecific cross was analysed as a family resulting from a cross between two heterozygous diploid parents. The family type code "CP" was used in the analysis.

Linkage groups were identified by stepwise lowering of the LOD score from 7 to 2. LOD scores of 2 and 3 were found to give unlikely groupings. The LOD score of 4.0 was the lowest stringency at which acceptable linkages were performed. A ripple was performed after the addition of every three markers and map distances were calculated using the Kosambi map function. JoinMap™ contructs maps in three cycles. In the first two cycles, markers which exceeded the JUMP threshold are excluded. In the third cycle the markers excluded are inserted,

TABLE 1
Segregation of AFLP and RFLP markers in the progeny of the UP1026 (*E. oleifera*) x T128 (*E. guineensis*) interspecific cross

Parental genotypes	Expected Mendelian ratio	AFLP band pattern on autoradiogram								Remarks
		Parents		Offspring						
Aa x Aa	3:1 (F_2)	O	G	1	2	3	4	5	6	*E. oleifera* and *E. guineensis* parent heterozygous
		–		–	–		–		–	
Aa x aa	1:1 (testcross)	O	G	1	2	3	4	5	6	*E. oleifera* parent heterozygous
		–		–			–		–	
aa x Aa	1:1 (testcross)	O	G	1	2	3	4	5	6	*E. guineensis* parent heterozygous
			–	–	–			–		

with no restriction on the JUMP threshold. In this study, the ordering produced in the second cycle was used for map construction.

Quantitative Data Analysis

Oil was extracted from ripened bunches for use in subsequent analysis. The criteria used to determine ripened bunches were as described by Corley and Tinker (2003), one loose fruit per bunch (irrespective of palm height). A total of three bunches per palm were used for oil extraction and as such three independent readings of oil quality were determined per palm. The following traits were then evaluated: (i) carotene content and (ii) iodine value (IV). The carotene content and IV are quantitative traits and they represent measures of oil quality. QTL mapping analysis was performed using interval mapping implemented by MapQTL version 3.0 (van Ooijen and Maliepaard, 1996). Genomic wide significant threshold levels to declare a significant QTL was determined as described by van Ooijen (1999).

RESULTS AND DISCUSSION

Analysis of the E. oleifera *(UP1026) x E. guineensis (T128) Mapping Family*

(1) AFLP Analysis

The *E. guineensis* parent (Palm T128) and *E. oleifera* parent (Palm UP1026) and a sample of F_1 individuals were tested against a large number of combinations of *Eco*RI primers and *Mse*I primers (both with three selective nucleotides). This was done in order to identify primer pairs which amplified bands that (i) were segregating in the mapping family and (ii) provided easy to read AFLP profiles. Each band was considered to represent a single locus of a dominant marker. On this basis, the presence of a fragment in a parent indicated that the parent was either homozygous dominant or heterozygous for that locus.

In the AFLP assay, a total of 26 AFLP primer pairs were used to screen the two parents and a small subset of the mapping family. The AFLP banding patterns obtained for one of these primer pairs is shown in *Fig. 1*. The number of bands observed in the population ranged from 13 to 99 (Table 2). A lower number of bands were usually obtained with primers which had CG dinucleotides. Primers with a high AT content usually gave higher numbers of bands. This indicates low frequency of CG dinucleotides in interspecific hybrids of oil palm, similar to that reported for other plant genomes (Moore *et al.*, 1993). *Fig. 1* shows how the AFLP markers are easily scored as segregating alleles by the presence or absence of an amplified DNA band among the progeny. All 26 of the AFLP primer pairs analysed revealed scorable polymorphisms, thus illustrating the efficiency of AFLPs for analysis of the mapping family. Since all the AFLP primer pairs tested were suitable for detecting segregation in the interspecific cross, they were all used for screening the entire mapping family. The data obtained also provided confidence in

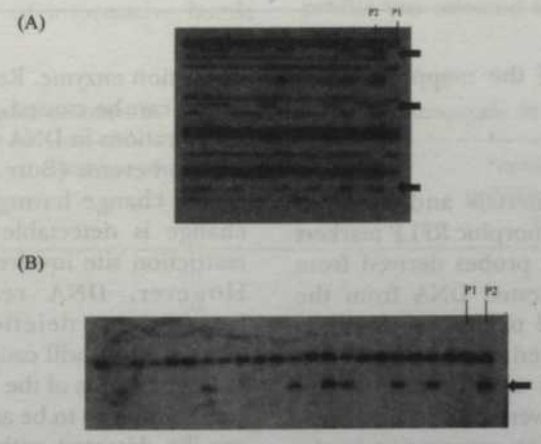

Fig. 1: Autoradiogram showing the segregating loci revealed by (A) AFLP markers and (B) RFLP markers. P1 is the female parent (E. oleifera), *P2 is the male parent* (E. guineensis) *and the rest are the F_1 progeny. Arrows indicate segregating markers*

