



UNIVERSITI PUTRA MALAYSIA

***DNA MARKERS AND MAPPING OF QUANTITATIVE TRAIT LOCI FOR
YIELD AND BUNCH QUALITY IN DELI DURA X YANGAMBI PISIFERA OIL
PALM (*Elaeis guineensis* Jacq.) POPULATION***

SENG TZER YING

IB 2015 12



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By

SENG TZER YING

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of
Philosophy**

July 2015

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy

DNA MARKERS AND MAPPING OF QUANTITATIVE TRAIT LOCI FOR YIELD AND BUNCH QUALITY IN DELI DURA X YANGAMBI PISIFERA OIL PALM (*Elaeis guineensis* Jacq.) POPULATION

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July 2015

Chair : Assoc. Prof. Faridah Qamaruz Zaman, PhD
Faculty : Institute of Bioscience

Increased modern farming of the oil biosynthesis efficient oil palm, *Elaeis guineensis* Jacq., has propelled it to be the world's largest source of edible oil today. However, further oil yield improvement by conventional breeding is increasingly limited by lengthy time and costs due to long reproductive cycles, large plant size and an evaluation period of 10-15 years. Molecular tools which allow rapid, large scale evaluation over a short time, independent of plant age, will be particularly valuable in the face of such constraints. Towards such a goal, the aim of this particular study was to construct a genetic linkage map of a high yield oil palm population using DNA markers and to identify Quantitative Trait Loci (QTLs) related to oil yield components. This was followed by configuration of Quantitative Trait Alleles (QTA) with favourable and unfavourable effects on their respective oil yield components. The mapping population was a high-yielding Fel-da breeding cross, coded DA41, represented by 118 progeny palms. Besides the genotypic data generated in this study, phenotypic data of 21 yield components were available from ongoing field trials. The DNA markers employed for genotype data were microsatellites (SSR), Amplified Fragment Length Polymorphism (AFLP) and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) markers. A total 804 segregating marker loci (489 SSRs, 307 AFLPs and 8 PCR-RFLP) were used for final linkage analysis and map construction. The map of DA41 (ARK86D X ML161P) was 2398.8 cM long with 512 marker loci (368 SSRs, 135 AFLPs and 9 PCR-RFLPs), at an average 32 markers and a range of 15-59 markers per linkage group, and an average map density of 5 cM. The linkage group length was 77.5 cM to 223 cM, with an average of 150 cM. Taking the yield components phenotype data on board resulted in the detection of 164 QTLs associated with oil yield components. The QTLs had an average confidence region of 15.4 cM and no marker interval exceeded 50 cM. In the DA41 population, cumulative QTL effects increased in tandem with the number of QTL markers, matching the QT+ allele for each of the traits tested.

The many QTLs detected per trait suggested that the traits studied are polygenic with many genes of individual small effects on independent loci. However, the scope of the study did not rule out or rule in epistasis between different QTLs affecting a particular trait. Furthermore, several QTLs probably also show pleiotropic effects as seen by QTL clustering of inter-related traits on almost all the linkage groups, confirming the complexity of the genetic architecture of not only oil yield but also its components in the oil palm. The overall picture suggests that certain regions of the chromosomes are richer in the genes that affect the expression of a particular yield component trait and encompass pleiotropic, epistatic and heterotic effects. Hence, it will not be surprising if a large proportion of the identified additive effects from QTLs actually arise from digenic interactions between loci. For practical applications from this work, it will be necessary to test these yield component QTLs in a broader array of genetic backgrounds and in different environments. Also, more closely linked markers or flanking markers to the QTLs should be sought because recombinations between the markers and QTLs can occur when transferring the results from one population to another. Clearly, while this study has generated results that can be used in initial marker-assisted selection (MAS) for oil palm breeding, such as in population selection and enrichment, more detailed knowledge of marker-trait association will further contribute to more precise applications.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**PETANDA-PETANDA DNA DAN MENGENALPASTI LOKUS SIFAT
KUANTITATIF BAGI HASIL DAN MUTU TANDAN KELAPA SAWIT
(*Elaeis guineensis* Jacq.) PADA POPULASI DELI DURA X YANGAMBI
PISIFERA**

