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Statistical Mapping of Quantitative Trait Loci Controlling the Time to First Callusing in Oil Palm (*Elaeis guineensis* Jacq.) Tissue Culture

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ABSTRAK

Sebanyak 400 penanda genetik (126 RFLP, 274 AFLP) telah berjaya dipetakan daripada 87 progeni F_1 dari kacukan Deli dura X Yangambi pisifera. Pemetaan tersebut telah menghasilkan peta genetik yang lebih padat dan meliputi genom yang berukuran 1,714cM dan 1,225cM bagi pisifera dan dura. Menggunakan Kosambi interval mapping, perisian MapQTL Versi 4.0, kajian selanjutnya telah dijalankan untuk mencari 'quantitative trait loci' (QTL) yang mempengaruhi tempoh pembentukan kalus (TFC) bagi kultur tisu sawit. Data kultur tisu menunjukkan taburan yang berterusan. Dalam kajian ini, tiga QTLs telah dikesan pada pisifera manakala dua untuk dura pada nilai signifikan 99% dan 95%. Ini menunjukkan lokasi QTL tersebut adalah signifikan secara statistik dalam mempengaruhi variasi bagi TFC. Oleh yang demikian, maklumat ini menunjukkan bahawa loci genomik memberi kesan terhadap kemampuan sawit untuk dikulturkan.

ABSTRACT

An additional 400 genetic markers (126 RFLPs, 274 AFLPs) were successfully mapped on the earlier developed linkage maps using 87 F_1 progenies derived from Deli dura X Yangambi pisifera cross. This resulted in a denser map with coverage length of 1,714cM and 1,225cM for pisifera and dura, respectively. Further exploration to search for quantitative trait loci (QTL) associated with time to first callusing (TFC) was carried out by Kosambi Interval Mapping using the computer program MapQTL Version 4.0. The tissue culture trait data showed a continuous distribution. In this paper, three likelihood QTLs were detected in pisifera and two QTLs in dura at 99% and 95% significant thresholds. These QTL locations can be designated as statistically significant for contributing to the variation of TFC. Therefore, the information points to a genomic loci affecting tissue culturability in oil palm.

INTRODUCTION

In the current tissue culture production of oil palm planting material, some genotypes are observed to be more amenable to tissue culture than others. The rate of callus formation has been reported to be variable between clones (Ginting and Fatmawati, 1995; Rival *et al.*, 1997; Wooi, 1995). This variability also extends to time to first callusing (TFC). This was clearly observed in TFC data collected in the current study where, some of the palms easily formed callus as early as 3 weeks and others took up to 33 weeks to form callus.

The development of molecular markers has made it possible to generate a linkage map for individual palm and consequently allows identification of QTLs based on the framework map. QTL mapping for tissue culture traits

have been reported in other crops such as rice (Taguchi-Shiobara *et al.*, 1997) and barley (Mano and Kotmatsuda, 2002). This has clearly showed that tissue culture traits can be mapped as QTLs in order to find out significant regions within the genome affecting the variation. Detection of QTLs opens up a fine view of quantitative genetic architecture and provides potential tools in marker-assisted selection.

The methodology of QTL detection is somehow laborious and involves complex mathematical calculations. However, computer software packages such as JoinMap and MapQTL have made mapping of QTLs possible for almost every species. Several QTL mapping approaches have been developed such as, Interval Mapping (Lander and Botstein, 1989) and MQM mapping (Jansen, 1993, 1994) which can be used together to improve the mapping resolution. A simple calculation for determining the significant LOD threshold for declaring a QTL was developed by Van Ooijen (1999) based on the result of stochastic simulation of a diploid species with a map density of one marker every 1cM. This helps to provide confidence in detection of QTLs and avoid false positives.

The objective of this study was to generate additional RFLP and AFLP markers to improve the dura (ENL48) and the pisifera (ML161) linkage maps developed by Chua *et al.* (2006). The dura linkage map constructed by Chua *et al.* (2006) consisted of 42 RFLPs and 36 AFLPs while the pisifera linkage map, had 65 RFLPs and 68 AFLPs. The QTL analysis was then carried out by using the updated framework maps to identify QTLs associated with TFC with the eventual aim to develop diagnostic tools for selection of ortets that are amenable to tissue culture.