TABLE 2
Segregation of AFLP markers in the pseudo-testcross revealed by a subset of 26 primer pairs used in analysis of the *E. oleifera* x *E. guineensis* progeny

Primer combination	Total no. of amplified bands	Segregation according to model		Total no. of segregating bands	No. of markers fitting goodness of fit to 1:1 ratio (P<0.05)
		Aa x aa (UP1026 heterozygous)	aa x Aa (T128 heterozygous)		
EACT/MCTA	57	-	11	11	9
EACT/MCAC	46	-	6	6	6
EACT/MCAA	99	-	11	11	8
EACT/MCTT	70	2	6	8	6
EACT/MCAT	75	2	11	13	9
EACT/MCTC	54	4	9	13	9
EACT/MCTG	45	-	4	4	4
EACT/MCAG	61	2	5	7	6
EACA/MCTC	52	1	7	8	7
EACA/MCAT	67	1	8	9	9
EACA/MCAA	86	4	8	13	10
EACC/MCTC	49	1	4	5	4
EACC/MCAC	34	-	2	2	1
EACC/MCAT	57	1	6	7	7
EACC/MCAA	67	1	9	11	8
EAAG/MCTG	87	-	12	12	10
EAAG/MCAG	54	-	8	8	6
EAAG/MCTC	78	2	4	6	4
EAAG/MCTA	78	5	15	20	11
EAAG/MCAC	70	-	8	8	8
EACG/MCAC	13	-	1	1	1
EACG/MCTC	34	-	1	1	1
EACG/MCAA	31	2	2	4	2
EAGG/MCAA	79	2	4	6	4
EAGG/MCAC	37	-	4	4	3
EAGC/MCAT	63	1	2	3	3
Total	1543	30	172	202	160
Mean	59.3	1.2	6.6	7.8	6.2

extending the analysis of the mapping family using RFLP markers.

(ii) RFLP Analysis

As described in the Materials and Methods section, screening for polymorphic RFLP markers was performed by testing probes derived from cDNA clones on the digested DNA from the parents and 10 individual palms. Parental and progeny DNA were digested with 14 restriction enzymes. A total of 288 cDNA probes from various cDNA libraries were tested for copy number and ability to detect segregation in the progeny. The results are tabulated in Table 3.

Of the 288 clones screened, 85, or 29.5%, showed polymorphisms with at least one restriction enzyme. Restriction polymorphism in plants can be caused either by base substitution or alterations in DNA structure due to insertion/deletion events (Burr *et al.*, 1983). In the case of a base change having occurred at a locus, this change is detectable with the enzyme whose restriction site involves the base that is altered. However, DNA rearrangements such as insertions, deletions, inversions and translocations will cause the spatial arrangement of large regions of the genome containing several restriction sites to be altered. As such, this change can be detected with several enzymes. In this study, the majority of the probes used were also found to be polymorphic with more than one restriction enzyme, suggesting that polymorphisms

resulted more often from insertion/deletion events compared to individual nucleotide substitution.

(iii) Segregation Analyses of AFLP and RFLP Markers in the Mapping Family

The segregation of AFLP markers was examined with 76 F_1 plants derived from the cross of *E. oleifera* x *E. guineensis*. Although there are 77 F_1 plants from this cross, a previous study (Rajinder and Cheah, 1999) had shown that one of the progeny palms is a contaminant. This palm was thus excluded from the analyses.

The inheritance of the AFLP markers in the progeny was examined using the pseudo-testcross mapping strategy described by Grattapaglia and Sederoff (1994). In this strategy, it is assumed that in a cross between two individuals, markers which are heterozygous in one parent and null in the other will segregate in the ratio 1:1 in their F_1 progeny following a testcross configuration (Aa x *aa* or *aa* x *Aa*). The term "pseudo-testcross" is used because the testcross mating configuration of the markers is only inferred after analyzing for segregation of the markers in the offsprings. Either the male or the female parent can be fixed as the heterozygous individual contributing the segregating bands. The term "one-way" or "two-way" pseudo-testcross is used when one or both parents involved in the analysis are heterozygous, respectively.

