

Original Research Article

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## Mapping of Quantitative Trait Loci (QTLs) for Oil Yield Traits using SSRs in African Oil Palm (*Elaeis guineensis* Jacq.)

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### ABSTRACT

Oil palm (*Elaeis guineensis* Jacq.) is a perennial monocotyledonous tree belonging to the family Palmae, with a diploid chromosome number,  $2n=32$ . Oil palm (*Elaeis guineensis* Jacq.) is an important edible vegetable oil crop which produces 4-6 tonnes of crude palm oil/ha. As oil palm crop is introduced in India from Africa, it is growing in India under different climatic conditions like high temperature, low humidity and less rainy days. There is a need to develop and strengthen the oil palm breeding program in India as there is a demand from the farmers to cultivate good yielding oil palm hybrids. In Parental analysis study, a total of 400 SSR markers of *Elaeis guineensis* were used to screen two parental genotypes. Out of 400 SSR markers analyzed for polymorphism, 19 SSR markers (4.75%) were polymorphic and these 19 polymorphic SSRs were used to genotype the 70 F<sub>1</sub> progenies of the 240D x 281D cross. So these identified markers were used for further studies such as linkage map construction and mapping QTL's for yield related traits by using simple interval mapping and composite interval mapping approaches.

#### Keywords

SSR markers, Dura oil palm 240D and 281D, Parental Polymorphism, QTL mapping.

#### Article Info

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### Introduction

Oil palm (*Elaeis guineensis* Jacq.) belongs to the family *Arecaceae* which contributes nearly 40 percent of edible vegetable oil production throughout the world<sup>1</sup>. The palm oil production is five times more than the annual oil yielding crops. In India, Andhra Pradesh (1.51

lakh ha area and 7.99 lakh tons production), Karnataka (0.38 lakh ha area and 1.01 lakh tons production), Tamil Nadu (0.28 lakh ha area and 0.05 lakh tons production), Mizoram (0.23 lakh ha and 0.09 lakh tons production) and Kerala are the principal oil palm growing states (Anupam *et al* 2015). Indonesia is the largest producer of palm oil followed by

Malaysia; however India is at its lag phase of growth in palm oil production. The oil palm genotypes are divided into *dura*, *pisifera* and *tenera* forms based on the shell thickness, which is a monogenic and co-dominantly inherited trait. Identification of these three fruit forms is a challenging task for oil palm breeders and growers. However, the fruit form determination can be possible only after 4±5 years by dissection of the fruit based on the thickness of shell and fibre ring, which requires a lot of time and space.

## Materials and Methods

### Plant Materials and Genomic Dna Isolation

In the present study, two DURA oil palm genotypes (240D and 281D) were selected which differ in yield and oil yield content to generate segregating populations. A total of 70 progeny palms from the cross 240D x 281D were raised at DURA block of IIPR, Pedavegi in the year 2000. The oil palm plantations were raised at ICAR-Indian Institute of Oil Palm Research (IIPR), Pedavegi, India (latitude 16° 48'N, longitude 81° 7'E).

The genomic DNA of 70 progeny oil palm genotypes was isolated by standard method as described by <sup>3</sup>Babu *et al* (2017) with few modifications such as repetition of chloroform: iso-amyl alcohol step to achieve good quality of DNA. The quality and quantity of genomic DNA was checked on 0.8% agarose gels along with uncut lambda DNA as a control. The DNA samples were normalized to a uniform concentration (25ng/μl) for SSR genotyping. Approximately 400 genomic SSR markers were used for genotyping of oil palm germplasm. The polymerase chain reactions (PCR) was performed in 20 μl reaction volume containing 2 μl of 10X buffer having 15 mM MgCl<sub>2</sub>, 0.2 mM of each forward and reverse primer, 2 μl

of 2 mM dNTPs, 0.2 μl of 1 U of *Taq* DNA polymerase (Invitrogen, USA), and about 25 - 50 ng of template DNA. The PCR amplifications was performed in a Thermocycler (MJ Research, USA) programmed for an initial denaturation of 3 min at 95°C followed by 35 cycles of 30s at 95°C, 30s of 50°C annealing temperature, extension of 1.0 min at 72°C, with a final extension of 10 min at 72°C, and hold at 4°C. The PCR products were fractionated on 3.0% Super Fine Resolution (SFR) agarose gel. The electrophoresis was carried out at 100 volts for 3hr at room temperature. Gels were stained with ethidium bromide and visualized using Bio Imaging System (BioRad).

