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FLANKING AFLP MARKERS FOR THE *Virescens* TRAIT IN OIL PALM

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ABSTRACT

Using the monofactorially inherited virescens *trait to determine the ripeness of oil palm fruit bunches, instead of the current counting of abscissed and fallen fruits, has the potential to facilitate harvesting and milling because of the starker colour change.* Virescens *fruits are characterized by being distinctly emerald green when young, turning to a bright orange on ripening, whereas the presently commercially produced* Nigrescens *fruits are dark purple young, and red/purple ripe. AFLP markers of 142 bp and 356 bp developed from the primer combination E-ACT/M-CAT and of 254 bp from E-ACT/M-CTA were found to be closely linked to the* virescens *trait in two crosses. The markers that flank the* virescens *gene on the genetic linkage map of oil palm may be useful for marker-assisted breeding for the* virescens *trait.*

Keywords: oil palm, *virescens*, fruit skin colour trait, AFLP, molecular marker.

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INTRODUCTION

DNA markers, harnessed in appropriate breeding strategies, can augment and render more efficient conventional breeding (Hayward and Hamilton, 1997; Westman and Kresovich, 1997). The potential of molecular markers in oil palm (*Elaeis guineensis* Jacq.) breeding (Shah *et al.*, 1994; Rohde *et al.*, 1995; 2002; Mayes *et al.*, 1996; 1997; Ashburner *et al.*, 1997; Lebrun *et al.*, 1998; Herrán *et al.*, 2000; Moretzsohn *et al.*, 2000; Billotte *et al.*, 2001; Perera *et al.*, 2001) is especially important as it is a large plant with a long generational interval. Markers that are linked to traits of high economic value, determined by only one or a few genes, expressed late in the plant's life and not convenient to be measured in the phenotype, are particularly valuable. Tracking the marker and selecting for it can be more effective in improving the trait than tracking for the gene through the phenotype. Shell thickness, fruit skin colour and the mantled fruit form are known to be monofactorially inherited and economically important in oil palm, or potentially so (Hartley, 1988). The objective of this study is to develop DNA markers for the oil palm fruit skin colour.

The oil palm produces fruits with two types of colouring - *Nigrescens* (*Nig*) which is dark violet (almost black) unripe, turning into a mixture of red and violet on ripening and *Virescens* (*Vir*) which is emerald green unripe, ripening to a bright orange (Hartley, 1988). Unlike the former, the latter lacks anthocyanins which produce purple and red colours to mask the natural fruit colour changes that occur during ripening. *Vir* is monofactorially dominant over *Nig* (Hartley, 1988). All the commercially grown oil palms are *Nig* which is naturally more common and which has been cultivated for a long time.

Vir, on the other hand, has received little breeding attention since it does not appear to confer any advantage. However, it may be a more convenient and accurate method for visualizing fruit ripeness without having to wait for the fruits to abscise and drop – and incurring the chore of picking them up in harvesting. The stark colour differences between ripe and unripe *Vir* fruits can also be used to improve milling with camera-based segregation of different

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ripeness fruits. Oil palm fruits are borne in bunches, each bunch carrying a few hundred to a few thousand fruits. The fruits ripen (slightly) differently but are harvested more conveniently as the bunch instead of gathering the individual fruits as they ripen, abscise and drop. If the bunch is harvested before any fruit abscission, its oil content is low. The bunch oil content increases as more fruits reach their full oil content and abscise, but the increase is asymptotic whereas exponentially more work is involved picking up the dropped loose (= abscised) fruits. However, too many abscissed fruits will result in an increase in free fatty acids in the oil. Therefore, the *Vir* marker may be useful in increasing harvesting efficiency, without sacrificing too much unproduced oil, as well as help ensure a better oil.