A total of 3,792 AFLP loci were scored in the progeny using 53 AFLP primer pairs. 363 of these AFLP loci turned out to be informative and were segregating in the population progeny. On average, the number of informative bands per primer pair was 7. The number of segregating bands obtained was also found to be significantly correlated with the total number of bands obtained (r = 0.70). This confirms our earlier results (Rajinder and Cheah, 1999) that an increase in pattern complexity of the total amplified DNA bands corresponds to a significant increase in informative markers for mapping.

Chi-square analysis was performed for each of the segregating bands scored to determine if segregation deviated from the expected 1:1 ratio. At a significance level of $P<0.05$, 309 of these markers (about 85%) segregated in the expected ratio. Approximately 15% of the segregating bands displayed skewed segregation ratios. This shows that a high number of AFLP markers in the oil palm is stably inherited from parents to offsprings following the rules of Mendelian inheritance. Generally, all of the segregating markers scored were in the pseudo-testcross configuration, and either the male parent was heterozygous and the fragment was absent in the female parent (*aa* x *Aa*) or vice versa (*Aa* x *aa*). Surprisingly, the F_2 type of segregation pattern, where bands were common to both parents at the same locus and segregating in the 3:1 ratio in the progeny (*Aa* x *Aa*), were not detected.

Of the 85 RFLP probes showing polymorphisms with at least one restriction enzyme, 60 were selected for screening the entire mapping family. In cases where more than one restriction enzyme showed polymorphisms with a particular probe, the probe/restriction enzyme combination that showed clear, single copy profile was selected to screen the family.

TABLE 3
Polymorphisms detected by cDNA probes in the interspecific hybrid mapping family

cDNA library (source of probes)	Number of probes screened	Number of probes showing polymorphism with at least one restriction enzyme
Kernel	20	10
Mesocarp	40	15
Germinated seeds	60	20
Flower	120	25
Subtracted flower	20	5
Root	7	2
Others*	21	8
Total	288	85

* Probes obtained from research collaborators within/outside MPOB

The 60 probes screened detected 72 segregating RFLP loci (Table 4). All the RFLP loci were scored using the pseudo-testcross strategy as described above for AFLP markers. The male or female parent was fixed as the heterozygous individual contributing to the segregating band. At a significance level of $P<0.05$, 68 of the 72 RFLP loci examined (94%), followed the expected 1:1 segregation ratio.

In this study, the majority of the segregating bands were found to be inherited from the male parent (palm T128, *E. guineensis*). Of the 435 (363 AFLP and 72 RFLP) segregating markers identified as segregating in the 1:1 ratio, 356 (290 AFLP and 66 RFLP) (82%) were heterozygous in the male parent and only 79 (18%) in the female parent, the Colombian *E. oleifera*. This confirmed that the male parent is more heterozygous than the female parent, *E. oleifera*. It is therefore concluded that it would be more appropriate to analyse this cross as a "one-way pseudo-testcross' in which the male, *E. guineensis* is considered to be the heterozygous parent and the Colombian *E. oleifera* the homozygous parent.

As for the female parent, a different set of AFLP enzyme combination (e.g. *PstI/MseI*) or a different marker system (e.g. RAPD) may have to be used to scan different regions of the genome to generate sufficient markers which are informative for mapping. The low level of heterozygosity detected in the Colombian *E. oleifera* could be explained by the fact that *E. oleifera* is found in scattered areas in the South American country (Rajanaidu, 1986). This could have encouraged inbreeding, resulting in a relatively high homozygous genome.

(iv) Linkage Analysis

Linkage analysis was carried out using JoinMap™ version 2.0. As explained in the previous section, there were sufficient markers generated to develop a genetic map for the male *E. guineensis* parent, T128, while insufficient markers were available to generate a map for the *E. oleifera* female parent.

A total of 290 AFLP markers and 66 RFLP markers were used to generate a linkage map for the male T128 parent at a LOD score of 5.0. A detailed description of the genetic map is in progress with a graphical representation of the map. Essentially the genetic map consists of 20 linkage groups containing at least 3 markers each. The total genetic distance covered by these markers was about 1,434 cM and the average distance between markers was about 5 cM. Generally, 297 of the markers analysed (about 83%) by the software could be linked to at least 2 other markers indicating good genome coverage. At a LOD score of 5.0, the RFLP and AFLP markers were found to be well distributed over all the 20 linkage groups.