## Results and Discussion

### Linkage Mapping

Mapping population was constituted of 70 F<sub>1</sub> progenies developed from a cross 240D x 281D parental palms. In a preliminary screening of 400 microsatellite markers, parents were found polymorphic for 19 SSRs. These 19 SSRs were considered reliable due to their co-dominant nature. The population was screened with this co-dominant subset of 19 putative polymorphic SSRs. Data for SSR markers was obtained in the form of A,B,H scoring which was then used for Linkage Map construction and QTL analysis. Linkage analysis and map construction were performed using Mapmanager software. Out of 19 SSRs, 13 SSRs were found linked with chromosome 1,6,8 and 15 consisted of 3 SSRs each, where as chromosome 8 consisted of 4 markers recorded. A total of 13 SSR were mapped to 4 linkage groups (C1,C6,C8,C15) of *Elaeis guineensis* genome (fig-1). Map was drawn with the help of QTL Cartographer after determining the best possible order by Mapmanager. The map covered four linkage groups of *Elaeis guineensis* with 13 polymorphic SSR primers.

## **Qtl Mapping for Yield Traits**

19 polymorphic SSR markers distributed on different chromosomes of *Elaeis guineensis* were used to map the QTLs associated with oil to dry mesocarp and oil to wet mesocarp on *Elaeis guineensis* in seventy (240D x 281D) progeny palms.

The genotypic and phenotypic data used in QTL cartographer software to identify the QTL's with these two approaches viz. simple interval mapping and composite interval mapping.

### **Simple interval mapping**

Simple interval mapping (SIM) analysis by WinQTL Cartographer 2.0<sup>4</sup>(Manly *et al* 2001) revealed a total of 3 QTLs for oil to dry mesocarp and oil to wet mesocarp in *Elaeis guineensis*. Out of these identified QTLs, 2 QTL's for oil to dry mesocarp, 1 QTL for oil to wet mesocarp were identified.

### **Oil to dry mesocarp**

In simple Interval mapping Two QTLs associated with oil to dry mesocarp in *Elaeis guineensis* were mapped on chromosome 1 at map position 38.7cM and 88.6cM respectively.

They showed the additive effect of 1.21 for qtl one and followed by 3.4 which had higher LOD score of 13.3 than qtl one which had LOD score of 9.3(Fig-2). These two QTLs one and two accounted for 2% and 9% of the phenotypic variation.

These two QTLs identified for oil to dry mesocarp had positive values for additive effect in simple interval mapping indicating

that the favoring alleles was from 240 Dura parent.

In earlier reports, Jeennor *et al* (2014) had reported one QTL associated with oil to dry mesocarp on linkage group 10 by using simple interval mapping method which had a LOD score of 3.8 and accounted for 25.9% phenotypic variance. They used MAPQTL 4.0 software programme<sup>6</sup>(Van Ooijen, 2002) for mapping the QTLs.

### **Oil to wet mesocarp**

In simple Interval mapping, One QTL associated with oil to wet mesocarp in *Elaeis guineensis* was mapped on chromosome 1 at map position 47.6cM which is having a LOD score of 2.1, additive effect of 1.10 and phenotypic variance of 3%(Fig-3). These two QTLs identified for oil to wet mesocarp had positive values for additive effect in simple interval mapping indicating that the favouring alleles was from 240 Dura parent. In earlier reports, Jeennor *et al* (2014) had reported one QTL associated with oil to wet mesocarp on linkage group 15 by using simple interval mapping method which had a LOD score of 3.0 and accounted for 28.5% phenotypic variance. They used MAPQTL 4.0 software programme (Van Ooijen, 2002) for mapping the QTLs.