Most DNA marker techniques fall into one of three basic categories depending on whether they are hybridization- or polymerase chain reaction (PCR)-based, and, if the latter, whether they involve sequencing (Karp and Edwards, 1995). Briefly, the categories are: (1) non-PCR techniques such as restriction fragment length polymorphism (RFLP) and mini-satellite or variable number of tandem repeats (VNTR) (hybridization-based); (2) random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellite primed PCR (MP-PCR) which includes unanchored single SSR primer amplification (SPAR), inter-SSR amplification (ISSR), randomly amplified microsatellite polymorphism (RAMP) and selective amplification of microsatellite polymorphic loci (SAMPL) (PCR-based); and (3) sequence-tagged site (STS) markers including single nucleotide polymorphisms (SNPs), cleaved amplified polymorphic sequences (CAPs), sequence characterized amplified regions (SCARs), allele-specific associated primers (ASAPs) and expressed sequence tags (ESTs) (targeted PCR and sequencing).

This study uses the AFLP technique - a fast, high multiplex assay that commonly shows up significant polymorphisms. With this technique, no prior knowledge about the genomic make-up of the organism is needed and a relatively small number of primer pairs suffice to visualize a large number of loci. Furthermore, the banding patterns are not particularly sensitive to the initial concentration of template DNA (Vos *et al*., 1995; Zhu *et al*., 1998).

MATERIALS AND METHODS

Leaf samples from each palm in two crosses segregating for *Nig* and *Vir* were collected from PAMOL Plantations, an oil palm company in Johor, south Peninsular Malaysia. The first cross, Pkg214/58 x Lb323/52/635, comprised 35 individuals (18 *Nig* and 17 *Vir*) and the second, Pkg235/970 x 7A/2, 79 individuals (36 *Nig* and 43 *Vir*). The first cross was part of a collection of crosses in the CBP (Combined Breeding Programme) and the second from a population of NPC (Non-Abscinding Palm Crosses).

Genomic DNA was extracted using a CTAB method, modified for use on stored mature oil palm leaf as described by Seng and Faridah (2006).

The material was assayed using both Bulk Segregant Analysis (BSA) (Michelmore *et al*., 1991) and the individual palm approach. For BSA, the DNA samples were bulked by phenotype to create '*Nig* bulk' and '*Vir* bulk' with equal amounts of DNA from each individual. In the first of two studies, DNA from 10 *Nig* and 10 *Vir* palms, picked at random, from the CBP and NPC crosses respectively was bulked separately to form four bulks. The number of palms bulked was deliberately kept small (10 palms each) to increase the likelihood of obtaining markers linked to target loci, even though the frequency of false positives is generally higher with smaller bulks (Michelmore *et al*., 1991). In the second study, DNA from all 18 *Nig* and 17 *Vir* palms from the CBP cross and from all 36 *Nig* and 43 *Vir* plants from the NPC cross were bulked for another four bulks. All the palms were used to maximize the number of recovered markers. This would also reduce the frequency of false positives, *i.e*. loci not linked to the trait of interest but detected as polymorphic between the bulks (Michelmore *et al*., 1991).

AFLP analysis was performed according to Vos *et al.* (1995) using the AFLP™ Plant Mapping Kit (PE Applied Biosystems) according to the manufacturer's instructions. The four fluorescent dyes used were FAM (blue), JOE (green), TAMRA (yellow) and ROX (red, as internal size standards). A total of 64 primer combinations from the AFLP Selective Amplification Start-Up Module (PE Applied Biosystems) were used in the first screening for the trait using bulk *Vir* and bulk *Nig*. In the second screening, the two bulked samples and three randomly-picked individuals from each cross were used to eliminate the false positives. From the second screening, primer combinations showing informative polymorphism were selected for final assay of the two crosses as follows: in the first study, for the CBP cross, 10 selected primer combinations were used directly on the *Vir* and *Nig* bulks of 10 palms each followed by assay of each individual palm. The four most informative primer combinations were then similarly used on the NPC cross. In the second study, for the CBP cross, the 10 primer combinations were reused on *Nig* and *Vir* bulks of all individuals followed by individual assay. This was repeated for the NPC cross using the four primer combinations mentioned above (*Table 1*).

The AFLP electropherograms in the range 50-500 bp were analysed using ABI PRISM *GeneScan*®

version 3.1. The fragments were re-checked manually against the corresponding AFLP gel images to eliminate or correct for spurious peaks from signal noise or misaligned lanes. Only fragments with an intensity of 50 or above arbitrary fluorescence units were scored and aligned accurately using the internal size standard (*GeneScan* ROX-500). The raw data were analysed using the ABI PRISM® *Genotyper*® version 2.1 software. Polymorphic bands that showed close correspondence to fruit skin colour were regarded as potential fruit colour-specific markers and confirmed by Chi-square test for independence.