Oleh

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Peningkatan pertanian secara moden kelapa sawit *Elaeis guineensis* Jacq yang bercekap biosintesis minyak, telah menjadikannya tanaman minyak yang paling lumayan di dunia hari ini. Walau bagaimanapun, hasil kelapa sawit secara pembiakan konvensional yang memakan masa dan mahal. Ini disebabkan kitaran pembiakan yang panjang, saiz pokok yang besar dan tempoh penilaian yang lama (10-15 tahun). Kaedah molekular membolehkan penilaian skala besar dalam masa yang singkat tanpa bergantung kepada faktor umur pokok. Tujuan kajian ini adalah untuk membina peta rangkaian genetik kelapa sawit berhasil tinggi dengan menggunakan penanda DNA (asid deoksiribonukleik) serta mengenalpasti Lokus Sifat Kuantitatif (QTLs) yang berkaitan dengan komponen hasil minyak. Analisis lanjut untuk konfigurasi QTA yang mempunyai kesan meningkat/menurun terhadap komponen hasil minyak turut diuji. Kacukan Felda DA41 yang berminyak tinggi terpilih bagi uji-kaji ini, sebanyak 118 pokok. Selain daripada data genotipik yang dihasilkan dalam penelitian ini, data fenotipik daripada 21 komponen hasil juga sedia-ada. Penanda DNA digunakan untuk data genotipik adalah mikrosatelit (SSR), Polimorfisme panjang fragmen teramplifikasi (AFLP) dan Reaksi berantai polymerase-Polimorfisme Panjang Berkas Restriksi (PCR-RFLP). Sejumlah 804 penanda lokus (489 SSRs, 307 AFLPs and 8 PCR-RFLPs) telah digunakan untuk analisis rangkaian dan pembinaan peta akhir. Peta DA41 (ARK86D X ML161P) adalah 2398.8 cM dengan 512 penanda lokus (368 SSRs, 135 AFLPs and 9 PCR-RFLPs), dengan purata penanda lokus 32 dan pada lingkungan penanda lokus 15-59 dalam setiap rangkaian kumpulan, dan purata densiti peta adalah 5 cM. Rangkaian kumpulan sepanjang 77.5 cM to 223 cM, dengan puratanya 150 cM. Sebanyak 164 QTLs yang berkaitan dengan komponen hasil minyak telah dikesan. QTLs mempunyai kepastian dalam lingkungan 15.4 cM dan tiada selang penanda melebihi 50cM. Dalam DA41, kesan terkumpul QTL meningkat apabila bilangan penanda QTL padan dengan allele QT+ untuk semua sifat yang diuji. Banyak QTLs yang dikesan

bagi setiap sifat mengesahkan bahawa sifat yang dikaji memang dikawal oleh banyak gen mempunyai kesan individu yang kecil terhadap lokus bebas. Walaubagaimanapun, epistasis antara QTLs juga perlu diambil-kira menyebabkan hubungan yang lebih kompleks antara QTLs dan jumlah ekspresi suatu sifat. Malah QTLs mungkin memberi kesan pleiotropic, dimana mereka mengawal sifat berkait yang berbeza, walaupun mempunyai kedudukan kromosom yang sama/dekat. QTL yang berkelompok sebegini dilihat dalam hampir kesemua kromosom, mengesahkan kerumitan seni-bina genetik yang mengawal hasil dan komponennya dalam kelapa sawit. Kelompok ini menggambarkan kawasan-kawasan tertentu dalam kromosom yang lebih kaya dengan gen yang memberi kesan terhadap ekspresi suatu sifat komponen tertentu. Kajian ini mengesahkan warisan kompleks pada sifat-sifat komponen hasil yang merangkumi kesan pleiotropic, epistatic dan heterotic pada QTLs hasil minyak. Oleh itu, tidak mengejutkan jika sebahagian besar kesan tambahan QTLs yang dikenalpasti juga disebabkan oleh interaksi digenic antara lokus dalam kelapa sawit juga. Lebih banyak eksperimen perlu dijalankan menggunakan pokok kelapa sawit yang mempunyai sumber genetik dan persekitaran yang berbeza, bagi menguji QTLs hasil. Selain itu, penanda rapat atau penanda pengapit bagi QTL diperlukan dalam kajian seterusnya. Ini disebabkan penggabungan semula yang mungkin berlaku dalam populasi lain antara penanda dan QTLs. Walaupun kajian ini berjaya menjana hasil yang boleh digunakan dalam proses pemilihan berasaskan penanda (marker-assisted selection, MAS), pengetahuan yang lebih terperinci dalam assosiasi penanda-sifat akan meningkatkan penggunaannya.