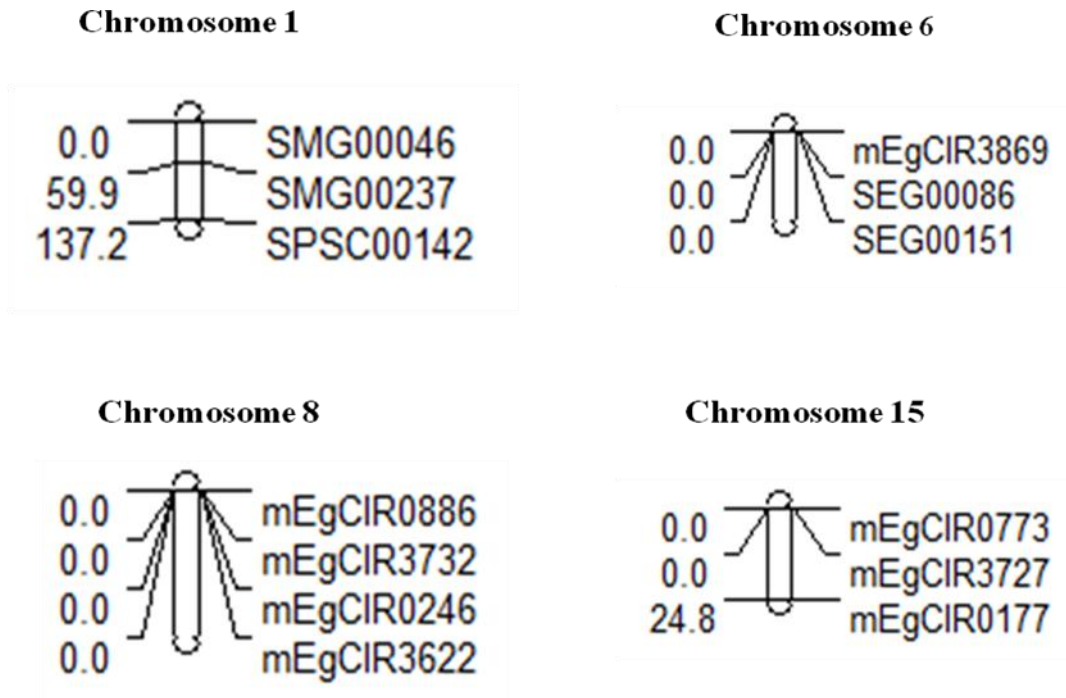
### **Composite interval mapping**

Composite interval mapping (CIM) analysis by WinQTL Cartographer 2.0(Manly *et al* 2001) revealed a total of 4 QTLs for oil to dry mesocarp and oil to wet mesocarp in *Elaeis guineensis*. Out of these identified QTLs, two QTL's for oil to dry mesocarp, two QTL for oil to wet mesocarp.

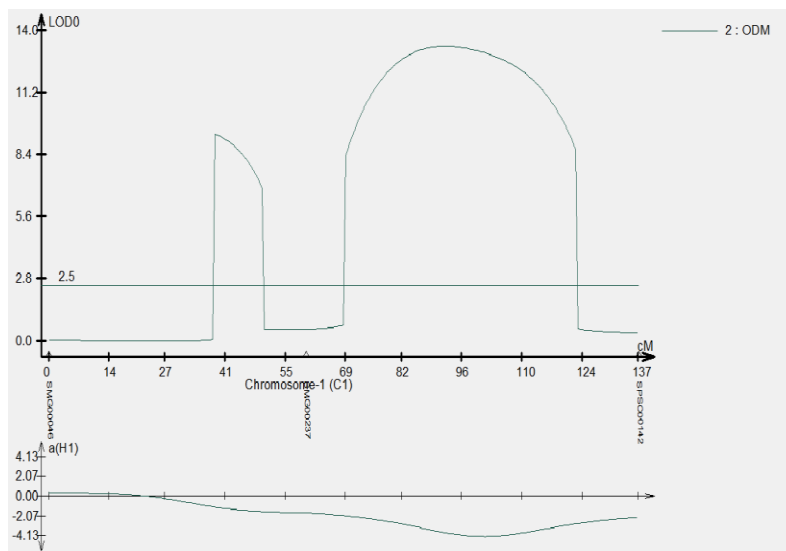
**Table.1** List of the primers that showed Polymorphism in both Parental and F<sub>1</sub> Analysis