MATERIALS AND METHODS

Plant Materials

The mapping material used in this study consisted of 87 F_1 palms produced by a cross between Ulu Remis Deli dura (ENL48) and Yangambi pisifera (ML161). The oil palm material was supplied by FELDA Agricultural Services Sdn. Bhd.

Tissue Culture Response: Time to First Callusing (TFC)

The tissue culture of the F_1 palms was carried out by the collaboration of seven major laboratories of the oil palm industry namely: Guthrie Biotech Laboratory Sdn. Bhd., IOI Corporation Bhd, United Plantation Bhd, Golden Hope Plantation Bhd., Ebor Laboratories, Applied Agriculture Research Sdn. Bhd. and Malaysian Palm Oil Board (MPOB). The cultures were observed closely for the first callus formation via TFC. The TFC, which is a phenotypic data, was transformed to $\arctan\sqrt{(x+1)}$ for improving the normality of variance prior to QTL analysis.

RFLP Analysis

RFLP procedures which included DNA extraction (Doyle and Doyle, 1990), digestion using various restriction enzymes (*Bam*HI, *Bcl*I, *Bgl*II, *Dra*I, *Eco*RI, *Hind*III, *Hin*dI, *Bst*NI, *Rsa*I, *Hae*III, *Taq*I and *Xba*I), electrophoresis, Southern blot and Southern hybridization were performed as described by Cheah *et al.* (1993). The oil palm cDNA probes used in the RFLP analysis were generated from different tissues and stages of development such as, young etiolated seedling, mesocarp, kernel, inflorescence, callus and embryoids which were kindly supplied by the MPOB Biological Research Centre (MBRC).

AFLP Analysis

Two restriction enzyme-combinations: *Eco*RI/*Mse*I and *Pst*I/*Mse*I were used for generating AFLP markers. The experimental assay for *Eco*RI/*Mse*I was carried out as described by the manufacturer in the manual (cat no. 10717-015 and 10719-011, Invitrogen™ Life Technologies) with some minor modifications as described by Rajinder *et al.* (1998). For *Pst*I/*Mse*I enzyme-combination, adapters and primers were synthesized by Invitrogen™ Life Technologies.

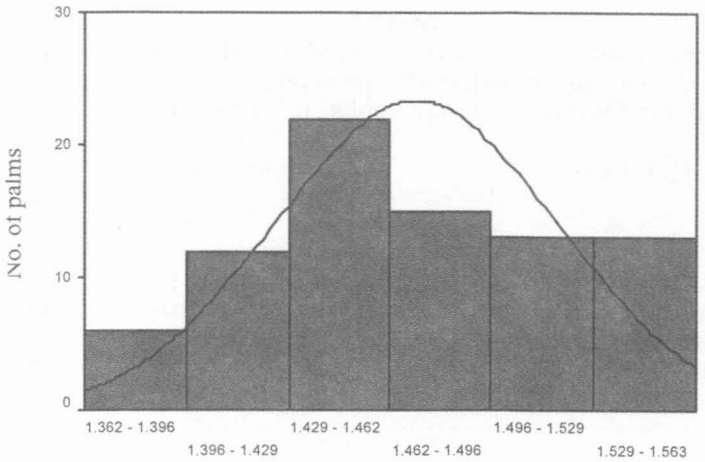

Fig. 1: Distribution of the time to first callusing trait (TFC) in F_1 mapping progenies

RFLP and AFLP Linkage Mapping

The RFLP and AFLP segregating data were expected to meet the Mendelian ratio in a pseudo-testcross strategy (Grattapaglia and Senderoff, 1994) and scored according to Lespinasse *et al.* (2000). The deviation from the hypothesis was tested using goodness of fit (X^2 test) at $p<0.05$. The linkage maps of dura and pisifera were constructed using JoinMap™ version 3.0 (Van Ooijen and Voorrips, 2001). Kosambi mapping function was applied for calculating the map distances in centiMorgan (cM). Linkage groups were determined at LOD score of 4.0 and recombination frequency 0.499. The order of markers arrangement was examined across the range from minimum LOD 1.0 to LOD 7.0.