The genetic map described above represent the relative order of genetic markers, and their relative distances from one another, along each chromosome of the oil palm. The oil palm has a haploid chromosome number of 16 (Maria *et al.*, 1995). As such more markers will definitely be required to resolve the map further to the basic chromosome number of 16.

(v) Quantitative Traits

A major objective in this study was to map QTLs associated with oil quality and yield in oil palm. The oil quality parameters analysed were carotene content and iodine value (IV). Oil that is high in carotene is desirable as there is a growing demand for natural sources of carotene for food colouring (Corley and Stratford, 1998). Iodine value, on the other hand, is a measure of the un-saturation of fats and oils. In order to allow for both edible and non-food usability of the oil, increasing the proportion of unsaturated fatty acids (particularly oleic acid) is desirable. Markers for these traits are important as they are potentially useful as early selection tools for oil palm breeding.

The two parents of the original cross could not be evaluated for these traits (carotene content and IV). Therefore, the trait values of the F_1 progeny cannot be compared to the parental generation. Interspecific hybrids typically display intermediate behaviour of these traits. However, since one of the individuals crossed is highly heterozygous, the F_1 is expected to be heterozygous, and a significant level of genetic variation is also expected to exist in the progeny. The existence of this genetic variation was explored in the QTL mapping experiment. Extreme phenotypes with trait values greater than and less than the standard deviation from the mean were observed for the carotene content and IV. The frequency distribution for the carotene content and IV did not differ significantly from normality assessed by Shapiro-Wilk statistics calculated by using PROC

UNIVARIATE (SAS, 1988). The mean, standard deviation and sample size for the carotene content and IV are shown in *Figs. 2* and *3* respectively. Sample sizes for the analysis of oil quality were less than the total number of individuals genotyped. This is because some of the individuals of the cross have yet to bear fruits, and, as such, oil analysis could not be carried out. The correlation estimated among the two traits were, however, not significant at χ=0.05 (r = 0.42).

(vi) QTL Analysis

QTL analysis was performed using interval mapping implemented by MapQTL version 3.0 (van Ooijen and Malieepard, 1996). In this study, for the interspecific cross, a linkage map was generated for the male parent, T128, only. The linkage map for the T128 male parent was constructed with about 300 markers in 20 linkage groups. This marker density was considered suitable for use in QTL loci detection.

For carotene content, a total of two putative QTLs were detected in two different linkage groups (2 and 15). However, a genomic region was declared significant only when the genomic wide empirical threshold level calculated at the P<0.05 significance level was above 3.1. The threshold level was calculated according to van Ooijen (1999). Only the QTL detected at linkage group 2 met this criteria. *Fig. 4* shows linkage group 2 with the significant QTL for carotene content. Generally, the genetic region controlling the QTL is located within the locus EAGG/

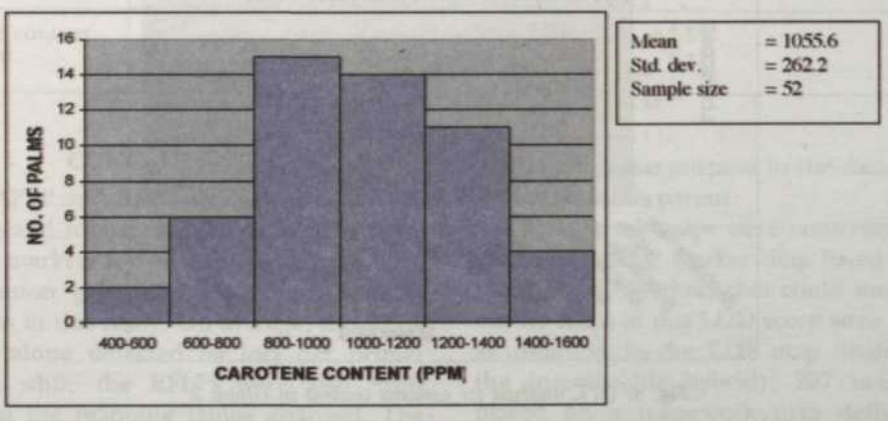

Fig. 2: Frequency distribution for carotene content in the interspecific hybrid F_1 progeny used for QTL mapping