Primer no.	Locus name	5'-3' Forward primer	5'-3' Reverse primer	Annealing temperature(°c)	Linkage group location
190	mEgCIR0773	GCAAAATTCAAAGAAAACCTTA	CTGACAGTGCAGAAAATGTTATAGT	52	15
215	mEgCIR2188	CGAAGTTGTTGGACATG	TTCCATCACAGGAGATATAG	52	9
168	mEgCIR0886	GATCTGCCGGTGCTCCTA	CTCAGTTTAGTCGATCCTTCCATTG	52	8
282	mEgCIR3544	AGCAGGGCAAGAGCAATACT	TTCAGCAGCAGGAAACATC	52	3
291	mEgCIR3693	TGCACACAGGCACACATA	AAAATGGGGTGTAGAGTTG	52	3
336	mEgCIR3869	CCAATGCAGGGGACATT	GAAGCCAGTGGAAGATAGT	52	6
339	mEgCIR3732	ATTTTATTTGGCTTGGTATA	ACTTTTCTATCTAATTCTTGAAGAT	52	8
352	mEgCIR3727	TATCTTATCTTTTACTCAACTA	CAGGTGACCAAGTGTAAT	52	15
62	SMG00175	TTTGTCTCCGTTTCCTTCTCT	CCCTAATCTCCTTCATCTCCTC	52	13
64	SEG00193	TGCCGTTGGTTAAGACTCC	GCGATGAGGAAGATGGTGAT	52	3
53	SMG00239	ACTGACATTTGGATCAGAGTT	GACTTCAATTTTACGCCTTTCT	52	7
86	SEG00086	GCAGCATTTCCCTCATTTC	TCGTATTCTAGGCTTCTCCCA	52	6
99	SMG00046	GCTCTTCTTCTATCATTTCATT	AAGACACAGTAGGGTGGTAGAT	52	1
109	SEG00151	ATCACAACAGCAGCAGCATC	CGCATCAAGAAACATGGAGA	52	6
41	SMG00237	CCAGGAGTGTAGAGAGTGAAGG	GGAAAAGAACAAGAGCAAACA	52	1
145	mEgCIR0177	TGAATGTGTGTGCAATGTGTAT	ATAGTCAATAATCGTAGGAAAATG	52	15
137	mEgCIR0246	GGTAAGAGATGAGATGGGTTGTC	AGGAATTAAGGGTTGTAGGTGAA	52	8
71	SPSC00142	AGAAACCCTCCAAACCATCC	GGCCAAATCATTTTTCCATC	52	1
300	mEgCIR3622	GCCAGTTAGGAATACAA	GTCACGCATTTTTCTTG	52	8

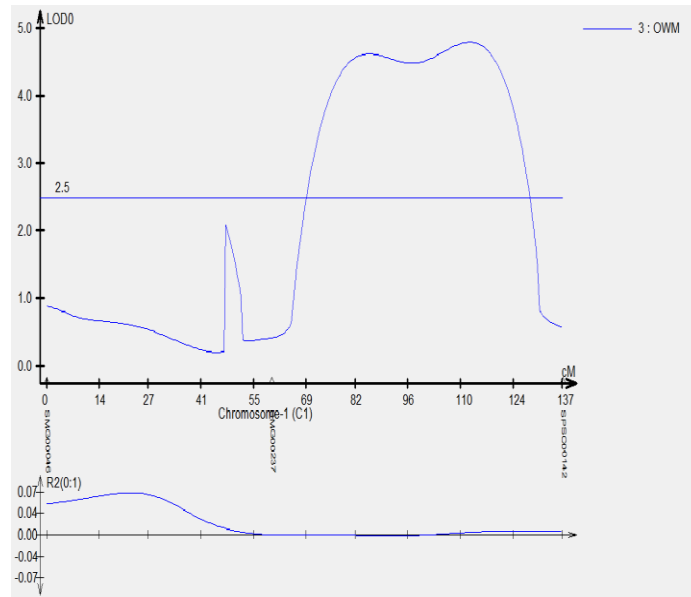
**Fig.1** Linkage map of *Elaeis guineensis* jacq. generated using 13 SSR markers in F<sub>1</sub> population derived from 240 x 281 Dura oil palm.