QTL Mapping

QTL analysis was performed using the Interval Mapping function, MapQTL version 4.0 (Van Ooijen, 2002). In addition, a multiple-QTL model (MQM) mapping was applied that included the significant markers to control for genetic background effects. Associations between TFC and marker were determined at both 95% and 99% genome-wide empirical thresholds. The significant thresholds were calculated based on the formula:

$$1-\alpha_c = \sqrt[n]{(1-\alpha_g)}$$

where, α_g is the genome-wide significance level; α_c is the chromosome-wide significant level and n is the number of linkage groups. The LOD score at $1-\alpha_c$ value was then obtained in the cumulative distribution function Table 1. The method is described in detail by Van Ooijen (1999).

RESULTS

Mapping Family

The mapping family used in this study was obtained from a dura X pisifera cross. It was reported by Chin and Suhaimi (1996) that the Ulu Remis origin dura combines well with Yangambi pisifera and the progenies gave good oil yield. As such the cross is of importance in the breeding programme and will be well maintained.

Distribution of TFC Data

The $\arctan\sqrt{(x+1)}$ transformed TFC data was found to be normally distributed at $p<0.05$ (Kolmogorov-Sminov test). The frequency distribution of transformed TFC trait of the 87 F_1 palms is shown in *Fig. 1*. The TFC data showed a continuous distribution, covering a

TABLE 1

Significant TFC-marker associations detected at the 95% (LOD3.1 for pisifera and, LOD3.0 for dura) and 99% (LOD3.9 for pisifera and, LOD3.8 for dura) genome-wide threshold level. Empirical thresholds were calculated according to Van Ooijen (1999)

Linkage map	QTL-markers	Linkage group	QTL Position (cM)	LOD	Variation (%)
ML161	FDB112/EACG/MCTA>330/FDB120	4	0.0-24.9	6.85	62.1
ML161	CB6F/TAGG/HCTA-550	5	78.0-80.9	5.48	70.2
ML161	TAAC/HCAA-650	8	71.7	3.22	17.4
ENL48	EACA/MCAG-99	8	0.0	3.87	78.1
ENL48	G142	19	16.3	3.02	18.6