ACKNOWLEDGEMENTS

I gratefully acknowledge the support, guidance and also encouragement of my chair supervisor, Assoc. Prof. Dr. Faridah Qamaruz Zaman. A special thank you goes to all co-supervisors, Prof. Tan Soon Guan, Dr. Rajinder Singh and Dr. Sharifah Shahrul Rabiah Syed Alwee, for their supervision and valuable comments that helped towards the completion of this study. Sincere thanks go to Dr. Enrique Ritter, Dr. Norbert Billotte, Ms. Ting Ngoot Chin, for assisting in my discovery of the research field, sharing their professional knowledge, giving valuable comments and constant encouragement.

My appreciation also extends to my laboratory colleagues in FGV Biotechnology Centre (FBC), especially Mrs. Jamilah Ismail, Mrs. Siti Hawa and Ms. Nurul Hafiza Ramli whose heartfelt support, patience, and assistance allowed the work to be completed successfully. I would like to thank all FBC staff for their helpfulness and willingness to share, thus making it possible to complete this work. Deep gratitude also goes to Mr. Chin Cheuk Weng, Mr. Nasruddin Mohamad, Mr. Mohd. Latif Kamarudin, Mr. Nardeman Samin and Mr. Yahor and the administration at breeding station, Pusat Penyelidikan Pertanian Tun Razak, Pahang for providing breeding materials.

Especial thanks go to Mr. Chang Kwong Choong (Andy) for editing my thesis and for the enduring encouragement. My special thanks go to a group of friends out of the lab, for their invaluable friendship, help, understanding, love and care. I wish to express my deepest gratitude and appreciation to my parents Seng Hoi Lum and Sing Mooi and also my siblings for their understanding, encouragement and endless love throughout my study. Last but not least, I thank my husband, Vengeta Rao, for a life of love, joy and courage. Also, my indebtedness to my two sons, Chenna and Manu, who were always so tolerant of the time that it took to finish this work and for their never failing love and support.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

Most of the abbreviations used are standard. However, attention is drawn to the following:

χ^2	Chi square values
ABI	Applied Biosystems (Perkin Elmer)
ABW	Average bunch weight
ACCase	Acetyl CoA-carboxylase
ACS	American Chemical Society
AFLP	Amplified Fragment Length Polymorphism
AMD	Average marker density
AMP-PCR	anchored microsatellite primed PCR
Ao	number of alleles observed per locus
AP-PCR	Arbitrarily Primed Polymerase Chain Reaction
ASAP	Allele-Specific Associated Primers
AVROS	Algemeene Vereniging van Rubberplanters ter Oostkust van Sumatra
BAC	Bacterial Artificial Chromosomes
BC	Backcross
BNO	Bunch numbers
BPRO	Breeding Populations of Restricted Origin
BSA	Bovine serum albumin
BWT	Bunch weight
BWT	Bunch Weight (kg)
CAP	Cleaved Amplified Polymorphic Sequence
CIM	composite interval mapping
CIRAD	Centre International Recherche Agricola et Developpement
cM	centiMorgan
CM	markers common to both parents
CRoPS	Complexity Reduction of Polymorphic Sequences
CTAB	Hexadecyltrimethyl ammonium bromide
D x T	<i>dura</i> x <i>tenera</i> cross
D	<i>dura</i> form
DAF	DNA Amplification Fingerprinting
DES	descent from P1: ARK86D or P2: ML161P
DNA	Deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
DPF	Dry Pericarp-to-Fruit (%)
<i>E</i>	expected number
e.g.	for example
EDTA	Ethylendiaminetetraacetic acid
eQTLs	expression QTLs
EST	Expressed Sequence Tags
EtBr	Ethidium Bromide
etc.	and the others
F(null)	null allele estimated frequency
F/B	ratio of total fruit weight to bunch weight