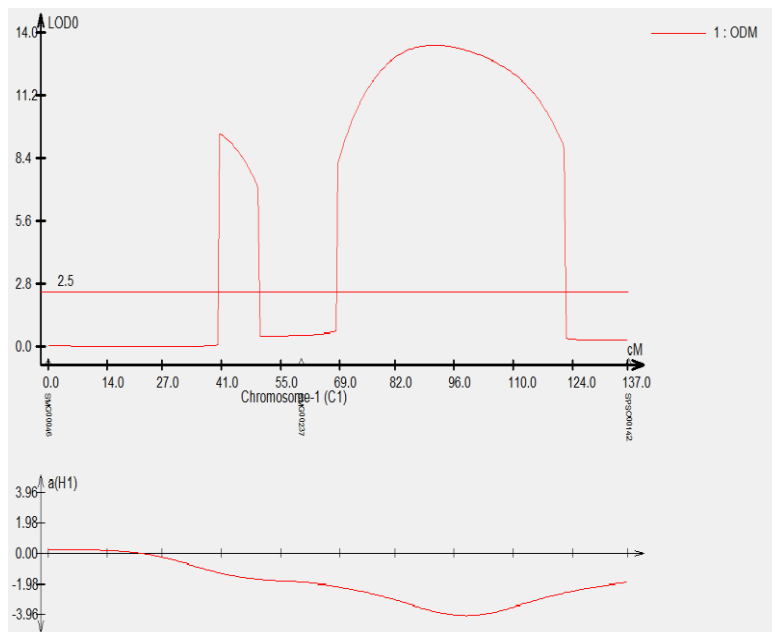
**Fig 2** QTLs distributed across the chromosome one for Oil to Dry Mesocarp using F<sub>1</sub> population derived from 240D x 281D Dura oil palm.(*Elaeis guineensis*) in Simple Interval Mapping.



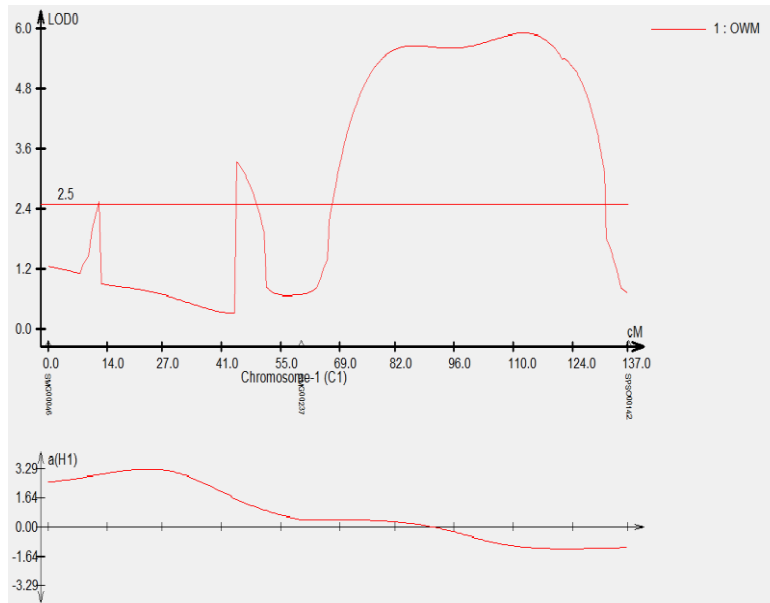
**Fig 3** QTLs distributed across the chromosome one for Oil to Wet Mesocarp using F<sub>1</sub> population derived from 240D x 281D Dura oil palm.(*Elaeis guineensis*) in Simple Interval Mapping



**Fig 4** QTLs distributed across the chromosome one for Oil to Dry Mesocarp using F<sub>1</sub> population derived from 240D x 281D Dura oil palm.(*Elaeis guineensis*) in Composite Interval Mapping.