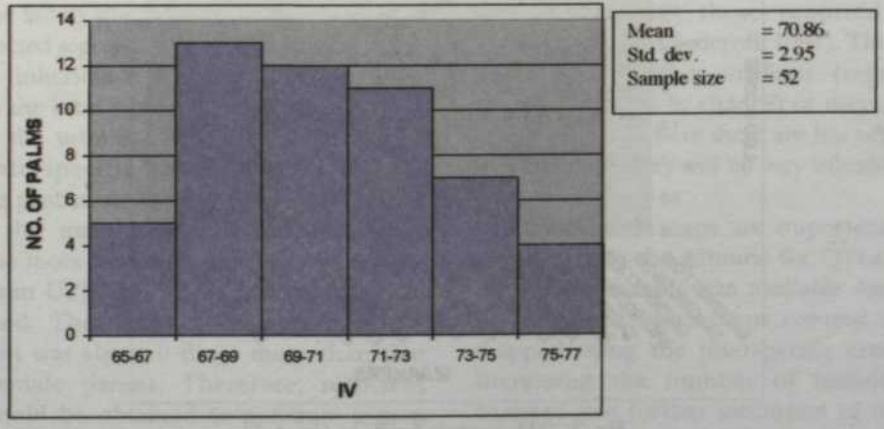

Fig. 3: Frequency distribution for IV in oil in the interspecific hybrid F_1 progeny used for QTL mapping

MCAA-340 and EACT/MCAT-590, in a region of about 20 cM. As for the IV parameter, a significant QTL was detected in linkage group 4 (*Fig. 5*). The region associated with the QTL was between the marker EACC/MCAT-195 and EAAG/MCAG-280, in a region spanning about 8 cM.

A summary of the QTLs detected in the analysis of the interspecific hybrid progeny is presented in Table 5. Generally the number of QTLs detected was low and this could be explained by the small size of the mapping family used. The cross has been redone and an additional 45 palms will be evaluated for both map construction and QTL analysis in the future. Furthermore, in this study stringent genomic-wide empirical thresholds were used to declare a QTL. The stringent threshold is important to reduce the number of false positives (van Ooijen, 1999). Nevertheless the QTLs detected do explain a significant proportion of the variation observed for these traits (28% for carotene content, and 24% for IV). The markers as such hold promise as candidates for incorporation into the oil palm breeding programme to improve on oil quality traits.

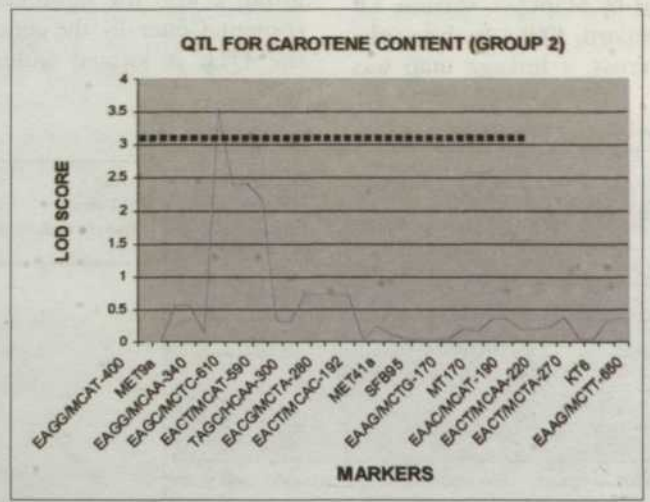

Fig. 4: QTL analysis for carotene content in Group 2

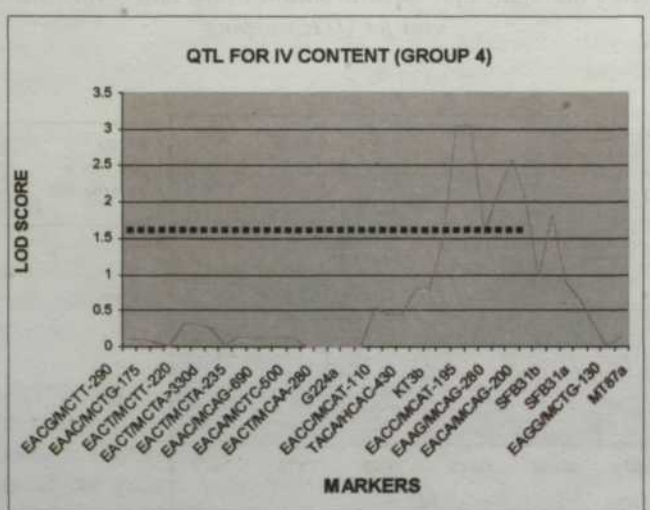

Fig. 5: QTL analysis for IV in Group 4

TABLE 4
Summary of RFLP and AFLP analyses of the interspecific hybrid mapping family