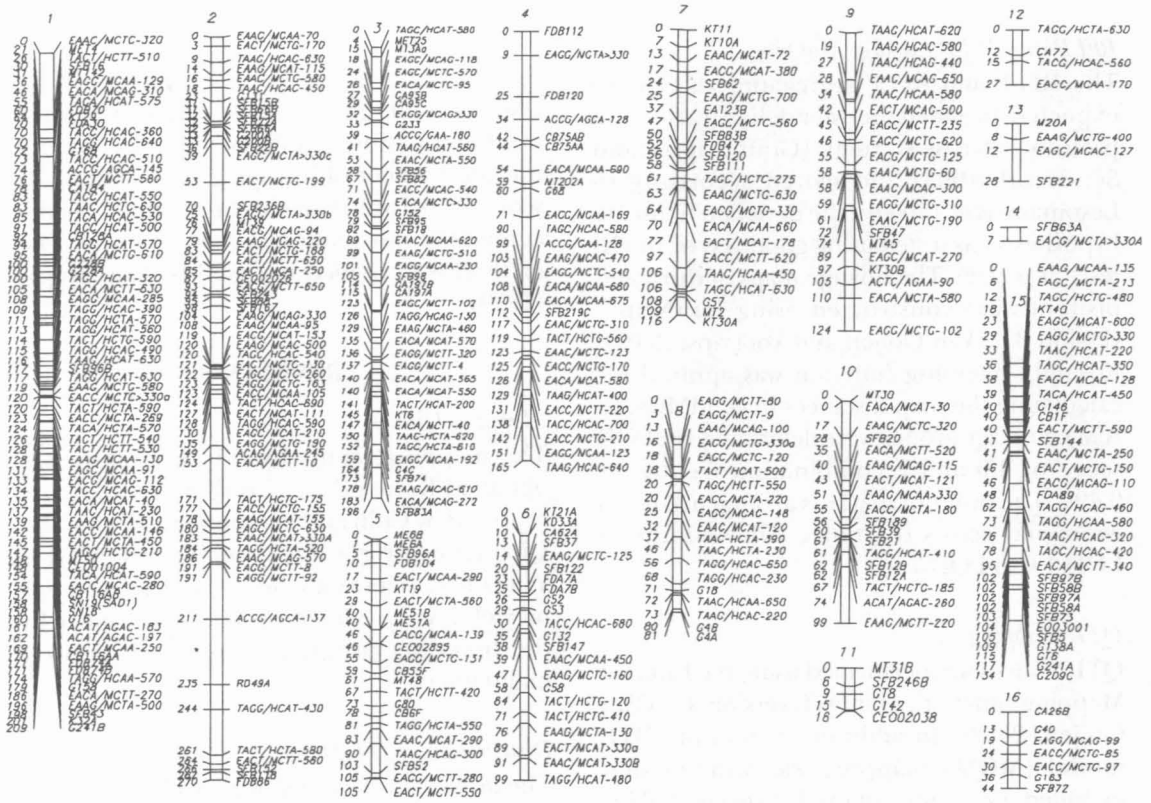

Fig. 2: Genetic linkage map of the pisifera (ML161) parental palm. Distances in Kosambi centimorgans (cM) are labeled on the left side of linkage groups

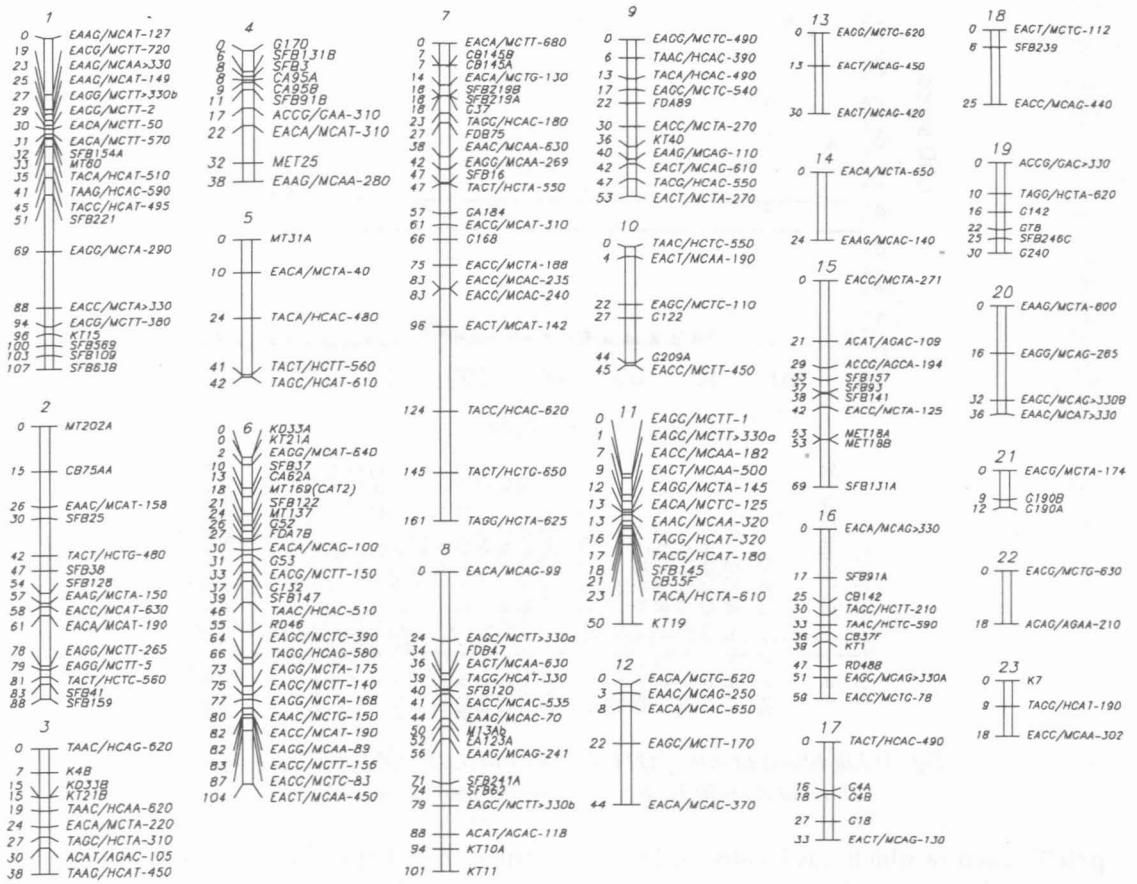

Fig. 3: Genetic linkage map of the dura (ENL48) parental palm. Distances in Kosambi centimorgans (cM) are labeled on the left side of linkage groups

wide range, 4 to 33 weeks with a mean of 12.10 ± 7.75.