**Fig 5** QTLs distributed across the chromosome one for Oil to Wet Mesocarp using F<sub>1</sub> population derived from 240D x 281D Dura oil palm.(*Elaeis guineensis*) in Composite Interval Mapping.



### Oil to dry mesocarp

Composite interval mapping (CIM) revealed that two QTLs associated with oil to dry mesocarp were mapped on chromosome 1 at map position 40.5cM and 88.6cM, respectively. They showed the LOD score of 9.5 for qtl one and 13.4 for qtl two (Fig-4). These both QTLs one and two accounted for 7% and 13% of the phenotypic variation.

These two QTLs identified for oil to dry mesocarp had positive values for additive effect of 1.13 and 3.54 in Composite Interval Mapping indicating that the favouring alleles was from 240 Dura parent. In earlier reports, Jeennor *et al* (2014) had reported one QTL associated with oil to dry mesocarp on linkage group 10 by using composite interval mapping method which had a LOD score of 3.8 and accounted for 25.9% phenotypic variance.

They used MAPQTL 4.0 software programme (Van Ooijen, 2002) for mapping the QTLs.

### Oil to wet mesocarp

Composite interval mapping (CIM) revealed that two QTLs associated with oil to wet mesocarp were mapped on chromosome 1 at map position 12cM and 44.7cM respectively. They showed the LOD score of 2.5 for qtl one and 3.3 for qtl two (Fig-5). These both QTLs accounted for 12% and 6% of the phenotypic variation.

These two QTLs identified for oil in wet mesocarp had positive values for additive effect of 2.92 and 1.56 in Composite Interval Mapping indicating that the favoring alleles was from 240 Dura parent. In earlier reports, Jeennor *et al* (2014) had reported one QTL associated with oil to wet mesocarp on linkage group 15 by using composite interval mapping method which had a LOD score of 3.0 and accounted for 28.5% phenotypic variance.

They used MAPQTL 4.0 software programme (Van Ooijen 2002) for mapping the QTLs.

In simple interval mapping and composite interval mapping two qtls for oil to dry mesocarp were detected. Whereas for oil to wet mesocarp in simple interval mapping one

qtl was detected and in composite interval mapping two qtls were detected. Here in both the methods all the qtls were detected on chromosome one only. In earlier reports Seng *et al* (2016) had reported QTLs associated with oil to dry mesocarp and oil to wet mesocarp both on linkage group 2. They had accounted for 11.99% and 15.07% of the phenotypic variance.

They mapped the QTLs using least square interval mapping with PROC NLIN computational analysis.

The QTLs detected in our present study cannot be directly compared to those of Rance *et al.* (2001), Jeennor *et al.* (2014) and Seng *et al* (2016) as there are no common markers between the maps and also the software used in our present study for mapping is WinQTL Cartographer 2.0 (Manly *et al* 2001) which is different from the software which they had used for mapping the QTLs.

In QTL mapping study two different methods were used i.e, simple interval mapping and composite interval mapping for QTL detection.

In Simple Interval mapping (SIM) analysis by WinQTL Cartographer 2.0 revealed a total of 3 QTLs for two yield traits in *Elaeis guineensis*. Out of these identified QTLs, two for oil in dry mesocarp, one for oil in wet mesocarp in *E.guineensis* in 70 progeny palms.

In Composite interval mapping (CIM) analysis by WinQTL Cartographer 2.0 revealed a total of 4 QTLs for two yield traits in *Elaeis guineensis*. Out of these identified QTLs, two for oil in dry mesocarp, two for oil in wet mesocarp in *E.guineensis* in 70 progeny palms. Results of our present study suggest that in terms of yield related QTLs, the most important linkage group is chromosome 1

with spanning major QTL for the entire yield related traits. Most prominent clustering signifying multifunctional QTL region was observed in the chromosome 1.

This multifunctional QTL region in the chromosome 1 contains at least one major QTL for two traits that are contributing towards yield such as oil to dry mesocarp, oil to wet mesocarp, in *Elaeis guineensis*.

QTLs identified in our study firstly need to be confirmed in other populations and then fine mapping of these yield related QTLs have to be done so that we can identify markers with close distance further to use them in marker assisted selection and breeding for yield related genotypes in *Elaeis guineensis* jacq.

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