Type of markers	No. probes/primer pairs evaluated	No. of polymorphic loci identified	No. of markers showing 3:1 segregation	No. of markers showing 1:1 segregation in the gametes of		No. of markers meeting goodness of fit to 1:1 or 3:1 ratio
				T128	UP1026	
RFLP	60	72	-	66	6	68
AFLP	53	363	-	290	73	309

TABLE 5
QTLs for carotene content and IV found to be significant at an empirical significant threshold level of 3.10 ($P<0.05$)

Trait	Linkage Group	Closest marker	LOD score	% variance explained by QTL
Carotene content	2	EAGG/MCAA-340	3.52	27.8
IV	4	EACC/MCAT-195	3.14	23.6

CONCLUSION

Both the AFLP and RFLP techniques proved to be reliable and robust techniques in generating molecular markers for oil palm. The high yield of information generated with these markers was obvious in this study. On average, the AFLP technique alone detected 59 loci per primer pair used, while the RFLPs were also easily detected in the mapping family analysed. The dominant AFLP markers and the co-dominant RFLP markers were also readily analysed in the interspecific hybrid progeny using the pseudo-testcross model. A majority of the markers (about 80%) in the family studied met the goodness of fit to the expected segregation ratios indicating stable Mendelian inheritance of the AFLP and RFLP markers in the interspecific hybrid cross analysed.

Using the two-way pseudo-testcross strategy in the interspecific cross, a data set for segregating probes was obtained for both parents. However, the male parent (Palm T128) was found to be more heterozygous than the female parent (Palm UP1026), as reflected in the data set collected. The data set generated for the male parent was about 6 times more than that for the female parent. Therefore, sufficient markers could be obtained to generate a map for the T128 male parent suitable for QTL detection. However, not enough markers were available for this purpose in the data set for the UP1026 female parent.

Framework maps were constructed for the RFLP and AFLP marker data based on a LOD score of 5.0. Markers that could not be placed on the maps at this LOD score were considered as unlinked. In the T128 map (male parent of the interspecific hybrid), 297 markers were placed on a framework map defining about 1,434 cM of total map distance. There were 20 linkage groups with an average length of 72 cM.

The proportion of unlinked markers in the mapping population was about 20% and is comparable with those reported elsewhere (Grattapaglia and Sederoff, 1994). These unlinked markers are either artifacts (segregating in Mendelian ratios by chance) or they sample parts of the genome where there are few other markers, in which case they will be very valuable (Marques *et al.*, 1998).

Framework maps are important tools with which to scan the genome for QTLs. In spite of the limited sample size available for this study, QTLs controlling carotene content and IV were mapped using the interspecific cross progeny. Increasing the number of individual palms analysed and further saturation of the map will very likely lead to the discovery of more QTL positions controlling traits of interest. It is for

this purpose that efforts were made to re-create the interspecific cross to plant out sufficient numbers of the progeny. At present there are an additional 45 palms that have come to fruiting and will be included in future analysis.

The map produced in this study is specific to the individual used as parents. Such "single-tree" genetic maps therefore may not be representative of the species. For this reason, several maps need to be generated for a number of individuals in order to create a consensus map (Gentzbittel *et al.*, 1995). In the formation of a consensus map, single tree maps are linked using landmark markers, or anchor probes. Co-dominant markers such as RFLP especially serve well as anchor probes, since the probes are highly robust and stable. RFLP probes can also be easily shared by different laboratories and easily identified in different genetic maps, if the nomenclature for naming the probes is retained. It is for this reason that considerable efforts were made in developing and applying the RFLP technique in this study, although it was considerably laborious and expensive. This same concept extends to QTL mapping. The QTLs mapped in this study are individual specific. Homologies of linkage groups or homologies of QTLs in different oil palm maps have not yet been established at this time. Such homologies will have to await the localisation of common RFLP or other types of markers, for example simple sequence repeats (SSR) on the different oil palm genetic maps. The most important result to come from this study are that the QTL analysis has yielded a number of significant QTLs for oil quality, which influence a significant proportion of the total phenotypic variance (excess of 20%). To the knowledge of the authors, this is also the first report on the detection of QTLs associated with oil quality for oil palm. It is envisaged that the markers identified in this study at the very least can assist in the design of a more effective breeding programme for improving oil quality.

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