RFLP and AFLP Linkage Mapping

A total of 299 and 173 segregating bands were scored from RFLP and AFLP analysis for the pisifera and dura parent, respectively. Linkage maps were constructed with density 395 loci/1,714cM for pisifera and 214 loci/1,225cM for dura. Correspondingly, the map distances between adjacent markers were 4.34cM and 5.72cM for the pisifera and dura maps, respectively. For pisifera, all the mapped loci were assigned into 16 linkage groups with size ranging from 7cM to 270cM *(Fig. 2)*. For dura, a map comprising of 23 linkage groups was constructed ranging from12cM to 161cM *(Fig. 3)*.

QTLs Associated to TFC

Using the Interval mapping analysis, three associated QTLs were identified in pisifera (ML161) and two in dura (ENL48) at the genome-wide significant threshold level calculated according to Van Ooijen (1999) (Table 1). Two highly significant QTLs, FDB112+EACG/MCTA>330+FDB120 and CB6F+TAGG/HCTA-550 were identified at

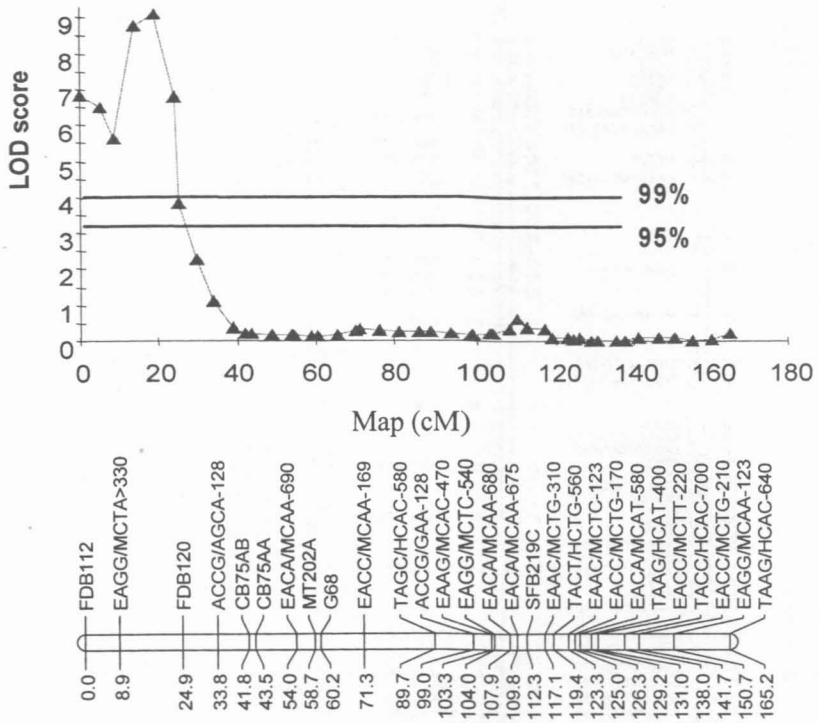

Fig. 4: Likelihood profile of QTL found at p<0.01 and p<0.05 genome-wide thresholds on linkage group 4 in the pisifera map (ML161)

p<0.01, each of which was located at linkage group 4 *(Fig. 4)* and linkage group 5 *(Fig. 5)* explaining 62.1% and 70.2% of the phenotypic variances, respectively. Another locus, TAAC/HCAA-650, located at linkage group 8 *(Fig. 6)* was found to be associated with TFC at p<0.05. In dura, two QTLs (EACA/MCAG-99 and G142) were detected at the end of linkage group 8 *(Fig. 7)* and at 16.3cM on linkage group 19 *(Fig. 8)*, respectively. Each of these QTLs accounted for 78.1% and 18.6% of the phenotypic variances of TFC, respectively.