TABLE 1. AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) PRIMER COMBINATIONS USED WITH COMBINED BREEDING PROGRAMME (CBP) AND NON-ABSCINDING PALM CROSSES (NPC) SAMPLES

Sample	CBP	NPC
Primer combinations	E-ACA/M-CAG	E-ACT/M-CAT
	E-ACA/M-CAT	E-ACT/M-CTA
	E-ACT/M-CTT	E-AAG/M-CAT
	E-AAG/M-CAC	E-AAG/M-CTA
	E-AAG/M-CTC	
	E-AAG/M-CTG	
	E-ACT/M-CAT	
	E-ACT/M-CTA	
	E-AAG/M-CAT	
	E-AAG/M-CTA	

RESULTS

Study 1: Bulk Segregant Analysis and 10 Individual Palms

The 10 primer combinations in *Table 1* used for screening produced 48–81 distinct fragments of 50 bp – 495 bp for the CBP cross. Three markers of 307 bp, 142 bp and 254 bp were obtained from the primer combinations E-ACA/M-CAT, E-ACT/M-CAT and E-ACT/M-CTA, respectively. They were found to be strongly linked to the fruit colour. On the other hand, the four selected primer combinations produced 48 – 69 fragments of 50 bp – 485 bp for the NPC cross. Three makers of 356 bp, 254 bp and 165 bp were obtained from the primer combinations E-ACT/M-CAT, E-ACT/M-CTA and E-AAG/M-CTA, respectively. They can be used to differentiate the *Vir* and *Nig* phenotypes.

Study 2: Bulk Segregant Analysis and All Individual Palms

The 10 primer combinations applied to the 35 CBP individuals resulted in 1146 fragments of 50 bp to 495 bp. The primer combinations generated 84-164 bands each, of which 79% were polymorphic. Primer combination E-ACT/M-CTA produced the most bands with potential association with the trait. Four out of nine such bands (44%) were significantly associated based on Chi-square test. Primer combinations E-AAG/M-CTA and E-AAG/M-CTC showed 36% while E-ACT/M-CAT and E-AAG/M-CTG showed 30% significant association and the rest were even lower (*Table 2*). Of the total fragments, 23 had significant association based on the Chi-square test at P< 5%. Of them, two - 142 bp and 245 bp, - from primer combinations E-ACT/M-CAT, E-ACT/M-CTT respectively and two - 129 bp and 154 bp - from E-AAG/M-CTA showed significant associations of >80% with the fruit colour trait. *Table 3* shows that a fragment of 142 bp (E-ACT/M-CAT) was the best marker in distinguishing fruit colour with only one plant showing a mismatch. Primer combination E-ACT/M-CTA generated a band of 254 bp which differentiated the different phenotypes with 77% accuracy while E-ACA/M-CAT (307 bp) only produced 67% accuracy.

In the NPC study, 22 bands showed significant association ($p \leq 0.05$) with primer E-ACT/M-CTA having maximum association. Specific bands produced by this primer showed the highest significant association (22%) between the bands and the trait. Primer combination E-ACT/M-CAT showed 19% association while E-AAG/M-CAT and E-AAG/M-CTA gave 8% and 14% association, respectively (*Table 4*). Of the 22 fragments obtained, eight showed more than 80% significant association to the fruit colour trait (*Table 5*). However, of these eight only one, of 356 bp (E-ACT/M-CAT) showed a mismatch rate of <10%. One marker of 254 bp produced by primer E-ACT/M-CTA showed 78% accuracy in differentiating the *Vir* and *Nig* phenotypes (*Table 5*).

