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BRV Ramaraju
M.Sc. (Ag), Department of Molecular Biology and Biotechnology, Advanced Post Graduate Centre, Acharya N.G. Ranga Agricultural University, Lam, Guntur, Andhra Pradesh, India.

JV Ramana
Department of Molecular Biology and Biotechnology Advanced Post Graduate Centre, Acharya N.G. Ranga Agricultural University, Lam, Guntur, Andhra Pradesh, India.

B Kalyana Babu
Department of Molecular Biology and Biotechnology Indian Institute of Oil Palm Research (IIOPR) Pedavegi, West Godavari, Andhra Pradesh, India.

Y Satish
Department of Plant Breeding Regional Agricultural Research Station, Lam, Guntur, Andhra Pradesh, India.

RK Mathur
Department of Plant Breeding Indian Institute of Oil Palm Research (IIOPR) Pedavegi, West Godavari, Andhra Pradesh, India.

Correspondence
B Kalyana Babu
Indian Institute of Oil Palm Research (Iiopr), Pedavegi, Eluru, West Godavari District, Andhra Pradesh, India

Study of parental polymorphism in oil palm (*Elaeis guineensis* JACQ.) using SSR markers

BRV Ramaraju, JV Ramana, B Kalyana Babu and Y Satish

Abstract
Oil palm (*Elaeis guineensis* Jacq.) is a perennial monocotyledonous tree belonging to the family Palmae, with a diploid chromosome number, 2n=32. It is recognized as the high yielding edible oil crop. 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. In the present study 400 microsatellites markers were used for studying the parental polymorphism between the two Dura oil palm genotypes 240D and 281D. Out of which 19(4.75%) microsatellites were found polymorphic. So these identified Nineteen markers between these two parents were used for further studies such as linkage map construction and mapping QTL's for various morphological traits and improve their yields.

Keywords: SSR markers, dura oil palm 240D and 281D, parental polymorphism, QTL mapping

Introduction
Oil palm (*Elaeis guineensis* Jacq.) belongs to the family *Arecaceae* which contributes nearly 40 percent of edible vegetable oil production throughout the world [1]. The palm oil production is five times more the 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) [2]. 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 taken for parental polymorphism by using 400 SSR markers. The oil palm plantations were raised at ICAR-Indian Institute of Oil Palm Research (IIOPR), Pedavegi, India (latitude 16^0 48'N, longitude $81^0$7'E).

The genomic DNA of oil palm genotypes was isolated by standard method as described by Babu *et al.* (2017) [3] 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 lamda 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 $MgCl_2$, 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 ^{0}C followed by 35 cycles of 30s at 95 ^{0}C, 30s of 50 ^{0}C annealing temperature, extension of 1.0 min at 72 ^{0}C, with a final extension of 10 min at 72 ^{0}C,

and hold at 4 ^{0}C. The PCR products was fractioned 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 & discussion

Parental polymorphism analysis

In this present study a total of 400 SSR markers of *Elaeis guineensis* were used to screen two parental genotypes (240D and 281D). Out of 400 SSR markers analyzed for polymorphism in two parental *Elaeis guineensis* genotypes (240D and 281D), 19(4.75%) were polymorphic. The Agarose gel showing parental polymorphism for selected markers are shown below in (Fig 1A and Fig 1B) along with the list of 19 ssr loci along with forward, reverse sequence, minimum temperature and linkage group location are given in the table below in (Table 1.)

Jeennor *et al.* (2014) [4] had done similar parental polymorphism analysis in which he had taken DURA X PISIFERA oil palm cross by using 211 SSR markers. His results revealed 97(46%) SSRs are polymorphic due to heterozygous populations. Another results of parental polymorphism were also reported by H.P. Bhagya *et al.* (2018) [5] where they used the genomic DNA of eight oil palm genotypes which were amplified using 110 SSR markers and out of which 42 (38.1 %) loci were found to be polymorphic.