DISCUSSION

A denser linkage map of dura and pisifera were produced by mapping of additional markers compared to the earlier map constructed by Chua *et al.* (2006). Additional 82 RFLP loci and 181 AFLP loci were successfully mapped and they produced a higher density pisifera linkage map. The additional markers have also linked up some of the linkage groups therefore, reducing the number to 16 linkage groups representing the basic chromosome number of oil palm (n=16). For example, linkage groups P2, P7, P15 and P17 from Chua *et al.* (2006) were linked together to form linkage group 2 in this study. Group 9 and group 15 were also formed by combination of groups P5 and P10 and; P6 and P8, respectively from the earlier map constructed. At the same time, the additional markers also formed new linkage groups such as groups 4, 14 and 16. The additional markers have widened the map coverage from 1,099.3cM to 1,714cM in this study. The total map length for pisifera reported here is close to the estimated genome size of 1,561cM calculated by Moretzsohn *et al.* (2000) for pisifera. The differences in map length may be due to the different marker systems, mapping programs and mapping functions used for constructing the linkage maps.

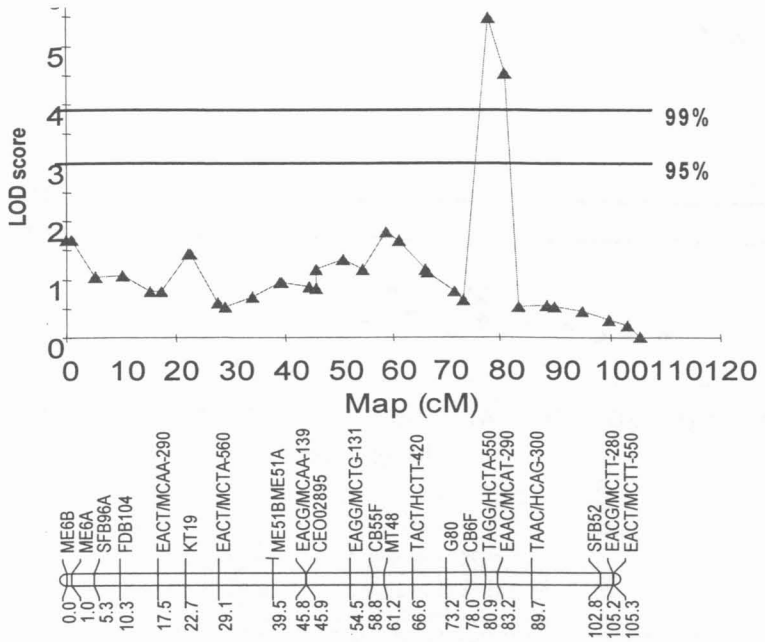

Fig. 5: Likelihood profile of QTL found at p<0.01 and p<0.05 genome-wide thresholds on linkage group 5 in the pisifera map (ML161)

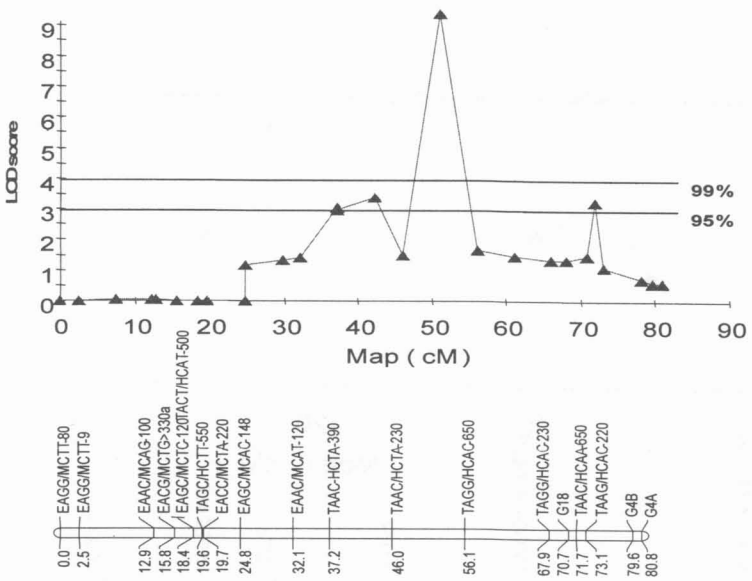

Fig. 6: Likelihood profile of QTL found at p<0.01 and p<0.05 genome-wide thresholds on linkage group 8 in the pisifera map (ML161)