DISCUSSION

Bulk segregant analysis, or BSA, in combination with the AFLP marker technique, is a very powerful tool for identifying markers tightly linked to, or co-segregating with, the genes controlling monogenic traits (Michelmore *et al.*, 1991; Uzun *et al.*, 2003; De Giovanni *et al.*, 2004). The BSA method evens out the background genetical difference so that the genetic difference for the trait is attenuated (von Stackelberg *et al.*, 2003) while the AFLP technique elicits large genetic polymorphisms with near complete coverage as well as even distribution across the genome (Zhu *et al.*, 1998; Vuylsteke *et al.*, 1999; Aggarwal *et al.*, 2002). These advantages were used to discover the AFLP markers linked to the gene determining fruit colour in the oil palm. Furthermore, fluorescence-labelled primers and automated fragment detection allowed for more AFLP markers of a broader size range to be scored without the errors inherent in visual scoring and

TABLE 2. NUMBER OF POLYMORPHIC BANDS, SPECIFIC BANDS, SPECIFIC BANDS SIGNIFICANT IN THE PEARSON CHI-SQUARE TEST, PERCENTAGE OF SIGNIFICANT SPECIFIC BANDS AND THEIR RESPECTIVE FRAGMENT SIZES OBTAINED WITH 10 SELECTED PRIMER COMBINATIONS FOR 35 PALMS FROM COMBINED BREEDING PROGRAMME (CBP) CROSS

Primer combination	No. of polymorphic bands	No. of specific bands	No. of specific bands (χ^2 significant)	% of specific bands (χ^2 significant)	Specific bands (bp)
E-ACA/M-CAG	72	9	-	-	-
E-ACA/M-CAT	83	6	1	16.67	306.58
E-ACT/M-CAT	87	10	3	30.00	54.42
					68.33
					142.13
E-ACT/M-CTA	60	9	4	44.44	58.21
					253.91
					126.36
					284.92
E-ACT/M-CTT	63	8	2	25.00	245.29
					320.54
E-AAG/M-CAC	101	7	-	0.00	-
E-AAG/M-CAT	124	9	-	0.00	-
E-AAG/M-CTA	146	14	5	35.71	128.73
					154.11
					277.81
					384.60
					85.56
E-AAG/M-CTC	91	14	5	35.71	97.14
					72.25
					158.87
					312.11
					346.00
E-AAG/M-CTG	73	10	3	30.00	77.14
					135.14
					180.33
Total	900	96	23	23.96	-

manual data interpretation (Coenye *et al.*, 1999; Dresler-Nurmi *et al.*, 2000; Arnold, 2004). Using an internal size standard (500-ROX) and an intensity standard for all AFLP profiles increased the reproducibility and accuracy of the data (Krauss and Peakall, 1998; Aggarwal *et al.*, 2002; Arnold 2004). In agreement with the general claim (Jones *et al.*, 1997; Mohan *et al.*, 1997; Uzun *et al.*, 2003), the AFLP markers were reproducible but with rigorous control needed for all the variable settings, standardized band scoring and good quality genomic DNA. As an initial marker screen, the BSA method was fast and reliable for mapping an uncharacterized gene to a chromosome arm in barley (Weerasena *et al.*, 2004). In the AFLP-based BSA study of von Stackelberg *et al.* (2003), two genetic maps of the regions around the *def* locus in pea with maximum lengths of 31 cM and 36 cM were produced, suggesting that the BSA technique delimits detectable polymorphisms to a maximum linkage length of not more than 40 cM around the gene of interest.

The combination of AFLP, or, indeed, any other marker system with BSA can, however, also produce bias. In this study, for example, in some instances, the polymorphic bands detected in the bulk samples were not detected in the corresponding individual palms, and the monomorphic bands present in the individual palms were absent in the bulk samples. The AFLP study of Fu *et al.* (2003) on oat, fringed brome and smooth bromegrass reported similar biases. Zhu *et al.* (1998), in their rice biodiversity study, found that the use of DNA mixtures for AFLP analysis decreased the sensitivity of detection. Likewise, van Treuren (2001) reported that many bands go undetected even though the intensity of bands from a bulk sample is directly related to the intensity from individual samples, making AFLP analyses of bulks less practical. The sizes of the fragments detected in both experiments in this study were similar, with the discrepancies in a few probably due to the different numbers of palms used in the two studies. Furthermore, the number of specific fragments detected in Study 1 was lower