F ₁	First cross / Filial 1
F ₂	Intercross between two F ₁
FAC	Fatty acid composition
FAM	5-carboxyfluorescein (ABI fluorescent label – blue)
FASSB	FELDA Agricultural Services Sdn. Bhd.
FB	Fruit-to-Bunch (%)
FELDA	Federal Land Development Authority Malaysia
FFA	free fatty acid
FFB	fresh fruit bunch
FIB	Fibre-to-Bunch (%)
FIPS	Family and individual palm selection
FIWP	Fibre-to-Wet Pericarp (%)
GBS	Genotyping by-sequencing
GCA	General Combining Ability
GDP	Gross Domestic Product
GLM	Generalized linear model
GMO	Genetically modified organisms
H _{exp}	expected heterozygosity
H _{obs}	observed heterozygosity
HVR	Hypervariable Regions
HWE	Hardy-Weinberg equilibrium
I-C	interval length (confidence)
INEAC	Institut National pour l'Etude Agronomique du Congo
IP1	individual markers (parent 1 specific)
IP2	individual markers (parent 2 specific)
IRD	Infrared dye
IRHO	Institut de Recherches pour les Huiles et Oleagineux
ISA/ISSR	Inter SSR Amplification
IV	iodine value
JOE	(ABI fluorescent label – green)
KB	Kernel-to-Bunch (%)
KF	Kernel-to-Fruit (%)
KLM	Kuala Lumpur Melanocca
KY	kernel yield
LGs	linkage groups
LiCl	Lithium chloride
LOD	logarithm-of-the-odds
M/B	Mesocarp-to-bunch
M/F	mesocarp-to-fruit
MABW	Mean Average Bunch Weight From Pool Over Years Weight
MAS	Marker-Assited Selection
MB	Molecular breeding
MBNO	Mean Bunch Number Weight from Pool Over Years Weight
MFFB	Mean Fresh Fruit Bunch from Pool Over Years Weight
MFW	Mean fruit weight
MFW	Mean Fruit Weight (g)
MIM	multiple interval mapping
MKW	Mean Kernel Weight

MPOB	Malaysia Oil Palm Board
MP-PCR	Microsatellite (repeat)-primed PCR
MPW	Mean Pericarp Weight
MRRS	Modified reciprocal recurrent selection
MSW	Mean Shell Weight
Na ₂ OAc	Sodium acetate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NE-1P	average non-exclusion probability for one candidate parent
NE-PP	average non-exclusion probability for a candidate parent pair
NGS	Next Generation Sequencing
NH ₄ OAc	Ammonium Acetate
NIFOR	Nigerian Institute for Oil Palm Research
<i>Nig</i>	<i>Nigrescens</i>
NIL	Near Isogenic Lines
O	observed number
O/F	oil-to-fruit
O/M	oil-to-mesocarp
OWM	oil-to-wet mesocarp
OB	Oil-to-Bunch (%)
OD	Optical Density
ODP	Oil-to-Dry Pericarp (%)
OWP	Oil-to-Wet Pericarp (%)
OY	oil yield
P	<i>pisifera</i> form
p	Probability
PCR	Polymerase Chain Reaction
pers. comm.	Personal communication
PIC	Polymorphism Information Content
PIR	Protein Identification Resource
PKO	Palm kernel oil
PNG	Papua New Guinea
POS	position of QTL from left flanking marker of the interval
PRL	probability for Null hypothesis of no QTL
PV	phenotypic variance
PVP	Polyvinylpyrrolidone
PVP-40	Polyvinylpyrrolidone with molecular weight 40,000
“q”	derived from male parent
“Q”	derived from female parent
QT+	quantitative trait plus
QTA	Quantitative Trait Allele
QTL	Quantitative Trait Loci
r	recombination estimates
R ²	coefficient of determination
RAD	Restriction Site Associated DNA
RAMP	Randomly Amplified Microsatellite Polymorphism
RAPD	Random Amplified Polymorphic DNA
RE	Restriction Endonuclease