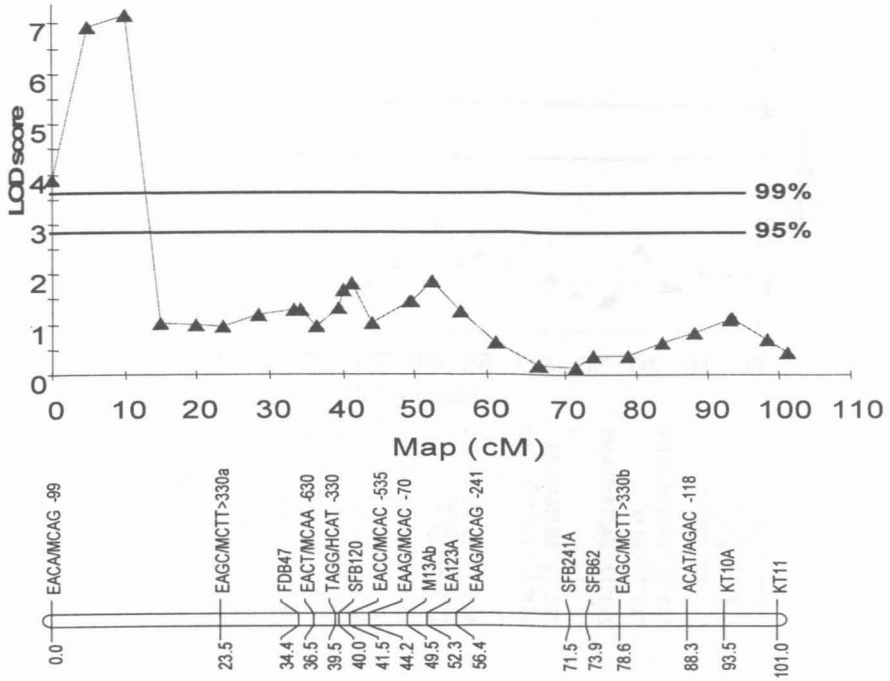

Fig. 7: Likelihood profile of QTL found at p<0.01 and p<0.05 genome-wide thresholds on linkage group 8 in the dura map (ENL48)

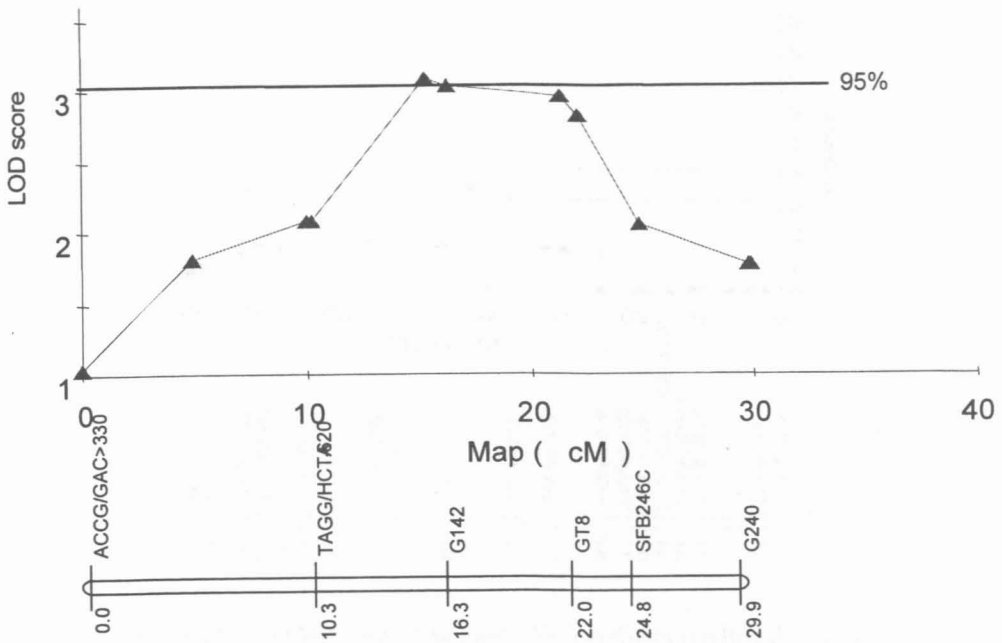

Fig. 8: Likelihood profile of QTL found at p<0.05 genome-wide threshold on linkage group 19 in the dura map (ENL48)

In the dura parental map, it was found that the additional mapped markers (44 RFLP markers and 93 AFLP markers) resulted in 23 linkage groups including some newly formed groups such as groups 12, 13, 14, 18, 20 and 22. Only linkage group 2 was formed by the integration of groups D10 and D11 from earlier map constructed by Chua *et al.* (2006). This resulted in an increase in the total map distance from 584.1cM to 1,225cM in this study. In order to further increase the density of the linkage maps especially for dura, mapping of SSR markers is in progress.