TABLE 3. SUMMARY OF SPECIFIC BANDS AND THEIR LENGTHS GENERATED BY SEVEN AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) PRIMER COMBINATIONS WITH COMBINED BREEDING PROGRAMME (CBP) BULKS AND 35 INDIVIDUAL PALMS

Primer combination	Specific band (bp)	Bulk *Vir*	Bulk *Nig*	CBP Line			
				Individual palms scored as *Vir* 18	%	Individual palms scored as *Nig* 17	%
E-ACA/M-CAT	306.58	1	0	12	66.7	4	23.3
E-ACT/M-CAT	54.42	0	1	0	-	6	35.3
	68.33	0	1	0	-	4	23.5
	*142.13	0	1	15	83.3	1	5.9
E-ACT/M-CTA	58.21	1	0	9	50	1	5.9
	126.36	1	0	12	66.7	4	23.5
	253.91	0	1	6	33.3	13	76.5
	284.92	1	0	13	72.2	5	29.4
E-ACT/M-CTT	*245.29	1	0	16	88.9	8	47.1
	320.54	1	0	14	77.7	4	23.5
E-AAG/M-CTA	85.56	1	0	12	66.7	4	23.5
	*128.73	1	0	15	83.3	6	35.3
	*154.11	1	0	15	83.3	4	23.5
	277.81	1	0	9	50.0	0	-
	384.60	1	0	9	50	3	17.6
E-AAG/M-CTC	97.14	1	0	6	33.3	0	-
	72.25	1	0	12	66.7	5	29.4
	158.87	1	0	6	33.3	0	-
	312.11	1	0	14	77.8	6	35.3
	346.00	1	0	8	44.4	1	5.9
E-AAG/M-CTG	77.14	1	0	10	55.6	3	17.6
	135.14	0	1	4	22.2	10	58.9
	180.33	0	1	1	5.6	8	47.1

Note: * specific markers showing >80% significance in differentiating the fruit colour trait.

than in Study 2 for both crosses, since the former required perfect correlation between the marker and phenotype whereas in Study 2, any fragment with >80% probability of distinguishing the phenotypes was considered.

Primer Combination E-ACT/M-CAT: A *Virescens*–Specific Marker

In this study, primer combination B12 generated a 142 bp fragment which may be a potential specific marker for *Vir* in the CBP cross. In Study 1, the genotype was completely associated with the *Vir* phenotype while in Study 2, it distinguished the *Vir* phenotype with only one mismatch. However, this marker was not detected in the NPC cross. Another marker of 356 bp was found to be *Vir*-specific; it was also obtained from the same primer in the NPC cross but not in the CBP cross. It correlated completely with the NPC *Vir* phenotype in Study 1 and distinguished the *Vir* trait with only three mismatches. The results clearly showed that these two markers have good potential in discriminating the fruit colour trait in oil palm. The study also illustrates that trait-linked molecular markers identified in one population are not necessarily found in another.

Primer Combination E-ACT/M-CTA: A *Nigrescens*–Specific Marker

The BSA results may be strongly affected by the dominant status of the trait locus and/or by the linkage phase between the marker and trait. Since the *Vir* allele is dominant and *Nig* recessive, the former is present in both dominant homozygotes and heterozygotes, both of which would occurred in the *Vir* bulk. The *Vir* and *Nig* bulks can only be differentiated by a (dominant) marker if the latter is linked in coupling phase. If this occurs, a band will appear for individuals with the dominant trait but not in recessive individuals. For the CBP cross, specific markers from most of the primer combinations were associated with the dominant trait. This was expected as *Vir* individuals are dominant homozygotes and heterozygotes (VV and Vv) whereas *Nig* individuals are recessive homozygotes (vv). However, rather surprisingly though not unexpectedly, the AFLP fragments from primer combination E-ACT/M-CTA were associated

TABLE 4. NUMBER OF POLYMORPHIC BANDS, SPECIFIC BANDS, SPECIFIC BANDS SIGNIFICANT IN THE PEARSON CHI-SQUARE TEST, PERCENTAGE OF SIGNIFICANT SPECIFIC BANDS AND THEIR FRAGMENT SIZES, FROM FOUR SELECTED PRIMER COMBINATIONS FOR 79 PALMS FROM NON-ABSCINDING PALM CROSSES (NPC) CROSS