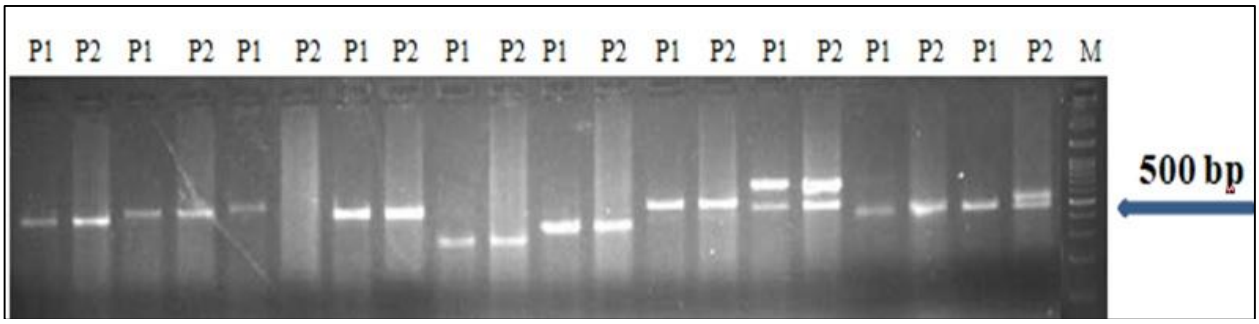

Fig 1A: Agarose gel showing polymorphism among parents P1-Parent 1(DURA-240), P2-Parent 2 (DURA-281) and M-100 bp DNA Marker

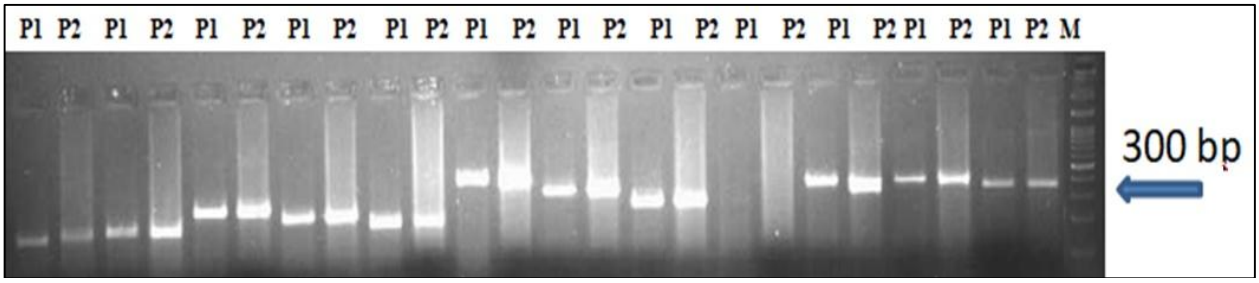

Fig 1B: Agarose gel showing polymorphism among parents P1-Parent 1(DURA-240), P2-Parent 2 (DURA-281) and M-100 bp DNA Marker

Table 1: List of the primers that showed Polymorphism in Parental Analysis.

Primer no.	Locus name	5'-3' Forward primer	5'-3' Reverse primer	Annealing temperature(°c)	Linkage group location
190	mEgCIR0773	GCAAAATTCAAAGAAAACTTA	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	GAAGCCAGTGGAAAGATAGT	52	6
339	mEgCIR3732	ATTTTATTTGGCTTGGTATA	ACTTTTCTATCTAATTCTTGAAGAT	52	8
352	mEgCIR3727	TATCTTATCTTTTACTCAACTA	CAGGTGACCAAGTGTAAT	52	15
62	SMG00175	TTTGTCTCCGTTTCCTTCTCT	CCCTAATCTCCTTCATCTCCTC	52	13
64	SEG00193	TGCCGTTGGTTTAAGACTCC	GCGATGAGGAAGATGGTGAT	52	3
53	SMG00239	ACTGACATTTGGATCAGAGTT	GACTTCAATTTTACGCCTTTCT	52	7
86	SEG00086	GCAGCATTTTCCCTCATTTC	TCGTATTCTAGGCTTCTCCCA	52	6
99	SMG00046	GCTCTTCTTCCTATCATTCATT	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

Conclusion

A total of 400 SSR markers of *Elaeis guineensis* were used to screen parental genotypes (240D and 281) in this study. Out of 400 SSR markers analyzed for polymorphism in two parental *Elaeis guineensis* genotypes (240D and 281), 19 (4.75%) were polymorphic. So these identified Nineteen markers between these two parents can be used for further

studies such as linkage map construction and mapping QTL's for various morphological traits and improve their yields.

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