Quantitative trait loci analysis has detected a region at linkage group 4 of the pisifera map which, comprised of three markers, FDB112, EACG/MCTA>330 and FDB120, covering the distance between 0.0 to 24.9cM. The likelihood QTL profile *(Fig. 4)* of these markers fall within a narrow region. Each of the markers showed highly significant linkage to TFC trait by sharing an average LOD score of 6.9. In addition, correlation analysis (SPSS version 11.0) found that FDB120 was significantly ($p<0.05$) correlated to EACG/MCTA>330A ($r=0.337$) and FDB112 ($r=-0.548$). Therefore, it is appropriate to consider these 3 markers which are in close proximity to one another as representing a single QTL (labeled as FDB112/EACG/MCTA>330/FDB120) as was also described by Rance *et al.* (2001). Similar observations were made in linkage group 5 where CB6F and TAGG/HCTA-550 were detected close to each other on positions 78.0cM and 80.9cM, giving rise to a QTL for TFC. Comparison of genotypes between the two markers, revealed a significant similarity at $p<0.05$ and were highly correlated ($r=-0.905$) to each other. Hence, it is suggested that single QTL position is encompassing the two closely linked markers.

The QTLs on linkage groups 4 and 5 of pisifera and, linkage group 8 of dura showed a large proportion of phenotypic variance. The values are slightly higher than that for rice which is 38.5% and 32.6% for number of regenerated shoots per callus and regeneration rate, respectively (Taguchi-Shiobara *et al.*, 1997). These values are also higher compared to the QTLs associated with tissue culture traits in barley (Mano and Kotmatsuda, 2002) which explained phenotypic variations ranging from 10.0% to 42.8%. The differences observed are probably due to the different types of family structure used for mapping, where in rice and barley F_5 and F_{10} lines were used, respectively. As reported by Austin and Lee (1996), single QTL detected in F_2 were found dissected into multiple QTL in F_6 where individual QTL with smaller effect was detected. However, the proportion of phenotypic variance of none-tissue culture traits explained by certain QTLs can be very high in some cases. For example, QTL for flower bearing in F_2 sugi was found to account for 81.2% of the total phenotypic variance (Yoshimaru *et al.*, 1998). The high variance explained indicates that the markers identified in the current study have a major effect on TFC.

The LOD genome-wide significant thresholds calculated from the method presented by Van Ooijen (1999) were used as a guide to search for statistically significant QTL regions in oil palm genome affecting TFC trait. This is a relatively simple approach with reasonable accuracy in predicting true QTLs. The LOD scores calculated for detection of QTLs in pisifera were 3.1 at $p<0.05$ and 3.9 at $p<0.01$. For dura, LOD values of 3.0 and 3.75 were calculated as the significant levels at $p<0.05$ and $p<0.01$, respectively. The values are in close agreement with the LOD 3.4 ($p<0.05$) and 4.2 ($p<0.01$) used by Rance *et al.* (2001) in determining true QTLs for yield components in oil palm.

CONCLUSION

Genetic linkage maps for the pisifera and the dura parental palms have been improved by mapping additional RFLP and AFLP markers. The two linkage maps of ML161 and ENL48 were constructed with map densities of 395 loci/1,714cM and 214 loci/1,225cM, respectively. Using the framework maps, quantitative trait loci (QTLs) associated with time to first callusing (TFC) have been

identified for oil palm tissue culture. Three statistically significant QTLs contributing to TFC were detected in the pisifera map and two QTLs in the dura map. The QTL regions marked by RFLP markers (which essentially are cDNA clones) will prove useful for future efforts at map based cloning of the genes influencing the trait concerned. The marker(s)-QTL detected in this study are currently being verified in other crosses of oil palm.

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