Primer combination	No. of polymorphic bands	No. of specific bands	No. of specific bands (χ^2 significant)	% of specific bands (χ^2 significant)	Specific bands (bp)
E-ACT/M-CAT	89	26	5	23.07	101.40 226.21 265.64 424.51 355.76
E-ACT/M-CTA	98	32	7	21.88	55.09 60.26 135.05 253.79 72.10 229.33 321.29
E-AAG/M-CAT	153	51	4	7.84	51.07 58.27 71.12 434.91
E-AAG/M-CTA	145	43	6	13.95	65.01 91.82 98.45 290.48 391.64 417.14
Total	485	152	22	14.47	-

with the recessive allele. The 254 bp fragment, generated from primer combination E-ACT/M-CTA, was 77% (CBP) and 76% (NPC) significant in distinguishing the fruit colour trait. Six *Vir* individuals from the CBP cross and nine from the NPC cross showed mismatches. The marker, nevertheless, was found in both crosses and can be used to augment breeding for this trait. According to Yu *et al.* (2000), if a linkage relationship between a marker and a locus of interest is confirmed in two populations, there is greater likelihood that the marker can be used in a third population, different from the first two.

There are many instances where application of molecular markers for indirect selection is limited in germplasm collections. In the studies of Beer *et al.* (1996) and Yu *et al.* (2000), the linkage relationships between a trait and molecular markers identified in a population were inconsistent when compared to in a population from distantly related germplasm. For example, use of the dominant RAPD marker, OA141100, that is linked to the Ur-3 rust resistance gene was limited to the Middle American gene pool (Miklas *et al.*, 1993); the OF10970 marker linked to the Ur-4 gene was only present in the Andean gene pool (Haley *et al.*, 1993) and the OAD19690 marker linked to the bc-2 gene only applicable in the Meso-American race within the Middle American gene pool (Haley *et al.*, 1994).

In a previous study on the oil palm genetic map constructed using a population created from a *Vir* selfed palm, Singh (2005) found a 252 bp fragment from the primer combination E-ACT/M-CTA. The fragment was positioned 52 cM from the *Vir* trait mapped with RFLP markers MET16 and KT3 into Group 3 (*Figure 1*). None of the AFLP markers screened were found to be closely linked to the *Vir* trait except for the marker produced by primer E-ACT/M-CAT>330b which was only 9 cM away. In this study, primer combination E-ACT/M-CAT generated a slightly bigger fragment (356 bp in the NPC cross), hence providing a potential diagnostic marker for the fruit colour trait. Likewise, as mentioned earlier, primer combination E-ACT/M-CAT also produced a marker with the potential to differentiate the fruit colour trait even though different-sized fragments were generated in two different crosses.

In this work, a small number of the samples showed mismatches, possibly due to cross contamination of the pollen during crossing or recombination between the marker and its linked allele during meiosis. The marker, though linked to the gene, can, nevertheless, be so far apart in

TABLE 5. SUMMARY OF SPECIFIC BANDS AND THEIR LENGTHS GENERATED BY FOUR AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) PRIMER COMBINATIONS WITH BULKS AND 79 INDIVIDUALS OF NON-ABSCINDING PALM CROSSES (NPC) PROGENIES

				NPC Line			
Primer combination	**Specific band (bp)**	**Bulk *Vir***	**Bulk *Nig***	**Individual palms scored as *Vir* 43**	*%*	**Individual palms scored as *Nig* 36**	*%*
E-ACT/M-CAT	*101.40	1	0	43	100	32	88.9
	226.21	0	1	20	46.5	27	75.0
	265.64	1	0	19	44.2	5	13.9
	*355.76	1	0	36	83.7	3	8.3
	424.51	1	0	30	69.8	16	44.4
E-ACT/M-CTA	*55.09	0	1	36	83.7	35	97.2
	*60.26	0	1	22	51.2	29	80.1
	*72.10	0	1	29	67.4	35	97.2
	*35.05	0	1	36	83.7	35	97.2
	229.33	0	1	5	11.6	15	41.7
	253.79	0	1	9	20.9	28	77.8
	321.29	0	1	11	25.6	24	66.7
E-AAG/M-CAT	51.07	1	0	42	97.7	24	66.7
	58.27	1	0	27	62.8	8	22.2
	71.12	1	0	34	79.1	19	52.8
	434.91	1	0	19	44.2	5	13.9
E-AAG/M-CTA	65.01	1	0	19	44.2	4	11.1
	91.82	0	1	21	48.9	26	72.2
	*98.45	0	1	36	83.7	35	97.2
	290.48	0	1	19	44.2	26	72.2
	*391.64	0	1	26	60.5	31	86.1
	*417.14	0	1	37	86.0	33	91.7