Rf	Restorer genes
RFLP	Restriction Fragment Length Polymorphism
RFs	recombination frequencies
RFU	Relative Fluorescent Units
RGAs	resistances gene analogues
RIL	Recombinant Inbred Line
RNA	Ribonucleic Acid
RNAase	Ribonuclease
ROX	Internal size standard (ABI fluorescent label – red)
rpm	revolutions per minute
S/F	shell-to-fruit
SAMPL	Selective Amplification of Microsatellite Polymorphic Loci
SCA	Specific Combining Ability
SCAR	Sequence Characterization Amplified Region
sCIM	simplified composite interval mapping
SCMV	Sugarcane Mosaic Virus
SDS	Sodium Dodecyl Sulphate
SF	Shell-to-Fruit (%)
<i>sh</i>	Shell gene
SM	single/simple marker analysis
SNP	Single Nucleotide Polymorphisms
SPAR	Single Primer Amplification Reaction
SPP	number of spikelets per panicle
SPSS	SPSS statistical software packages
SSR	Simple Sequence Repeat
STMS	Sequence-Tagged Microsatellite Sites
STR	Short Tandem Repeats
STS	Sequence Tagged Sites
T x T	<i>tenera</i> x <i>tenera</i> cross
T	<i>tenera</i> form
TAE	Tris-acetate EDTA
TAMRA	(ABI fluorescent label – yellow)
TBE	Tris-borate EDTA
TE	Tris-EDTA
TGW	1,000 grain weight
TM	total number of markers for linkage group
Tris	Tris (hydromethyl) methylamine
UV	Ultraviolet
V0	mean value of the trait across all palms without the band
V1	mean value of the trait across all palms with the band
Vdif (V1-V0)	difference between the mean
<i>Vir</i>	<i>Virescens</i>
VNTR	Variable Number Tandem Repeat
WAIFOR	West African Institute for Oil Palm Research
WFI	Water-to-Fiber (%)
WM/F	wet mesocarp-to-fruit
WPF	Wet Pericarp-to-fruit (%)
WWP	Water-to-Wet Pericarp (%)

CHAPTER 1

AN OVERVIEW

1.1 Introduction

The oil palm, *Elaeis guineensis* Jacq., is the world's most productive oil crop and has been an important crop for mankind for more than 5000 years (Zeven, 1967). The cultivation has contributed greatly to the economic development of otherwise backward rural areas by providing cash employment and higher earnings over the traditional, largely sustenance, agriculture. Palm oil is an important export commodity in some of the countries where the crop is grown, and is the second largest contributor to Malaysia's Gross Domestic Product (GDP). Besides Malaysia, oil palm cultivation has expanded rapidly in other parts of South East Asia, especially Indonesia, and, to a smaller extent, Africa and South and Central America.

Although the oil palm is the most productive oil crop, from intrinsic high oil yields coupled with breeding and agronomic improvements, the national Malaysian yield has stagnated for the past 20 years with fresh fruit bunch (FFB) production at ~20t/ha/yr, palm oil at ~4t/ha/yr and palm kernel (KY) ~1.0 t/ha/yr (Malaysian Palm Oil Board, 2013). With the rapid expansion of the world population particularly in the third world where dietary fat intake is still very low, edible oils and fats consumption is likely to increase tremendously, prompting increased production. For this to happen, simply expanding the area cultivated would be the easiest, but practically difficult for want of land and the increasingly strident calls for conservation. For example, in 2010 it was estimated that any future expansion in the oil palm area in Malaysia would only be to 1.3M ha (Malaysian Prime Minister's Department, 2010). This leaves the only option of increasing yield to solve this problem. There certainly has been yield improvement over, say, the last 50 years – about 70 percent due to breeding improvement and 30 percent due to better agronomic practices (Rosenquist, 1985; Davidson 1993; Corley and Tinker, 2003). The most important single breeding effort for yield improvement was the gain in bunch oil content from 16% (in the Dura type of oil palm) to 26% (in the tenera or DxP type) following discovery of the shell thickness gene and subsequent universal adoption of DxP planting materials (Hardon *et al.*, 1985, 1987). Following the advances with the shell gene, further yield gains were made through classical breeding for better duras and pisiferas to cross, i.e., with good general combining ability (GCA) and specific combining ability (SCA). Oil production as high as 14.9 t/ha/year from FFB yield of 45 t/ha/year and oil content of 35 percent have been recorded in experimental plantings (Rajanaidu and Kushairi, 2003). These impressive experimental yields are, nevertheless, still below the theoretical maximum of 18.2 t/ha/yr oil from 45t FFB (Corley 1983, 1985, 1998).