Note: *Specific markers showing >80% significant association in differentiating the fruit colour trait.

recombinant units that it did not faithfully co-segregate with the *Vir* trait. Also, two band sizes were detected using the same primer combination in the different crosses. This might be due to a limitation of the technique itself. For example, a mutation in the restriction enzyme recognition site would render the sequence recognizable or unrecogniable by the digestion enzyme in the different crosses. Similarly, an addition, insertion, deletion or duplication might also result in the above.

Although AFLP markers are technically more complex than other PCR-based markers, they combine high sensitivity and reproducibility and are generated in large numbers in a single assay. Hence, they are sometimes considered more efficient in studies aimed at identifying molecular markers linked to genes of interest (Reamon-Büttner and Jung, 2000). In other crops, various AFLP markers have been shown to be linked to many different traits; prominent among these are leaf rust resistance (Cervera *et al.*, 1996), sugar cane mosaic virus (SCMV) in maize (Xu *et al.*, 1999; Dussle *et al.*, 2002), seed coat colour in *Brassica* species (Negi *et al.*, 2000; Liu *et al.*, 2005), sex-specific marker in hemp (Flachowsky *et al.*, 2001; Peil *et al.*, 2003; Rode *et al.*, 2005), closed capsule mutant trait in sesame (Uzun *et al.*, 2003) and powdery mildew resistance in tomato (De Giovanni *et al.*, 2004).

The AFLP markers generated in this study, ranging from 140 to 350 bp, are generally too short to be reliable as specific markers in MAS programmes (Bradeen and Simon, 1998; Shan *et al.*, 1999; Negi *et al.*, 2000; Brugmans *et al.*, 2003), but it is possible to convert them to sequence-specific primers, such as STS, SCAR, STM and CAP markers. Markers larger than 350 bp can be easily converted into SCAR markers (Sardesai *et al.*, 2002; Liu *et al.*, 2005) but not the smaller ones of 140 bp and 250 bp (Liu *et al.*, 2005). The approach of PCR walking, or inverse-PCR, was used for the markers of 150 to 300 bp to isolate the fragments adjacent to the AFLP marker for conversion to SCAR markers (De Jong *et al.*, 1997; Devic *et al.*, 1997; Bradeen and Simon, 1998; Negi *et al.*, 2000). A cut-off limit of 200 bp for AFLP to CAPS conversion was also used by De Jong *et al.* (1997). Dussle *et al.* (2002) were able to convert two short AFLP bands (150–300 bp) into PCR-based inserts/deletes (indel) and CAPS markers. However, the yet smaller fragments (60–183 bp) can be transformed into STS markers (Reamon-Büttner and Jung, 2000) as was done by von Stackelberg *et al.* (2003) to three AFLP markers in pea. Brugmans *et al.*