As noted above, classical oil palm breeding is time consuming and costly - due to long generation cycles, large plant size and an evaluation period of 10-15 years. For such crops, the new science of molecular breeding (MB) - using molecular markers to facilitate the breeding process (Mohan *et al.*, 1997; Hash and Bramel-Cox, 1999; Kumar, 1999) – beckons. Having markers for characters with easily detectable phenotypes can simplify the recovery of genes of interest linked to the traits, hence the vogue for Marker-Assisted Selection (MAS). MAS generally refers to using molecular markers, near to, or flanking, to a gene which effect on the phenotype is of interest, to identify and then select for/against the gene (Kumar, 1999). The markers are thus signposts for the whereabouts of the gene and indicate the part of the genome to manipulate, i.e., where to introduce/remove genes in the crop (Hash and Bramel-Cox, 1999; Young, 1999; Mackill, 2003). Once a gene is tagged with a marker, pre-selection for the gene (through the marker) can be made in even very young seedlings. Only the plants with the marker/trait of interest are retained/rejected. Furthermore, MAS can greatly reduce the number of breeding cycles by offering greater precision in palm selection in each cycle. The key to accelerating oil palm breeding through MAS is genetic linkage information, usually presented as linkage maps.

As maybe gathered from the above, oil yield is the most important economic trait in oil palm, hence the search for marker(s) for the shell gene which has veered most mapping work to *tenera x tenera* (Mayes *et al.*, 1997; Singh *et al.*, 2005), *dura x tenera* (Rhode, 2003; Billotte *et al.*, 2005) and *tenera x pisifera* (Moretzsohn *et al.*, 2000) crosses. However, away from the shell thickness hunt, direct higher oil yield in the oil palm (as opposed to more oil from less shell), like in most other crops, is a composite trait which final expression is the sum of a number of components that result in higher FFB yield and better fruit quality traits for oil in the bunch (Sparnaaij *et al.*, 1963). Thus, direct oil yield in oil palm is a complex trait under polygenic control, multiplicatively interrelated and highly influenced by the environment. Therefore, while a useful first step, MAS, based merely on markers for the single gene of shell thickness alone, is unlikely to contribute much to oil yield of the crop.

Hence, the main aims of this study are to detect and map QTLs affecting the oil yield components in oil palm and in passing, re-assess the interrelationships between oil yield and its components. The study was preceded by a survey of the genetic variation in existing potential mapping populations to obtain an indication of the potential improvement possible through breeding, and to identify suitable planting materials for mapping. The availability of reliable phenotype data for the traits of interest (oil yield components), from FASSB recording, for all selected individual palms in the proposed mapping population, was obviously also a key requirement. Three molecular marker assay systems with a slew of advantages – AFLPs, RFLPs and SSRs - were employed to genotype the mapping population.

1.2 Objectives

The objectives of this study were:

- i) To select of mapping population and survey the genetic variation of oil yield components in the population.
- ii) To identify polymorphic AFLPs, RFLPs and SSRs markers.
- iii) To construct genetic linkage maps for both parental palms, ARK86 and ML161 and construct a high density map by integrating both parental maps
- iv) To identify markers possibly associated with the quantitative traits linked to the oil yield components in oil palm.
- v) To determine the allelic configurations of each QTL and their specific effects (positive or negative) on the trait and compute the cumulative effects of all the QTL+ alleles for each trait.

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