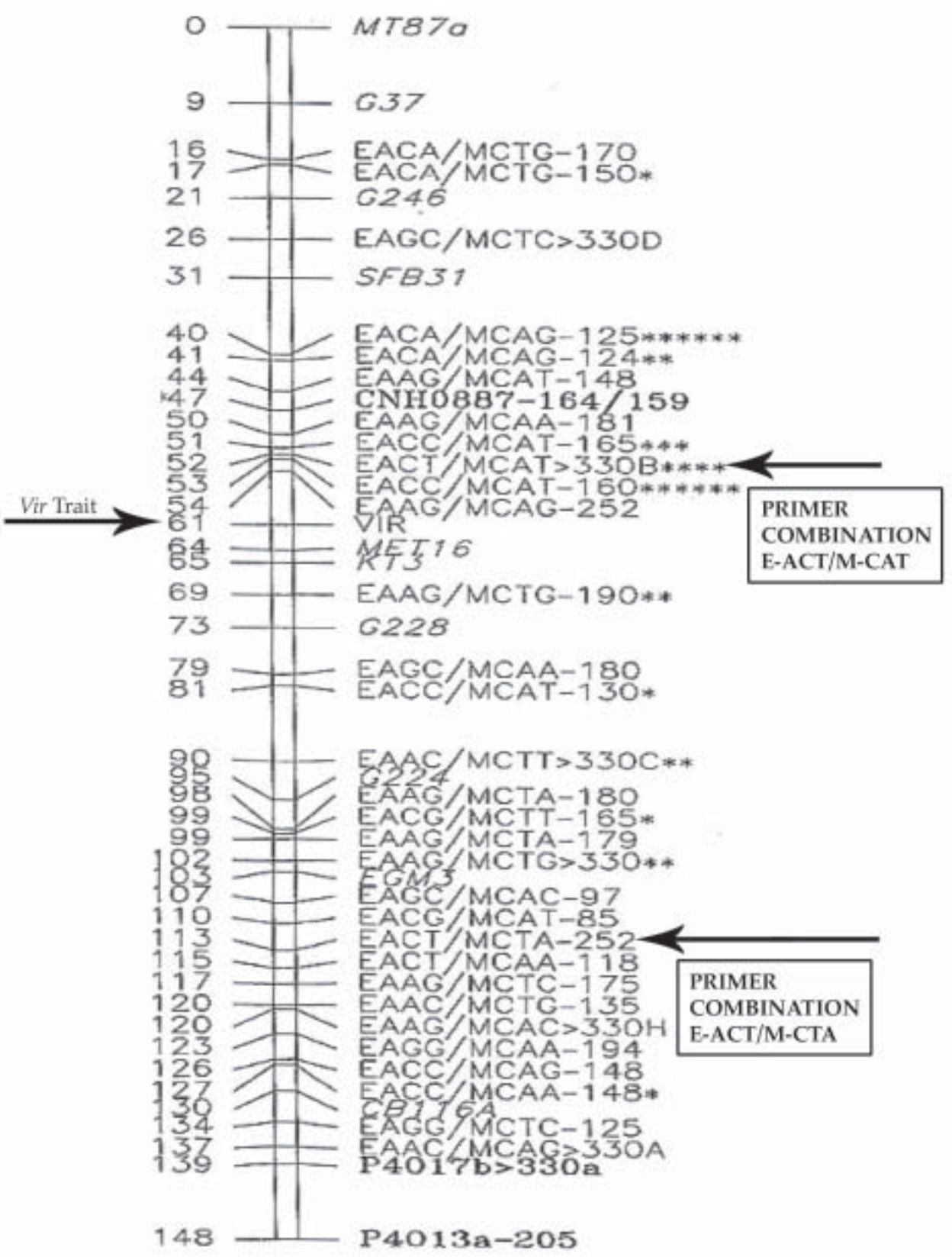

Figure 1. Group 3 in the genetic linkage map of the oil palm with primer combinations E-ACT/MCAT and E-ACT/M-CTA from this study shown.

Source: Singh, S (unpublished).

(2003) demonstrated it possible to convert AFLP markers, whether small (131 bp) or large (359 bp), into SNP markers.

CONCLUSION

Three AFLP fragments (E-ACT/M-CAT – 142 bp and 356 bp; E-ACT/M-CTA – 254 bp) generated from two AFLP primer combinations (E-ACT/M-CAT and E-ACT/M-CTA) were found to be putative markers for the fruit colour trait in oil palm. The 254 bp fragment was strongly associated with the trait in both the crosses used in this study, suggesting its potential application for selecting palms for fruit colour. Furthermore, the position of this and the 356 bp fragment suggest that they flank the *Vir* gene on the linkage map of oil palm which implies that in combination they would provide a powerful tool for marker-assisted selection.

The sequence data of these fragments could be useful to create sequence-specific markers to the genes encoding for the fruit skin colour trait that are applicable in a marker-assisted selection (MAS) programme to breed *Vir* plants.

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